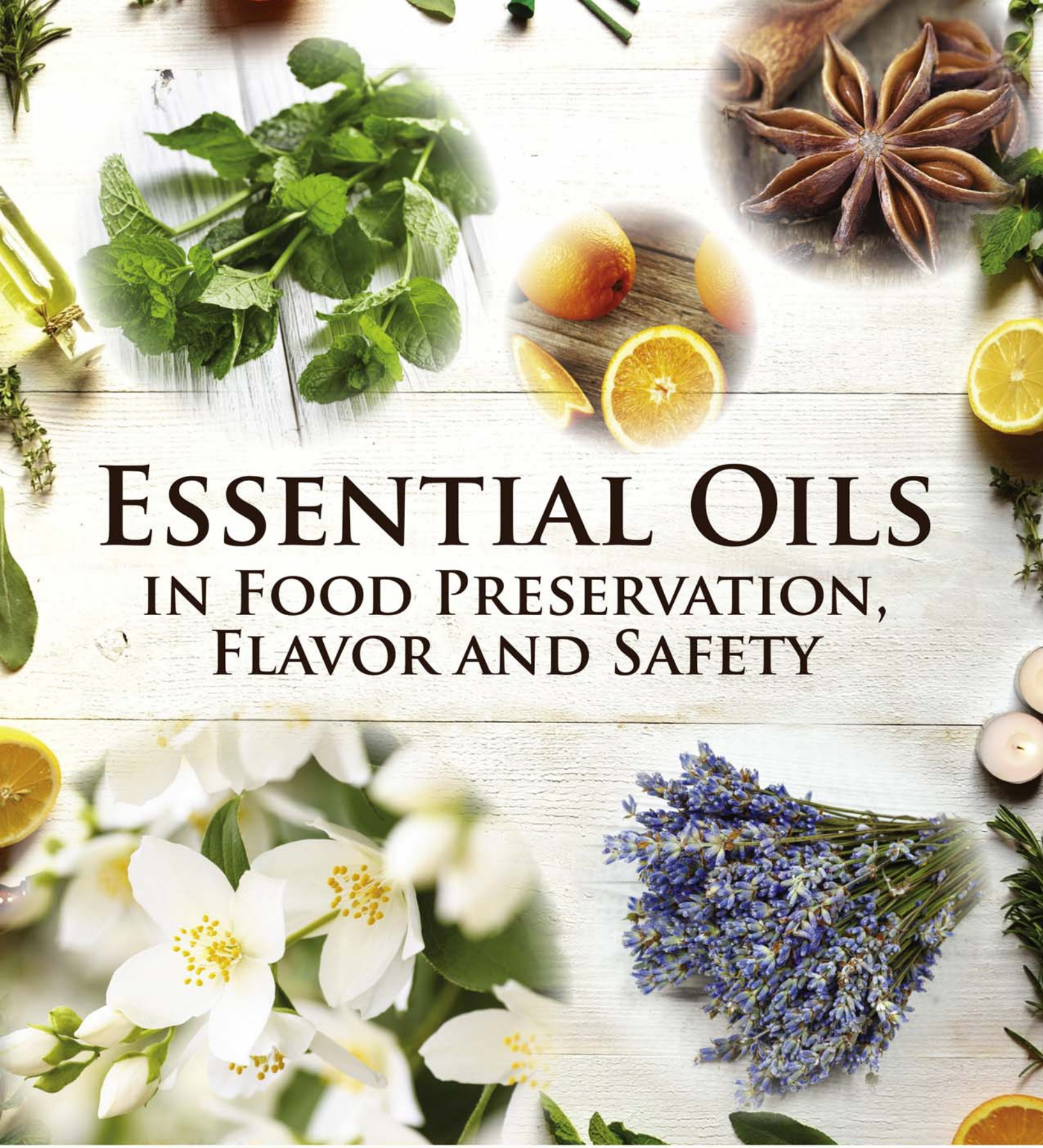

Essential Oils in Food Preservation, Flavor and Safety

Edited by

Victor R. Preedy

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Preface

Essential oils are derived from flowers, roots, bark, leaves, fruits, seeds, and other parts of plants. Their extraction or manufacture entails the use of local domestic methods or more commercial large scale industrialized processes. Some of the usage of essential oils in food science is based on traditional practices or folklore. In contrast, more modern research has entailed the discovery and characterization of novel lipid fractions with key biological features. On the other hand, details on the uses of essential oils in enhancing food favor or food production (including preservation and safety) are somewhat fragmentary or sporadic. Moreover, some of the documented material on essential oils has paid little attention to the scientific basis for their use. There are many essential oils; some are derived from common plants while others are rarer or more exotic. Even the definition of essential oils is variable, with different extraction methods producing divergent chemical profiles. These limitations and issues have, however, been succinctly addressed in **Essential Oils in Food Preservation, Flavor and Safety** which has details on over 80 essential oils.

This is an authoritative synopsis of many of the complex features of the essential oils as applied to food science; ranging from production and harvesting to their antispoilage properties. This book embraces a holistic approach and is divided into two distinct Parts:

Part 1, General Aspects
Part 2, Named Essential Oils

Chapters in Part 2 have three subsections:

1. Botanical aspects
2. Usage and applications
3. Usage and applications in food science

The unique subsection **Usage and Applications in Food Science** emphasizes the fact that essential oils are frequently used to impart flavor and aroma or prolong storage. The use of essential oils in counteracting food-borne pathogens and other areas of food science are also extensively documented in this section.

Part 1 has 16 chapters starting with a description of essential oils, what they are, and how the term essential oil is defined. There follows material on characterization, authentication, adulteration, cultivation, extraction methods, food pests, microorganisms, arthropods, agricultural production systems, food preservation including meat, poultry, beverages, nanoformulations, microencapsulation, organoleptic properties, food packaging, edible films, and other food applications.

Part 2 has material on eighty three common and rarer oils, as follows: African cardamom, agarwood, ajwain, Amazon rosewood, angelica, aniseed, anise myrtle, *Annona* species, basil, bay laurel, bergamot, bitter gourd, bitter orange, black cumin, black pepper, caraway oils, cardamom, carrot, carvone, cedar, celery, cinnamon, clove, coriander, costmary, cumin, curry leaf, curry plant, dill, eucalyptus, fenugreek, frankincense, garlic, ginger, grape, grapefruit, hyssop, iris, jasmine, juniper, lavender, lemongrass, lemon myrtle, licorice, lime, lovage, Mexican oregano, mint, mugwart, myrtle, neem, negundo chaste tree, nutmeg, onion, oregano, patchouli, pune-sa, rockroses, rose, rosehip, rosemary, rose pepper, rose-scented geranium, saffron, sage, sandalwood, Shirazi thyme, spiked ginger lily, star anise, summer savory, sweet fennel, sweet flag, sweet orange, tagetes, tangerine, tarragon, Tasmanian pepper leaf, thyme, turmeric, vetiver grass, wormwood, yellow and white mustard, and ylang–ylang.

The contributors are authors of international and national standing, leaders in the field, and trendsetters. Emerging fields of food science and important discoveries relating to essential oils are covered in this book. This represents essential reading for food scientists and those optimizing food production and safety in the various steps from “field to plate”.

Professor Victor R. Preedy

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Part I

General Aspects

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Chapter 1

Essential Oils: What They Are and How the Terms Are Used and Defined

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INTRODUCTION

An Historical Overview

Essential oils are aromatic and volatile liquids obtained from plant material, including flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plants (Hyldgaard et al., 2012). Throughout history, these oils have been regarded with great interest, although many of their uses have been lost with time, it is generally accepted that human beings have been extracting them from aromatic plants since the dawn of humanity. The applications of essential oils for different purposes are varied and include not only their use in cooking to enhance the taste and health benefits of food, but also their application in the manufacture of perfumes and cosmetics.

The ancient Egyptians used essential oils in medicine, perfumery, and in the art of embalming and preparing bodies for burial through mummification. In ancient Asia, the Vedas codified the uses of perfumes and aromatics for both liturgical and therapeutic purposes. Indeed, throughout history, numerous civilizations have used essential oils and fragrances for various purposes, including for religious ceremonies, in the production of perfumes, or as therapeutic agents against infectious diseases. The Phoenicians, Jews, Greeks, Romans, and other cultures situated around the Mediterranean basin as well as the Mayas and Aztecs in the Americas all possessed a fragrance culture of great refinement (Sonwa, 2000).

After the fall of the Roman Empire and with the advent of both Christian and Muslim civilizations, the art and science of fragrance were brought to the Arab world, where it reached a high level of sophistication. In the Middle Ages, this knowledge of fragrances was brought back to Europe by the Crusaders returning from the Holy Land and was developed further by alchemists as well as in the monasteries. Alchemists sought to create the "elixir of life" in order to live indefinitely while the monasteries used essential oils for obtaining various medicinal products to heal different diseases or for making aromatic derivatives such as soap and perfume. During the Renaissance, the use of essential oils in perfumery and cosmetics was extended throughout the world (Sonwa, 2000).

Concept and Definition

Essential oils are odiferous, highly volatile substances present in plants. Because of their volatility, these substances can be isolated by means of steam distillation from an aromatic plant of a single botanical species and can be detected by both smell and taste. Individual essential oils are known by the name of the plant from which they are derived and the odor is similar to that of the part of the plant from which they are obtained, although the aroma is generally more intense.

There are various definitions of essential oils, but the most accurate is probably that proposed by Schilcher, Hegnauer, and Cohn-Riechter, which was summarized by Sonwa (2000): "Essential oils are products or mixtures of products, which are formed in cytoplasm and are normally present in the form of tiny droplets between cells. They are volatile and aromatic". They are composed of "mixtures of fragrant substances or mixtures of fragrant and odorless substances", where a fragrant substance is defined as a "chemically pure compound which is volatile under normal conditions and which owing to its odor can be useful to society".

For its part, the International Organization for Standardization (ISO) has defined essential oils as "product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase—if any— by physical processes", going on to specify that "the essential oil can

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undergo physical treatments, which do not result in any significant change in its composition." Still other groups, societies, and companies have established their own criteria of concept and quality. For example, Association Française for Normalization, Generally Recognized As Safe, Fragrance Materials Association, the International Fragrance Association, Bundesinstitut für Risikobewertung, Research Institute for Fragrance Materials, and Scientific Committee on Consumer Safety have all established norms to regulate the maximum quantities and uses of certain oils as well as the single compounds therein (Sonwa, 2000; Turek and Stintzing, 2013). Nevertheless, the ISO standards are generally accepted worldwide.

As defined above, essential oils are complex mixtures, usually formed by numerous constituents, generally liquid, but sometimes solid. At room temperature, these mixtures usually range from colorless to slightly yellowish in color when freshly distilled, with an aromatic smell, very clean to the touch, and easily absorbed by the skin. In contrast with fatty vegetable, animal or mineral oils, a drop of essential oil on paper disappears quickly, taking between a few minutes and a few days, depending on the temperature. In addition, essential oils usually have a low density, except in particular cases such as cinnamon, clove, and sassafras oils, which are denser than water. They have a poor solubility in water, but are soluble in most common organic solvents, including ethanol and diethyl ether, and mix well with vegetable oils, fats, and waxes.

Essential oils have a high refraction index and rotatory power, which could be of interest for their identification and quality control; however, the latter tends to be variable because of the different codices currently used around the world.

Variability of Essential Oils

Almost 3000 different essential oils have been described. Of these, about 300 are used commercially in the flavoring and fragrances market (Burt, 2004). However, the high variability in the chemical composition of aromatic plants presents a potentially serious problem for the perfume manufacturing industry. For this reason, much research has focused on the various factors contributing to this variety other than strictly genetic ones. For example, investigators have described distinct races of the same species, for example, *Melaleuca bracteata*, that are rich in different chief constituents, each of which produces a different essential oil with either methyl eugenol, methyl isoeugenol, and elemicin. Other aromatic plants, such as sweet flag (*Acorus calamus*), wormwood (*Artemisia absinthium*), sweet basil (*Ocimum basilicum*), lemon balm (*Melissa officinalis*), thyme (*Thymus vulgaris*), camphorwood (*Cinnamomum camphora*), peppermint (*Mentha piperita*), or tansy (*Tanacetum vulgare*) have been widely studied and different chemotypes and chemical races have been described (Evans, 2009). It has likewise been discovered that other factors can modify the chemical composition of essential oils, such as climate, rainfall, or geographic origin of the plant.

Presence and Functions in the Vegetable Kingdom

Essential oils are usually formed as secondary metabolites. In many cases they are stored in nondifferentiated cells (Lauraceae) or secreted organs, such as glandular hairs (Lamiaceae and Asteraceae), secretory ducts (schizogenous in Myrtaceae and schizolysigenous in Rutaceae), or cavities (Conifers). Sometimes, the essential oil is not formed in the plant itself, but it is produced through hydrolysis of some compounds present in the plant, as is the case in valeriana or garlic (Evans, 2009; Franz and Novak, 2010).

With respect to their localization, essential oils can be formed in all parts of the plant, including the aerial parts, usually made up of flowers, leaves, and stems (chamomile, peppermint, lavender); bark (cinnamon); fruits (anise); seeds (nutmeg); as well as in the radix and rhizomes (curcuma and ginger).

The role of essential oils in a given plant varies: in some cases they are used for pollination, in other cases they are employed as a defense mechanism, often as a repellent or irritant. Moreover, there are different theories about their possible role as antioxidants in that they donate hydrogen in oxidative reactions, especially in the presence of light. They are also thought to be antifungal and antibacterial, protecting the plant from possible pathogenic attack (Evans, 2009).

Obtaining Essential Oils

Essential oils can be obtained by means of water distillation, water and steam distillation, or steam distillation alone. In fact, these are the most traditional and commonly used methods. When the solubility of a certain essential oil in water is high, as in the case of geranium, rose, or lavender, another process, namely cohobation, can be used. In this protocol, in order to avoid the loss of hydrosoluble compounds, the condensed water is returned from the separator back to the still. Other processes for obtaining essential oils include maceration and enfleurage, as well more modern techniques such as extraction with solvents or supercritical fluids. Maceration can be used when the yield from distillation is poor, while enfleurage and solvent extraction is suitable for expensive, delicate, and thermally unstable materials (Chamorro et al., 2012).

In general, essential oils are obtained through steam distillation or water distillation from different parts of the plant, including the whole plant or just the wood, bark, roots, leaves, flowers, fruits, or seeds (Hyldgaard et al., 2012). The plants can be fresh, partially dehydrated, or dried, but in the case of flowers, they must be fresh. Although these processes will be reviewed in other chapters, some reference to the extraction method should be made here, since the term essential oil is generally synonymous with steam distillation, with the expected compounds being volatile derivatives in all cases. However, in processes in which solvents are used, the chemical composition varies with respect to the chemical composition of the same essential oil obtained through distillation. Moreover, the final composition is different when the plant material is put together with water and suffers high temperatures during a significant period. When water and plant material are kept in different recipients and the water vapor passes through the plant material, the contact is short and the oil can be collected just a few minutes after commencing the process (Chamorro et al., 2012).

One alternative to distillation that avoids chemical alteration to the final product is supercritical CO_2 extraction. This process tends to leave labile compounds and a wide range of other principles unaltered, for example, when purified by means of this procedure, the final product may contain nonvolatile flavonoids (Herzi et al., 2013). Likewise, when an essential oil is obtained by means of solvent extraction, including enfleurage, the extractive liquid may dissolve compounds such as resinoids and other nonvolatile derivatives. Different results can be obtained with other processes, such as cold pressing or squeezing of the outer peel of the fruits (principally citrus) in presses, then decanting or centrifuging the oils to separate the plant material and the aqueous remains. In all these cases, the chemical composition of the essential oils is quite different from that the obtained through distillation; however, they are grouped together under the same general concept (Chamorro et al., 2012).

Control and Analyses

Different methods have been used to control and analyze essential oils (Zellner et al., 2010). Currently, however, the identification of essential oil components is usually carried out with the aid of gas chromatography–mass spectrometry (GC–MS) equipped with flame ionization detector (FID) and MS detectors, a capillary column (30 m × 0.25 mm, film thickness 0.25 μm), and a split. Test conditions may vary depending on the column and the sample. For example, GC–MS experiments are often performed with injector and detector temperatures of 250 and 270 °C, respectively, with the oven initially at 50 °C for 2–3 min and then increasing by 3–10 °C/min until it reaches 200–240 °C, with a flow of 0.7–1.0 μL/min. Normally, helium is used as a carrier gas at a flow rate of 0.7–1.0 mL/min, the samples are diluted (1/10, v/v), and the volume injected is 0.2 μL in the split mode (split ratio 1:44). Results are usually processed with a library mass spectral search program (Viuda-Martos et al., 2007; Chamorro et al., 2012).

Still, the evolution of analytical techniques has increased both the quality and capacity of analysis. For example, Filippi et al. (2013) used a comprehensive two-dimensional GC to analyze essential oils from vetiver employing a GC–MS system equipped with a GC × GC with a cryogenic dual jet/loop modulator and a secondary oven installed in the main GC oven. In a similar vein, Pellati et al. (2013) used GC–MS and GC–FID techniques for the phytochemical analysis of essential oils. Thus, elemental analyzer–isotope ratio mass spectrometry (IRMS) and GC–combustion (C)–IRMS were used to determine the $\delta^{13}C$ composition of bulk samples as well as some specific components. The determination of the $\delta^{13}C$ isotopic fingerprint by means of GC–C–IRMS represents an effective, rapid, and convenient method for authenticity assessment of target components from essential oils.

CHEMICAL COMPOSITION

Essential oils are composed of lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300. In the very first definitions of essential oils, these were strongly identified with terpenes, principally mono- and sesquiterpenes. However, with the identification of other types of compounds, specifically allyl and isoallyl phenols, the original definition changed. Different terpenes with a higher number of carbons have also been found, principally diterpenes. Other substances have also been identified in volatile oils obtained by distillation. This is due to the fact that many other plant metabolites such as fats, coumarins, anthraquinones, and certain alkaloids are distillable, whereas some compounds are derived from glycosides, which are transformed during the distillation process. In general, the main components of essential oils are formed by mono- and sesquiterpenes. In some cases, the major derivatives are hydrocarbons (e.g., turpentine, formed by α- and β-pinene) whereas in others, the main components are oxygenated (e.g., cloves, formed by eugenol). In a reduced number of species, the predominant derivatives are aromatic principles; these include thyme with thymol and carvacrol, peppermint with menthol, and anise with anethol (Bakkali et al., 2008; Sell, 2010; Chamorro et al., 2012).

Terpenes

Essential oils formed by terpene derivatives are usually composed of mono- and sesquiterpenes, which may be hydrocarbons or oxygenated, as described above. However, they can also be derived from aliphatic or alicyclic derivatives and some may have aromatic structures. Examples of essential oils from this extensive group include turpentine (*Pinus* spp.) with α- and β-pinene, geraniol from damask rose (*Rosa damascena*), tea tree oil (*Melaleuca alternifolia*) with terpinen-4-ol, coriander (*Coriandrum sativum*) with linalool, peppermint (*M. piperita*) with menthol, lemon (*Citrus limon*) with limonene, caraway (*Carum carvi*) with carvone, wormwood (*A. absinthium*) with thujone, eucalyptus (*Eucalyptus globulus*) with cineol, and chenopodium (*Chenopodium ambrosioides*) with ascaridole as examples of monterpenes (Figure 1). Examples of sesquiterpenes include sandalwood (*Santalum album*) with santalol, German chamomile (*Matricaria recutita*) with bisabolol, and ginger (*Zingiber officinale*) with zingiberol (Figure 2).

Allyl phenols

Some aromatic plants have an essential oil formed principally by aromatic derivatives derived from allyl or isoallyl phenol. Plants containing these compounds are rarer than those containing terpenes, but that only serves to make this kind of compound selective. Some relevant examples of essential oils with these types of compounds are those from aniseed (*Pimpinella anisum*), star anise (*Illicium verum*), and fennel (*Foeniculum vulgare*), all with *trans*-anethol; cinnamon (*Cinnamomum verum*) with *trans*-cinnamic aldehyde; and clove (*Syzygium aromaticum*) with eugenol (Figure 3).

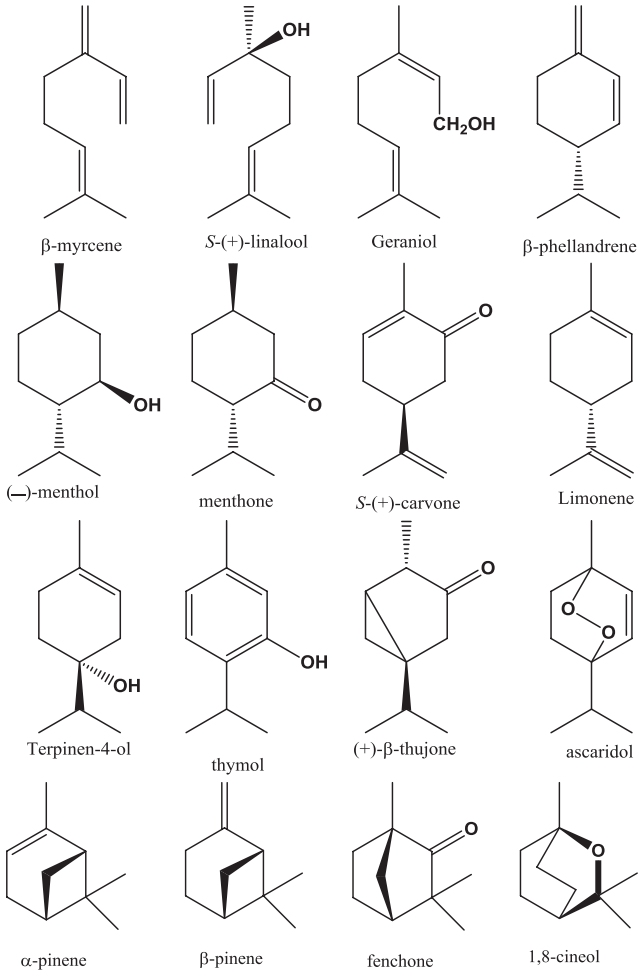

FIGURE 1 Examples of aliphatic, mono- and bicyclic, hydrocarbon, and oxygenated monoterpenes.

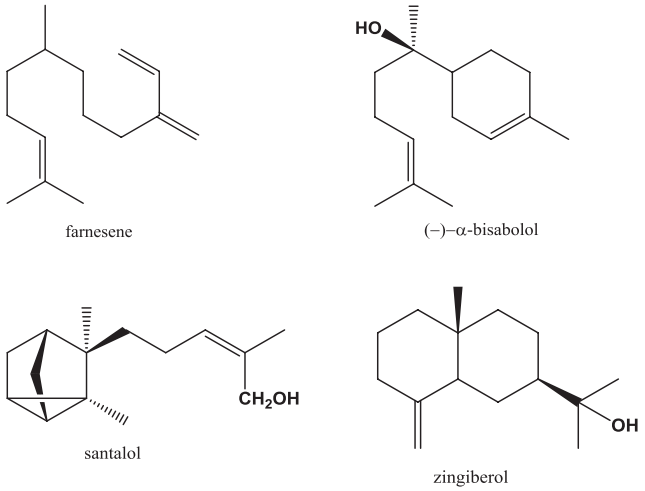

FIGURE 2 Examples of aliphatic, mono- and bicyclic, hydrocarbon, and oxygenated sesquiterpenes.

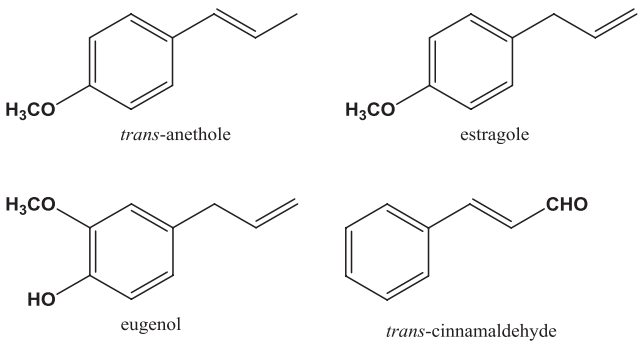

FIGURE 3 Examples of allyl and isoallyl phenols.

Other Constituents

Other volatile compounds may also be present in essential oils. In the special case of essential oils obtained from procedures other than distillation, such as enfleurage, extraction with solvents, or pressing, nonvolatile compounds may be present. In many cases, these are precursors of known derivatives, such as sesquiterpene lactones or glycosides, which in some cases are hydrolyzed or transformed into volatile compounds during the distillation process whereas in the case of extraction, they may be present in their original form in the extract. Other compounds found in essential oils include ligustilide from lovage (*Ligusticum levisticum*) and coumarins, which although present in low quantities can be detected in some relevant aromatic plants or species, such as lavender and lavandin (*Lavandula* spp.) which can contain up to 0.3% coumarin. Other examples are furanocoumarin in the essential oil of bergamot (*Citrus bergamia*), which often contains bergapten; sinalboside (glucosinolate) from white mustard (*Brassica alba*); salicylates from wintergreen (*Gaultheria procumbens*); and amygdalin from bitter almond (*Prunus communis* var. *amara*), which liberates benzaldehyde and hydrogen cyanide (Figure 4).

USE OF ESSENTIAL OILS

The use of essential oils is extremely diverse depending on the source, quality, extraction procedure, etc. Essential oils have proven industrial applications in the manufacture of perfumes, cosmetics, soap, shampoos, or cleaning gels. Another interesting aspect of these oils is their potential as therapeutic agents in aromatherapy or as active principles or excipients of medicines. Another significant application of essential oils is in the agrofood industry, both for producing beverages and for flavoring foods.

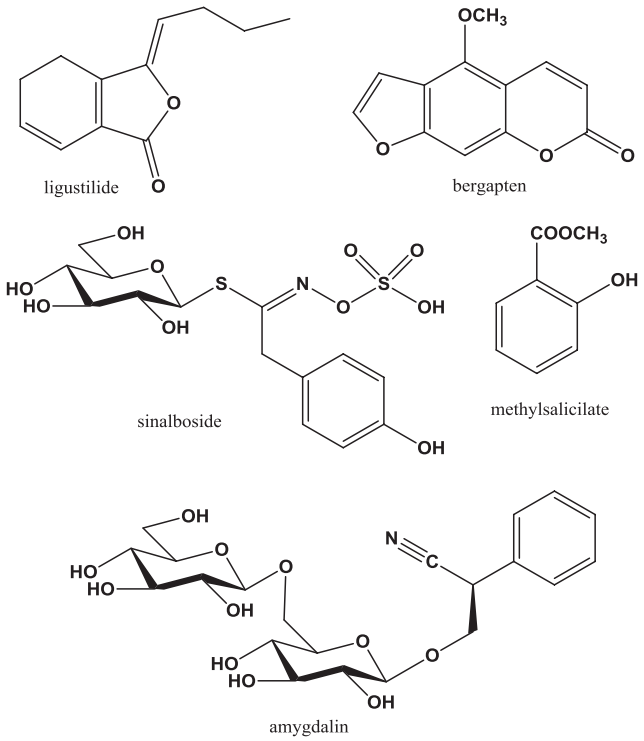

FIGURE 4 Other compounds detected in essential oils.

Cosmetics

The use of essential oils in the cosmetics, soap, detergent, and perfume industry is of great interest from an economic point of view. The world production of essential oils for the preparation of perfumes has clearly increased, with specific groups of aromatic plants being highly sought after in the market. Concrete chemotypes of salvia, lavender, and thyme are especially prized for obtaining fine and novel perfumes. To this end, production technology and an adequate selection of the raw source material are essential elements for improving the quality of the final product.

Medicine and Pharmaceutics

Essential oils are used in pharmaceutics for their potential as medicinal agents (Harris, 2010; Lis-Balchin, 2010). This is especially the case of the essential oils from eucalyptus (*E. globulus*), peppermint (*Mentha×piperita*), anise (*P. anisum*), sage (*Salvia officinalis*), clove (*S. aromaticum*), and tea tree (*M. alternifolia*). These oils are used as an expectorant for treating cough and bronchitis (eucalyptus), as antimicrobial agents (sage, tea tree, and clove), as a decongestant of the respiratory tract (peppermint), and as a carminative (anise), among other uses. Thus, clove oil is used in dentistry for its antiseptic and analgesic properties while tea tree oil is used in dermatology as an antiacne agent because of its antimicrobial activity against Gram-positive bacteria (Buchbauer, 2010). In addition, some essential oils are used in pharmaceutics for aromatizing pharmaceutical preparations and improving their taste.

Indeed, aromatherapy may be the principal application of essential oils as medicinal agents. The administration of essential oils obtained from different sources is carried out through a variety of application methods. The term "aromatherapy" was established by Gattefossé in the 1920s and was revived by Maury in the 1960s. Since the 1980s, its popularity has increased steadily. At present, it is fairly well established in Australia, Canada, France, Germany, New Zealand, Switzerland, the United Kingdom, and the United States (Lis-Balchin, 2010; Boehm et al., 2012).

The most common application method of essential oils is topical in diluted forms, often together with a carrier oil as part of massage therapy (Boehm et al., 2012). They can also be inhaled after adding a few drops to steaming water or by means of an atomizer or humidifier. In addition, they can be applied as ointments, creams, and compresses (Boehm et al., 2012). However, oral application of essential oils through encapsulation or other programmed release methods has been introduced as an effective method for obtaining the beneficial effects of these substances (Karlsen, 2010). They may be drunk in the form of tea with sugar or in soft capsules, which allows for more accurate dosage while avoiding several undesirable effects. Nevertheless, it is possible that the toxicity of essential oils may be higher when administered in this fashion.

Food

Essential oils are used in a wide variety of consumer goods such as confectionery food products, soft drinks, and distilled alcoholic beverages. In addition to their widespread use as a flavoring material (Kettenring and Geeganage, 2010), they are used in the nutritional and agricultural fields for their reported antibacterial, antifungal, antiviral, nematicidal, insecticidal, and antioxidant properties (Turek and Stintzing, 2013; Lopez-Reyes et al., 2013). Because of this, their use as antioxidants and preservatives in food has been suggested (Tiwari et al., 2009; Hyldgaard et al., 2012), either incorporated into the foodstuff packaging material (Kuorwel et al., 2011) or as plant and crop protectants (Adorjan and Buchbauer, 2010). Many essential oils have antioxidant (Dandlen et al., 2010) and antimicrobial properties (Lang and Buchbauer, 2012), but their application as food preservatives requires a good knowledge of their properties, including the sensitivity of the target microorganisms, the specific mode of action, their antimicrobial potency, and the effect of food matrix components on their antimicrobial properties (Hyldgaard et al., 2012).

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Chapter 2

Methods for the Characterization, Authentication, and Adulteration of Essential Oils

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List of Abbreviations

GC–MS Gas chromatography–mass spectrometry
SSR Simple sequence repeat
RAPD Random amplified polymorphic DNA
SFE GC–MS Supercritical fluid extraction GC–MS
MDGC Multidimensional gas chromatography
GC–C–IRMS Gas chromatography–combustion–isotope ratio mass spectrometry

INTRODUCTION

Essential oils extracted from plants are used in cosmetics, in many foods, and for their fragrance, flavoring, and preservative properties. The bulk is employed for the fragrance or flavor industries, with only a small percentage for therapeutic purposes. It is imperative that therapeutic oils are unadulterated. Completely pure natural essential oils should be obtained straight from the grower without intervention by a vendor. Ideally, essential oils should be clearly labeled with the botanical name, cultivation method, country of origin, the plant tissue employed for distillation, and main constituents. However, very few people using essential oils know that the oils they are using may be adulterated. Unscrupulous manufacturers of essential oils may resort to adulterating the oils with the intent to make the price competitive and maximize the profit. Extraordinarily cheap essential oils and labels such as "for external use only," "not for internal use," and "dilute prior to topical application" should alert one to the possibility of adulteration.

The adulterants are: (1) vegetable carrier oils, alcohol, and synthetic oils used as diluents; (2) cheaper oils of the same species but of different geographical origins; (3) cheaper essential oils extracted from another part of the plant; (4) cheaper essential oils from related species; and (5) isolated natural, or (semi) synthetic compounds. Frankincense may be adulterated with gum resin, alcohol, and other solvents. Many commercial samples of lavender oil are composed of lavandin, camphor, linalyl acetate, propylene glycol, and petrochemicals. Synthetic oils have appeared in products claimed to be natural oils. Noxious chemicals with health risks, including phthalates and benzyl alcohol, have been detected in essential oils.

In view of the aforementioned adulteration of plant essential oils, it is of paramount importance to examine the various methods available for authentication and adulterant detection which are mentioned below.

METHODS OF AUTHENTICATION

Gas Chromatography–Mass Spectrometry (GC–MS) for *Hyssopus cuspidatus* Essential Oil

An investigation of the chemical composition of the essential oil of *Hyssopus cuspidatus* Boriss from Xinjiang, China employing GC–MS led to the identification of 50 compounds. The principal constituents comprised oxygenated

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sesquiterpenes (1.25%), octane (1.85%), monoterpenes (26.14%), and oxygenated terpenes (66.33%). The authenticity of *H. cuspidatus* may be confirmed by the physicochemical parameters of the compounds (Zhou et al., 2010).

Determination of Enantiomeric Composition for Essential Oils of Indian Origin

Use of 10% heptakis(2,3-di-O-methyl-6-O-tert-butyldimethylsilyl)-beta-cyclodextrin as a chiral stationary phase to ascertain the enantiomeric ratios of linalool in a diversity of authentic essential oils originating from India has been reported by Chanotiya and Yadav (2009). A complete enantiomeric excess for (3S)-(+)-linalool was typical of leaf oils from *Lippia alba* and *Cinnamomum tamala* while below 90% excess was found in *Zanthoxylum armatum* leaf, *Zingiberroseum* root/rhizome, and *Citrus sinensis* leaf oils. By contrast, an enantiomeric excess of (3R)-(−)-linalool was observed in essential oils of basil (100% for *Ocimum basilicum*) and bergamot mint (72–75% for *Mentha citrata*). Thus enantiomeric compositions could be used to assess the authenticity of essential oil (Chanotiya and Yadav, 2009). In addition, Kreis and Mosandl (1992) reported the stereoanalysis of chiral compounds present in lavandula essential oil. An example is shown in Table 1.

Supercritical Fluid Extraction GC–MS (SFE GC–MS) Involving Use of Multidimensional GC to Resolve Enantiomers for Essential Oils of Lavandula

An approach based on SFE was adopted to investigate, both qualitatively and quantitatively, the compositions of the predominant volatile aroma constituents in essential oils of different varieties of Lavandula. Under optimal conditions of SFE, relative standard deviations (RSDs) from three replicates below 2% and recoveries up to 59% could be achieved. For separation of the target enantiomeric compounds, multidimensional gas chromatography was deployed. The data disclosed an enantiomeric purity exceeding 90% for every compound in the different varieties examined, lending credence to the natural constant enantiomeric composition of the compounds of interest. The data can be of value in authenticity investigations and in choosing natural sources of enantiomerically homogeneous compounds (Flores et al., 2005).

TABLE 1 Enantiomeric Distributions of Chiral Monoterpenoids From Authentic Samples of Lavandula Oils

	trans-Linalol oxide (2R, 5R)	cis-Linalol oxide (2R, 5S)	Linalyl acetate (R)	Lavandulol (R)	Terpinen-4-ol (S)	Linalol (R)
No.	2	3	11	13	16	17
1	86.4	88.5	>99	93.2	–	97.4
2	–	–	>99	98.5	97.8	94.5
3	88.6	86.0	>99	98.0	–	96.6
4	84.6	86.7	>99	89.8	98.0	95.1
5	90.2	91.5	>99	>99	94.2	97.5
6	96.1	91.5	>99	>99	98.2	97.3
7	95.8	92.9	>99	98.3	98.0	96.9
8	>95	>95	>99	98.7	98.3	98.2
9	85.4	90.0	>99	>99	98.1	96.1
10	87.5	95.8	>99	>99	98.4	97.1
11	–	–	>99	>99	98.1	95.2
12	76.7	82.9	98.8	>99	98.1	97.2
13	86.2	89.3	>99	96.2	89.1	95.1

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Enantioselective Capillary Gas Chromatography and Online Methods of Isotope Ratio Mass Spectrometry

Mosandl (2004) wrote an excellent review on enantioselective capillary gas chromatography and online methods of isotope ratio mass spectrometry in the authentication of food flavor and essential oil compounds, covering papers published in the 10 years preceding his review.

Enantioselective Capillary Gas Chromatography and Isotope Ratio Mass Spectrometry, Coupled Online with Capillary Gas Chromatography on an HP5 Column for Various Essential Oils

Enantioselective capillary gas chromatography conducted using a Supelco beta-DEX 225 column heptakis(2,3-di-O-acetyl-6-O-tert-butyldimethylsilyl)-beta-cyclodextrin SPB 20poly-20% diphenyl, 80% dimethylsiloxane) and isotope ratio mass spectrometry, coupled online with capillary gas chromatography on an HP5 column have been exploited for analysis and authenticity investigations of essential oils. The essential oils examined included those of the following plants: lemon (*Citrus limon*), lemongrass (*Cymbopogon citratus* and *Cymbopogon flexuosus*), citronella (*Cymbopogon nardus* L.–Ceylon type and *Cymbopogon winterianus*—Java type), *Litsea cubeba, Lippia citriodora*, lemon myrtle (*Backhousia citriodora*), lemon gum (*Eucalyptus citriodora*), and precious lemon balm oil (*Melissa officinalis* L.). Isotope data ($\delta^{13}C$(Pee Dee Belemnite (PDB)) and $\delta^{2}H$(Vienna standard mean ocean water (V-SMOW)) for citral (neral + geranial) and citronellal from isotope ratio mass spectrometry online coupled with capillary gas chromatography (GC-Py-IRMS) and chiral data for citronellal in these essential oils can yield information on the origin of essential oils and disclose adulterants. Principal components analysis of specific compounds was carried out for discriminating essential oils from *L. cubeba*, *C. citratus*, and *C. flexuosus* (Nhu-Trang et al., 2006a).

Online Gas Chromatography Pyrolysis Isotope Ratio Mass Spectrometry (HRGC–P–IRMS) for the Flavor Compounds Decanal, Linalool, Linalyl Acetate, E-2-Hexenal, and E-2-Hexenol in Essential Oils

Hör et al. (2001) applied the HRGC–P–IRMS technique to find the $\delta^{2}H$(SMOW) values of the aforementioned flavor compounds in foods and essential oils. Procedures such as simultaneous distillation extraction, solvent extraction, and liquid/liquid extraction used for preparing samples had negligible effects on the $\delta^{2}H$ values. Only the $\delta^{2}H$ data recorded for linalool did not permit distinction between synthetic and natural products. For decanal, linalyl acetate, E-2-hexenal, and E-2-hexenol, the data appeared to be useful for differentiation purposes (Table 2).

TABLE 2 Application of HRGC–P–IRMS Technique to Distinguish Between Synthetic and Natural Flavor Compounds in Essential Oils

	$\delta^{2}H$(SMOW) Values: ^{2}H Abundance
Synthetic decanal samples	From −90 to −156 per 1000
Natural decanal samples	From −138 to −262 per 1000
Synthetic linalyl acetate samples	From −199 to −239 per 1000
Natural linalyl acetate samples	From −213 to −333 per 1000
Synthetic E-2-hexenal samples	From −14 to −109 per 1000
Natural E-2-hexenal samples	From −263 to −415 per 1000
Synthetic E-2-hexenol samples	From −41 to −131 per 1000
Natural E-2-hexenol samples	From −238 to −348 per 1000
Synthetic linalool samples	From −207 to −301 per 1000
Natural linalool samples	From −234 to −333 per 1000

Only the $\delta^{2}H$ data recorded for linalool did not permit distinction between synthetic and natural products.
Data compiled from Hör et al. (2001).

TABLE 3 δ^2H(V-SMOW) Values Of Various Components in Essential Oils

	δ^2H(V-SMOW)
Carvacrol	−61 per 1000
Thymol	From −49 to 7 per 1000
p-Cymene	From −300 to −270 per 1000
γ-Terpinene	From −285 to −248 per 1000
Thymol	From −259 to −234 per 1000
Carvacrol in authentic oregano oils	From −223 to −193 per 1000
p-Cymene in authentic oregano oils	From −284 to −259 per 1000
Aromatic Compounds in Authentic *Satureja montana* subsp. Montana Essential Oil	
Carvacrol	−226 per 1000 (SD = 1.7 per 1000)
p-Cymene	−283 per 1000 (SD = 3.0 per 1000)
γ-Terpinene	−273 per 1000

SD, standard deviation.
Data compiled from Nhu-Trang et al. (2006b).

Isotope Ratio Mass Spectrometry Online Coupled with Capillary Gas Chromatography (GC-Py-IRMS)

GC-Py-IRMS on column INNOWAX has been exploited in studies on the authenticity of phenolic essential oils (Table 3, Nhu-Trang et al., 2006b).

Gas Chromatography–Combustion–Isotope Ratio Mass Spectrometry (GC–C–IRMS), in Combination with GC–MS and GC Flame Ionization Detector (FID) for *Rosa damascene* Essential Oil

Nineteen commercial samples of *R. damascena* essential oil collected from various localities, together with an authentic sample of essential oil extracted from fresh flowers, were analyzed by GC–MS and GC–FID. Elemental analyzer (EA)–IRMS and GC–C–IRMS were employed for analysis of the δ^{13}C composition of bulk samples and of some specific components. It was discovered that essential oils from Turkey and Bulgaria contained mainly citronellol, geraniol, and nonadecane, while Iranese essential oils were abundant in the aliphatic hydrocarbons nonadecane. The δ^{13}C values of bulk samples fell within the range −28.1 to −26.9‰, characteristic of C_3 plants. The δ^{13}C values of specific components were found within the range for natural aromatic substances from C_3 plants, with the exception of geranyl acetate, which demonstrated elevated values (up to −18‰) caused by adulteration with an oil from a C_4 plant (*Cymbopogon martinii*, palmarosa) which has a lower cost and existing in the bulk of essential oils (Pellati et al., 2013).

Headspace–Solid Phase Microextraction Coupled to GC–C–IRMS for Citrus Oils

Schipilliti et al. (2010) evaluated the authenticity of some mandarin essential oils using GC–C–IRMS. Schipilliti et al. (2013) examined the authenticity of Italian liqueurs, bergamot, lemon, and mandarin, by using headspace–solid phase microextraction coupled to GC–C–IRMS. Their carbon isotope ratios were compared with those of genuine cold-pressed peel oils. Direct enantioselective gas chromatography was used to determine the enantiomeric distribution of selected chiral volatiles and GC–MS was employed for qualitative analyses (Schipilliti et al., 2013).

Multi Dimensional Gas Chromatography (MDGC) and GC-C-IRMS for Bitter Orange Flower Oil (or Neroli) and Lime Oils

Five samples of Egyptian neroli oils, manufactured in 2008 and 2009 in the same factory, and claimed to be authentic, were used in the study of Bonaccorsi et al. (2011). GC–FID and GC–MS–linear retention index were employed to analyze

the composition, and enantioselective GC utilized to investigate the enantiomeric distribution of 12 volatile compounds. GC-C-IRMS was employed to ascertain the $\delta^{13}C$(VPDB) values of some alcohols, esters, and monoterpene and sesquiterpene hydrocarbons. The variation of the composition depended on the time of manufacture. Linalool dropped while linalyl acetate rose from March to April. The RSD for the $\delta^{13}C$(VPDB) remained small (max. 3.89%), assuring sample authenticity (Bonaccorsi et al., 2011).

Bonaccorsi et al. (2012) conducted an investigation on the authenticity of lime oils (*Citrus aurantifolia* Swingle and *Citrus latifolia* Tanaka), by employing MDGC to study the enantiomeric distribution of camphene, limonene, linalool, α-phellandrene, β-phellandrene, β-pinene,terpinen-4-ol, α-terpineol, sabinene, and α-thujene. GC–C–IRMS was used to ascertain the isotopic ratios of β-caryophyllene, geranial, germacrene B, limonene, neral, α-pinene, β-pinene, α-terpineol, and trans-α-bergamotene. The concurrent deployment of the two techniques facilitates detection of adulteration in citrus essential oils. In fact, in some cases detection of adulteration is possible only with one of the two techniques. The merit of their simultaneous usage is the need to analyze only a small number of constituents hence minimizing the amount of data to be handled. Moreover, the traditional method of analysis which relies on the assessment of the entire volatile fraction may not be sensitive enough to disclose the oil quality when there is only a slight level of adulteration (Bonaccorsi et al., 2012).

GC–FID and GC–MS for *Zanthoxylum armatum* Leaf Essential Oil

The predominant types of compounds detected in the leaf oils comprised acyclic and menthane monoterpenoids, simple alcohols, aldehydes, and ketones. The unique composition was characterized by a richness of nonterpenic acyclic ketones, exemplified by 2-undecanone and 2-tridecanone, and the meager content of undec-10-en-1-al and p-phellandren-8-ol. Other components encompassed oxygenated monoterpenes like 1,8-cineole, linalool, terpinen-4-ol, and alpha-terpineol. Sesquiterpene hydrocarbons, in particular trans-caryophyllene, a-humulene, and germacrene D, were present. On the contrary, the oil distilled from the leaves on the second day of distillation was characterized by an abundance of 2-tridecanone and trans-caryophyllene compared with fresh foliage. Moreover, a rich content of 2-undecanone and 2-tridecanone is characteristic of *Z. armatum*. Thus, the two acyclic ketones may be utilized in authenticity testing (Bisht and Chanotiya, 2011).

Ultra-High Performance Liquid Chromatography–Time-of-Flight–Mass Spectrometry (UHPLC–TOF–MS) Profiling and ^{1}H Nuclear Magnetic Resonance (NMR) Fingerprinting for Lemon Oil

A metabolomic strategy which depends on UHPLC–TOF–MS profiling and ^{1}H NMR fingerprinting to reveal variances in metabolites has been designed for lemon oil samples that find application in the flavor and fragrance industry. Flavonoids, furocoumarins, fatty acids, and terpenoids present in the mixtures were markers with a differentiating role. Quantitative NMR unveiled low levels of citropten and an abundance of bergamottin in samples from Italy compared with Argentinian samples (Marti et al., 2014).

Near Infrared (NIR) Spectroscopy for Sandalwood Oil

NIR spectroscopy in conjunction with multivariate calibration models such as principal component regression and partial least square regression is qualitative and quantitative analytical tool for detecting adulterants in sandalwood oil with the advantages of speed, sensitivity, and nondestructiveness. Following appropriate preprocessing of the raw near infrared data, the models were constructed by cross-validation. To find the optimal number of factors, the smallest root mean square error of cross-validation and calibration was utilized. The coefficient of determination and the root mean square error of prediction in the prediction sets were employed as the parameters for evaluation (Kuriakose et al., 2010).

Kuriakose and Joe (2013) conducted a study that employed NIR spectroscopy to investigate the authenticity of samples and quantitate adulteration of sandalwood oils. Quantitative analysis of data was executed using full spectrum or sequential spectrum. The optimum number of partial least square components was procured according to the smallest root mean square error of calibration (RMSEC = 0.00009% v/v). The smallest root mean square error of prediction (RMSEP = 0.00016% v/v) in the test set and the largest coefficient of determination ($R^2 = 0.99989$) were employed for assessment for the best model. Locally weighted regression was introduced to collect nonlinear information and for comparison with the linear partial least square regression model (Kuriakose and Joe, 2013).

NIR Spectroscopy for Various Essential Oils

Cross-validation models can be used to predict with accuracy virtually all of the constituents of essential oils. In various cinnamon (*Cinnamomum zeylanicum*) and clove (*Syzygium aromaticum*) essential oils, which demonstrated analogous

compositions, 23 components (accounting for the bulk of the oil) were correctly predicted. Likewise, 20 components in *Cinnamomum camphora*, 32 components in *Ravensara aromatica*, and 26 components in *Lippia multiflora* that made up the bulk of the oils, were also correctly predicted. For almost all of the components, the modeled and reference values obtained by GC–FID exhibited a high correlation and a variance below 5%. The model was used to disclose erroneous commercial labeling of *C. camphora* oil as *R. aromatica* oil (Juliani et al., 2006).

Simple Sequence Repeat (SSR)

The certification labels protected designation of origin (PDO) and protected geographical indication (PGI) are required by the European Commission for assuring the authenticity of food products. Regulations for labeling, production, and commercialization of olive oil were laid down in European Economic Community Regulation No. 510/2006. Genotype is important in the establishment of PDO and PGI labels. The analysis of 21 olive oil samples was conducted with the help of nine nuclear and two shortened simple sequence repeats. An array of simple sequence repeat markers can be used to accurately attribute an olive oil to a certain cultivar (Vietina et al., 2011).

Random Amplified Polymorphic DNA (RAPD) Method

A study of 84 commercial Mediterranean oregano samples collected between 2001 and 2007 disclosed the prevalence of adulteration. Materials from plants with an oregano-like flavor (*Satureja montana* and *Origanum majorana*), and plant materials devoid of essential oils from *Cistus incanus, Rubus* sp. and *Rhus coriaria*, were added as adulterants. The RAPD methodology devised by Marieschi et al. (2009) using 13 differentiating primers facilitated the sensitive detection of materials from *C.incanus, Rubus* sp., and *R. oriaria* added as adulterants and expedited analysis of big batches of samples (Marieschi et al., 2009).

Attentuated Total Reflectance (ATR)-Mid-infrared Portable Handheld Spectrometer for Peruvian Sacha Inchi Seed Oils

Thermally regulated ZnSe ATR mid-infrared benchtop and diamond ATR midinfrared portable handheld spectrometers were used by Maurer et al. (2012) to investigate Peruvian seed oils abundant in omega-3 fatty acids. A soft independent model of class analogy and partial least squares regression were utilized for the analysis of the spectral data. Polyunsaturated fatty acid concentrations resembling levels seen in flax oils were revealed. Partial least square regression showed good correlation between reference tests and spectra from infrared devices with correlation coefficients exceeding 0.9, facilitating speedy analysis of composition of fatty acids. Results indicated existence of adulteration (Maurer et al., 2012).

SUMMARY POINTS

- The bulk of plant essential oils are utilized by the fragrance or flavor industries, with only a small percentage for therapeutic purposes.
- In order to lower the price of the essential oils and hence attract more customers, adulterants are added by the producers to the oils.
- Adulterants include diluents and cheaper oils from other plant tissues, related plant species, or plants of the same species but from other countries. Some of the adulterants detected such as phthalates and benzyl alcohol are hazardous to health.
- Various types of methodology are available for authentication and adulterant detection. They include GC–MS, determination of enantiomeric composition, SFE GC–MS involving use of multidimensional GC to resolve enantiomers, enantioselective capillary GC, and online methods of IRMS.
- Other methods comprise enantioselective capillary gas chromatography and IRMS coupled online with capillary gas chromatography, NIR spectroscopy, SSR, RAPD, and various other methods.

DISCLAIMER

This article was written in a personal capacity (E.F.F.) and does not represent the opinions of the United States Food and Drug Administration, the United States Department of Health and Human Services, or the United States Federal Government.

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Chapter 3

Cultivation of Essential Oils

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INTRODUCTION

The odorous volatile principles of plant and animal sources are known as volatile oils or essential oils. As they evaporate when exposed to air at ordinary temperatures or become volatile in steam, they are called volatile oils. They represent the essence or active constituent of plant, hence they are known as essential oils. Essential oils, especially from plant sources, and their preparations have been traditionally used in food and beverages since time immemorial. Essential oils are used in perfumery, food, and condiment industries, the household industry, and making confectioneries and beverages as well as in the manufacture of pharmaceutical and cosmetic products. Because of their multipurpose applications, the production and utilization of essential oils is increasing continuously. Therefore, systematic cultivation strategies of essential oil-bearing plants based on modern scientific knowledge are necessary to maintain a regular supply of quality plant materials required for essential oil production. The present chapter illustratively deals with the cultivation influencing factors and contemporary cultivation methods of some important essential oil-bearing plants.

IMPORTANT SPECIES FOR ESSENTIAL OIL CULTIVATION

The occurrence of essential oils is very widespread in the plant kingdom. According to market data, there are about 400 species, from 67 plant families, which are cultivated on a large commercial scale for production of essential oils. The most important families from this point of view are Asteraceae (syn. Compositae), Lamiaceae (syn. Labiatae), and Apiaceae (syn. Umbelliferae). Each includes more than 15 species producing essential oils on a large scale. Other families of importance are Fabaceae, Rutaceae, Rubiaceae, Zingiberaceae, Pinaceae, Myrtaceae, Piperaceae, and Burseraceae (Duke et al., 2002; Kalia, 2005).

FACTORS INFLUENCING CULTIVATION OF ESSENTIAL OILS

Cultivation of aromatic plants takes cognizance of several factors, interacting with one another for their favourable growth. These are briefly explained with relevant evidence.

Environmental Factors

Growth and development of aromatic/essential oil-bearing plants and often the nature and quantity of essential oils are affected by temperature, rainfall, day length and radiation characteristics, and altitude.

Generally, formation of essential oils enhances at higher temperatures, although very hot days may lead to an excess physical loss of oil. Continuous rain can lead to loss of essential oils from leaves and roots by leaching (Evans, 2002).

It has been shown that under long-day conditions peppermint leaves contain menthone, menthol, and traces of menthofuran; plants grown under short-day conditions contain menthofuran as a major component of the volatile oil. Furthermore, a long photoperiod for young leaves activates the reduction pathway with conversion of menthone to menthol. In studies on the day–night changes in the relative concentrations of volatiles from flowers of *Nicotiana sylvestris* and other species a marked increase (about 10-fold) in aromatic compounds including benzyl alcohol was detected at night, whereas no increase in the other essential oil constituents (e.g., linalool, caryophyllene) was noted (Loughrin et al., 1990).

The type of radiation which plants receive is also important. With sweet basil (*Ocimum basiclicum*) Johnson et al. (1999) found that in relation to herbs grown under glass and receiving no ultraviolet (UV)-B radiation, supplementary UV-B radiation increases levels of both the phenylpropanoids and terpenoids (essential oil constituents) of the leaves. Depletion of the

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TABLE 1 Favourable Altitudes for Cultivation of Essential Oil-Bearing Plants

Aromatic Plant	Botanical Name	Altitude (meters)
Clove	*Eugenia caryophyllus*	Up to 900
Camphor	*Cinnamomum camphora*	1500–2000
Cardamom	*Elettaria cardamomum*	600–1600
Cinnamon	*Cinnamomum zeylanicum*	250–1000
Saffron	*Crocus sativus*	Up to 1250

ozone layer and the consequent effect of increased radiation on the Earth's surface has been a matter of global environmental concern. Concerning aromatic plants, Karousou et al. (1998) raised two chemotypes of *Mentha spicata* that were subjected to increased UV-B radiation equivalent to l5% ozone depletion over Patras, Greece. In one chemotype, essential oil production was stimulated by the treatment while a similar nonsignificant trend was noted with the other.

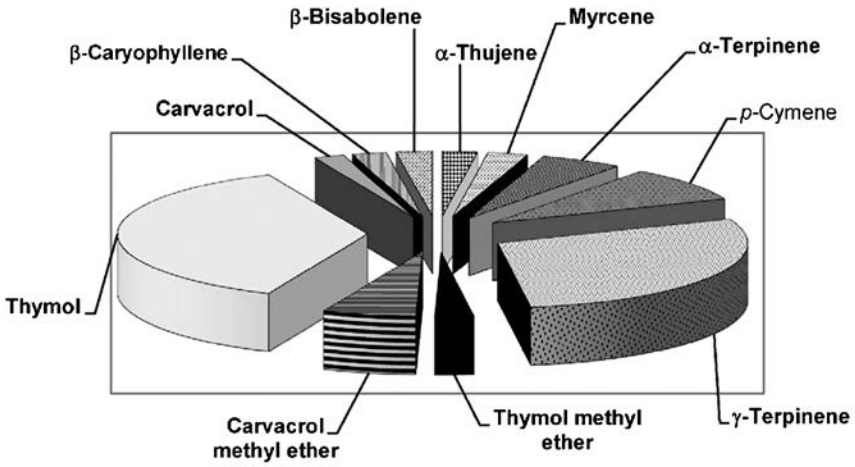
Altitude is another environmental factor. The essential oil content of thyme (*Thymus vulgaris*) and peppermint (*Mentha piperita*) decreases with altitude (Evans, 2002). A few examples of aromatic plants indicating altitude for their successful cultivation are mentioned in Table 1.

Harvesting-Related Factors

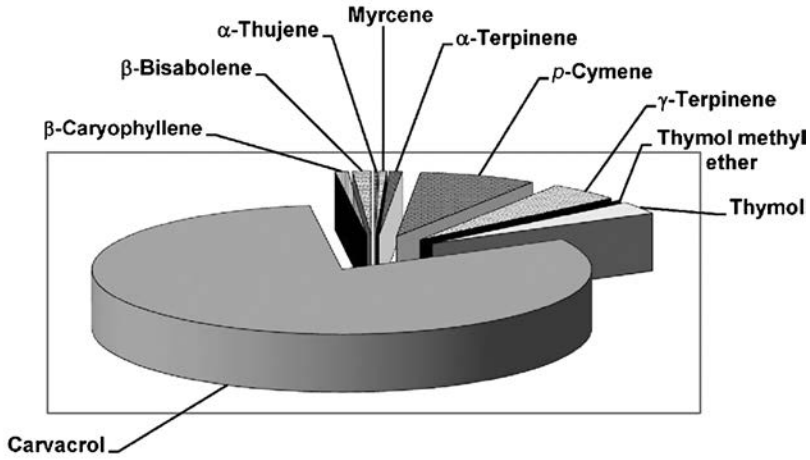
- **Season:** The season in which the aromatic plant is harvested is usually a matter of considerable importance. In a study, the influence of harvesting season on essential oil yield and composition was recorded for Greek oregano oil harvested in two consecutive seasons. Essential oil concentration per plant increased significantly from about 1.5–2.0% in the dry warm season to about 5.5% in the next wet cold season and it was higher in the inflorescences compared with leaves. Carvacrol (the main constituent of oregano essential oil) content was higher in the drier and warmer season (70.75–84.88%), whereas several other compounds tended to accumulate at higher levels during the wetter and colder season along with comparatively lower levels of carvacrol (56.46–75.12%) in both leaves and inflorescences (Karamanos and Sotiropoulou, 2013).
  The time of the day when harvesting is also important. A study conducted on the rose-scented geranium, *Pelargonium graveolens*, in Kiyombe (Rwanda) investigated the effects of the time of day on essential oil yield. The geranium plants were manually harvested at 10:00 am, 12:00 noon, 2:00 pm, and 4:00 pm and dried under shade for 17 h prior to essential oil isolation by steam distillation. The geranium plants harvested in the early afternoon (2:00 pm) possessed the highest essential oil concentration (0.22%) (Malatova et al., 2011).

- **Geographical location:** Geographical location is a major cause of variation in essential oils as it governs the environmental conditions. The essential oil from bay laurel (*Laurus nobilis*) shows highest yield at the end of August (Portugal), July (China), spring (Israel), containing highest level of 1, 8-cineole (Evans, 2002). Bonfanti et al. (2012) studied the effect of geographical variation on composition and sensory properties of oregano essential oil. The oregano oil from typically cultivated Sicilian plant (*Origanum vulgare* subsp. *hirtum* (Link) Beguinot) exhibited thymol and γ-terpinene as the main compounds followed by *p*-cymene, α-terpinene, carvacrol and thymol methyl ethers, myrcene, and carvacrol. The commercial sample (source not disclosed), instead, showed a different profile, where carvacrol was the principal constituent (>77%), followed by *p*-cymene, γ-terpinene, and thymol (Figure 1). Therefore, the commercial sample may be similar to Greek oregano oil that is generally characterized by carvacrol as dominant constituent (Kokkini and Vokou, 1989). Differences were also detected in their sensory profiles. The intensity of sensory attribute "typical oregano flavor" was found satisfactory for the Sicilian oil sample; instead the commercial sample had less freshness and a lower typical oregano flavor, associated with a higher level of off-flavor.
  Aromatic plants can be categorized by the geographical regions where they are cultivated and harvested. Analyzing the climatic and ecological conditions of the cultivation sites: tropical, Mediterranean, and temperate essential oil producing crops can be distinguished (Hornok, 1992; DerMarderosian, 2001). Some of the species characteristic of the above-mentioned regions are enumerated in Tables 2 and 3.

- **Age:** The age of the plant undergoing harvesting is also of considerable importance and governs not only the total quantity of essential oil produced but also the relative proportions of the components of the essential oil. A few examples of such ontogenetic variations are given in Table 4.



CULTIVATED SAMPLE



COMMERCIAL SAMPLE

FIGURE 1 Comparison between the chief constituents of cultivated and commercial samples of oregano oils.

Fertilizers

Fertilizers are used to maintain soil fertility for improving plant growth and development. In case of *Oregano* species, Omer (1999) detected positive nitrogen-effects on Egyptian oregano (*Origanum syriacum*) grown in pots, and Ozgüven et al. (2006) noticed a significant increase in fresh and dry weight of the same species at a rate of 40 kg ha^{-1} in the field. Field studies on Greek oregano (*O. vulgare* subsp. *hirtum* (Link) Ietswaart) demonstrated that nitrogen application significantly influenced a number of vegetative traits thus inducing both biomass and essential oil yield at a rate of 80 kg N ha^{-1} (Sotiropoulou and Karamanos, 2010). In a study on Greek oregano, essential oil yield showed significant hike at the nitrogen rate of 80 kg ha^{-1} in both dry worm and cold humid seasons and was comparatively higher in the wetter season. Such a response was ascribed to the positive nitrogen effects on herbage yield (Karamanos and Sotiropoulou, 2013).

The effect of nitrogen fertilization, season of cultivation and stage of harvest on the growth, foliar essential oil composition, and yield of dill (*Anethum graveolens* L. "Ducat") was studied in two consecutive crops. Seeds were sown in October (autumn–winter crop) and January (spring crop). Nitrogen (NH_4NO_3) was applied at four levels (50, 150, 300, 450 ppm) in a randomized design. The plants were harvested at 158 (autumn–winter crop) and 83 (spring crop) days after sowing. The plant foliage was weighed and the essential oils were isolated and analyzed. The fresh weight of foliage per plant progressively increased with increasing nitrogen up to 450 ppm in the autumn/winter, but was maximal at 300 ppm nitrogen in the spring. The essential oil concentration of the foliage was low and was unaffected by nitrogen

TABLE 2 Essential Oil Species Cultivated in Tropical Regions

Sl. No.	Common Names	Botanical Names
1	Cananga (ylang–ylang)	*Cananga odorata*
2	Citronella	*Cymbopogon nardus*
3	Lemon grass	*Cymbopogon citrates* *Cymbopogon flexuosus*
4	Palmarosa	*Cymbopogon martinii*
5	Clove	*Eugenia caryophyllus*
6	Vetiver	*Vetivera zizanioides*
7	Cinnamon	*Cinnamomum zeylanicum*
8	Ginger	*Zingiber officinale*
9	Nutmeg	*Myristica fragrans*
10	Pepper	*Piper nigrum*
11	Eucalyptus	*Eucalyptus globulus*
12	Sandalwood	*Santalum album*
13	Cassia	*Cinnamomum cassia*
14	Geranium	*Pelargonium graveolens*
15	Camphor	*Cinnamomum camphora*
16	Cajuput	*Melaleuca cajeputi*
17	Jasmine	*Jasminum officinale*
18	Cubeb	*Piper cubeba*
19	Patchouli	*Pogostemon cablin*

application in the autumn/winter, but was significantly higher at 300 ppm nitrogen than at other nitrogen levels in the spring. The main constituents of the foliar essential oil were α-phellandrene, β-phellandrene, dill ether, α-pinene, β-pinene, α-thujene, myrcene, and π-cymene. In both crops α-phellandrene was the chief constituent. In the spring the concentration of all the essential oil constituents identified (except π-cymene) was highest at 300 ppm nitrogen, whereas in the autumn–winter crop the concentrations of α-phellandrene, β-phellandrene, and dill ether were also higher at 300 ppm nitrogen, but the other constituents were not influenced by higher nitrogen. Therefore, for the autumn–winter crop 450 ppm nitrogen was optimum for biomass and essential oil yield whereas in the spring 300 ppm nitrogen was recommended (Tsamaidi et al., 2012).

Cultivars

A cultivar is a plant(s) selected for desirable characteristics that can be maintained by propagation. Verma et al. (2013) studied the influence of cultivar on essential oil yield and composition of rose-scented geranium, that is, *P. graveolens*. Three cultivars namely Bourbon, CIM–Pawan, and Kelkar were cultivated in Western Himalayan region of India. Their yields and chief chemical composition are summarized in Table 5. The chemical composition of Kelkar was found to be different from the other two cultivars.

A similar study was performed on essential oils of 17 cultivars of fresh ginger from North-Eastern India (Kiran et al., 2013). Assam fibreless cultivar showed highest yield of essential oil (4.17%) and higher monoterpene hydrocarbon content (38.65%) than sesquiterpene hydrocarbon (25.38%), which was unique among all the studied cultivars. Among all these cultivars, Assam Tinsukia had the highest citral content (23.66%) and Meghalaya mahima had the highest zingiberene content (29.89%).

TABLE 3 Essential Oil Species Cultivated in Mediterranean Regions

Sl. No.	Common Names	Botanical Names
1	Rose	*Rosa damascena*
2	Bitter orange	*Citrus aurantium*
3	Lemon	*Citrus limon*
4	Fennel[a]	*Foeniculum vulgare*
5	Lavender[a]	*Lavendula officinalis*
6	Sage[a]	*Salvia officinalis*
7	Thyme[a]	*Thymus vulgaris*
8	Dill[b]	*Anethum graveolens*
9	Anise	*Pimpinella anisum*
10	Caraway[a]	*Carum carvi*
11	Peppermint	*Mentha piperita*
12	Spearmint	*Mentha spicata*
13	Clary sage[a]	*Salvia sclarea*
14	Chamomile[b]	*Matricaria recutita*
15	Angelica	*Angelica archangelica*
16	Lovage	*Levisticum officinale*
17	Coriander[b]	*Coriandrum sativum*
18	Tarragon	*Artemisia dracunculus*
19	Rosemary	*Rosmarinus officinalis*
20	Bay laurel[a]	*Laurus nobilis*

[a]*Also cultivated in temperate regions.*
[b]*Also cultivated in tropical regions.*

TABLE 4 Examples of Ontogenetic Variations of Some Essential Oils

Essential Oil	Biological Source	Ontogenetic Variations
Peppermint oil	*Mentha piperita*	Relatively high proportion of pulegone in young plants, replaced by menthone and menthol as leaves mature
Spearmint oil	*Mentha spicata*	Progression from predominance of carvone in young plants to dihydrocarvone in older plants
Clove oil	*Eugenia caryophyllus*	Contain about 14–21% of oil; mother "blown" cloves contain very little oil
Coriander oil	*Coriandrum sativum*	Marked changes in oil composition at the beginning of flowering and fruiting
Camphor oil	*Cinnamomum camphora*	Camphor accumulates in heartwood as the tree ages; ready for collection at 40 years
Yarrow oil	*Achillea millefoltum*	During flowering monoterpenes predominate in oils from leaves and flowers. Oil obtained during vegetative period contains mainly sesquiterpenes

TABLE 5 Effect of Cultivar on Yield and Composition of Essential Oil From *Pelargonium graveolens*

Cultivar	Yield (%)	Major Constituents (%)
Bourbon	0.05–0.20	Geraniol (14.1–34.6) Citronellol (15.2–31.3) Linalool (2.9–9.2) Citronellyl formate (4.4–9.2) Isomenthone (4.5–6.6) Gerenyl formate (3.8–6.2) 10-epi-γ-eudesmol (4.7–6.7)
CIM Pawan	0.10–0.25	Geraniol (11.9–31.9) Citronellol (16.1–30.2) Citronellyl formate (5.2–8.9) Linalool (3.7–6.4) Isomenthone (4.0–6.3) 10-epi-γ-eudesmol (4.4–5.2) Gerenyl formate (4.3–5.0)
Kelkar	0.03–0.12	Citronellol (51.0–63.4) Isomenthone (9.8–17.8)

CULTIVATION OF SELECTED ESSENTIAL OIL-BEARING PLANTS

In this section, contemporary cultivation methods of certain important essential oil-bearing plants are illustratively discussed in brief (Wallis, 1985; Hornok, 1992; Evans, 2002; Kokate et al., 2008a,b). The methods of propagations are summarized in Table 6.

Turpentine Oil

Turpentine oil is commercially cultivated in India, the USA, France, and Russia. Different species are cultivated for the commercial production of turpentine oil. *Pinus echinata*, *Pinus maritima,* and *Pinus sylvestris* are grown in the USA, France, and Russia, respectively. In India, *Pinus insularis* and *Pinus longifolia* (also known as *P. roxburghii*) are cultivated commercially. *P. longifolia* grows naturally in India. Climatic conditions suitable for cultivation of this plant are rainfall between 100 and 175 cm, altitude 600–2400 m, and temperature 30–38 °C. It grows on a variety of soils like limestone, band of quartz and clay, sandstone, and even on bare rocks, and grows naturally by seed germination. In nurseries, plants are grown by sowing the seeds in March and April. The seedlings are transplanted at a distance of 1.5 m in July and once again after two years just before the beginning of monsoon. Plants of 20–25 years of age are utilized for the collection of turpentine oil.

Peppermint/Mentha Oil

Mentha species are cultivated in various parts of the world. It grows wild in Europe while it is cultivated in Japan, England, France, Italy, the USA, Bulgaria, Russia, and India. For the cultivation of peppermint (*M. piperita*), well-drained fertile sandy loam soil with neutral pH is required. Other requirements for cultivation include rainfall in the range of 95–105 cm, altitude of 250–400 m, and temperature between 15 and 25 °C. Cultivation is done by vegetative propagation method by using suckers. The treatment of mercury compounds as fungicides encourages better sprouting. Suckers are placed at a distance of 10 cm in January/February. The distance maintained between two rows is approximately 50–60 cm and the plants should be kept free from weeds by hoeing frequently. Foliar spray with fertilizers is advantageous. The plant is very susceptible to pests; therefore, effective pesticide treatment is necessary for pest control. Harvesting is done when the crop reaches at the flowering stage.

Caraway Oil

Caraway oil is obtained from the dried ripe fruits of *Carum carvi* and is indigenous to Netherlands and central Europe. It is cultivated in Central Asia, Europe, and many other countries and is grown in dry and temperate climate. It needs humus soil. For cultivation, the dried seeds are sown by the broadcast method in rows. The total height of the plant is not more than a meter. The fruits are collected before ripening. The entire plant is dried and the fruits are threshed out.

TABLE 6 Propagation Means of Essential Oil-Bearing Plants

Seeds	Vegetative Means	Both Seeds and Vegetative Means
Turpentine	Peppermint	Nutmeg
Caraway	Bitter orange peel	Cinnamon
Cardamom	Garlic	Black pepper
Coriander	Ginger	Vetiver
Ajowan		Rosemary
Dill		Palmarosa
Fennel		Citronella
Clove		Lemon grass

Cardamom Oil

Cardamom oil is obtained from the dried ripe fruits of *Elettaria cardamomum*. It occurs wild in Sri Lanka, Myanmar, and Malaysia and is cultivated in these countries as well as in India and Guatemala. For cultivation cardamom needs 150–600 cm rainfall, 600–1600 m altitude, and 50–100 °F temperature range. It grows on variety of soils but the soil should be full of moisture and also well drained. Mulching is necessary for its cultivation. Cardamom is cultivated by sowing the seeds. The seeds are very hard and take four months to germinate. They are either sown by the broadcast method or selected seeds are sown in prepared seed beds. The seedlings are transplanted into nursery beds and finally in the fields. The germination of seeds is poor (30–40%). Fresh seeds should always be used for cultivation. The seeds are sown and covered with soft earth. After germination, about 3- to 4-month-old seedlings are transplanted into nursery bed by keeping a distance of 15–40 cm in between. The seedlings, being delicate, are provided with sheds in the beginning. When they are about 130 cm in height or bear about 8–10 leaves and are 1–1.5 years old, they are transplanted in the forest fields just before monsoon. The cardamom plants start bearing from third year of planting. The fruits are picked up just before their ripening, so as to prevent the capsules from splitting on the floors. The collected capsules are dried either in sun or in artificial dryers. During drying the fruits shrink and that appearance persists in the final product. Sun-dried fruits are preferred because the seeds remain intact and also the characteristic aroma of cardamom oil can be maintained.

Coriander Oil

Coriander oil is obtained from the dried ripe fruits of *Coriandrum sativum*. It is cultivated throughout the European countries, principally in Russia, Hungary, and the Netherlands. It is also cultivated in India, Egypt, and Morocco. It needs light-to-heavy black soil and is sown by the drilling method. It is rotated with wheat, grain, onion, and also grown with cotton and sugarcane as a mixed crop. The crop is ready for harvesting after 100 days of growth.

Ajowan Oil

Ajowan oil is obtained from the dried ripe fruits of *Trychyspermum ammi* syn. *T. copticum.* It is indigenous to Egypt and is cultivated in Iraq, Iran, Afghanistan, Pakistan, and India. It is cultivated by the broadcast method in October–November. Thinning, weeding, and manuring are done as usual. Irrigation is essential for cultivation and harvesting is done in February–March.

Dill Oil

Dill oil is obtained from the dried ripe fruits of *A. graveolens*. It is indigenous to Southern Europe and is cultivated in England, Germany, Romania, and Mediterranean countries. It is cultivated by sowing the fruits and needs sandy loam soil, devoid of gravel, with slightly acidic nature. It is a cold weather crop; sowing is done in October and the crop is harvested in March–April. The fruits are collected at full maturity and dried thoroughly.

Fennel Oil

Fennel is obtained from the dried ripe fruits of *Foeniculum vulgare*. It is indigenous to Mediterranean countries and largely cultivated in Romania, Russia, Germany, France, India, and Japan. It is cultivated by the dibbling method. Quality fruits of good germination rate are sown just before the spring. Free branching of the herb and specific arrangement of leaves (in hair-like segments) on the stems require plenty of space in between two pants and rows, as well. Four to five seeds are sown at a time in holes at a distance of 25 cm apart. Well-drained and calcareous soil in a sunny situation is favorable for the cultivation of fennel. The crop is kept free from weeds and provided with suitable fertilizers. When the fruits are ripe, the crop is harvested and dried in the sun. Fruits are separated by threshing.

Orange Peel Oil

Orange peel oil is obtained from orange peel which is dried or fresh outer part of the pericarp of ripe or nearly ripe fruits of *Citrus aurantium*. It is indigenous to India and commercially cultivated in Spain, Caribbean islands, the USA, Morocco, and Sicily. It grows in loamy soil, with adequate arrangements of irrigation. The plants are cultivated by sowing the seeds or by grafting or cutting. Generally, the grafting method is adopted for cultivation. The plant starts bearing fruit after 5–6 years and, when the fruits are just ripe with orange spots, they are carefully picked by hand. The peels are treated to extract the oil or dried and preserved for further use.

Nutmeg Oil

Nutmeg oil is obtained from the dried kernels of the seeds of *Myristica fragrans*. It is indigenous to the Moluccas islands and cultivated in Indonesia, Caribbean islands, India, and other tropical countries. The plant is a dioecious tree. The seed is obtained from the female plants. The plant thrives well in warm humid climate and grown in locations from sea level to an altitude of 900 m. The requirement for rainfall is about 200–250 cm. Sandy loam, clay loam, and lateritic soils are satisfactory for cultivation. It is cultivated by sowing the seeds. Only freshly collected seeds are suitable for cultivation. Vegetative propagation is also possible. The seedlings are raised in the nursery beds. The seeds take about 2–3 months for germination. When the seedlings are about 16–18 months old, they are transplanted by keeping the distance of 8–9 m in between. The plant essentially needs shade for its protection. If sufficient rainfall is not available, irrigation is necessary. Fertilizers and manures are provided to the plants from time to time. The sex of the plant is determined and male trees are reduced to 10% of the total population. Fruits are produced by the plant throughout the year, but the number is maximum from December to May. The fruits are suitable for picking when the husk of the fully ripe nut splits, or else they are allowed to drop on the ground after dehusking. The red feathery aril (mace) covering the seed is detached from the seed shell by hand and the shells are dried in the sun, which takes about 4–8 weeks. Alternatively, they can be dried by artificial heat. After drying the shells are broken up and kernels are removed for extraction of nutmeg oil. Commercially, plantation is only economical when the plants are more than 10 years old.

Cinnamon Oil

Cinnamon oil is obtained from the dried inner bark of the shoots of the coppiced trees of *Cinnamomum zeylanicum*. It is indigenous to Sri Lanka and the Malabar Coast of India. It is also found in Jamaica and Brazil. Although the plant can be propagated by planting the cuttings and layers, commercially it is generally propagated by seed as it is the easiest method to adopt. It is a crop of tropical countries. It needs sandy or siliconous soils with an admixture of humus. The favorable altitude is 800–1000 m and a sheltered situation with an annual rainfall of 200–250 cm is ideal for cultivation. The seeds are sown in well-prepared nursery beds located at suitable places in June and July. The seeds are sown at a distance of 10 cm and covered with a small layer of soil and watered properly. It takes approximately 20 days for germination of seeds. Seedlings are provided with the shades and are allowed to grow for about 10–12 months. Transplantation is done in October/November or in rainy season by keeping the distance of 2 m in between two plants. Shades are provided with the pedals of coconut leaves. The field is weeded 2–4 times in a year. For a healthy growth, suitable fertilizers and manures are applied properly. The trees are coppiced to induce the formation of shoots. The trees are allowed to grow further unless they turn to uniform brown by formation of cork. Harvesting is done in rainy season when it is easy to peel off the bark from shoots. The bark is dried in shade over the mats prior to oil extraction.

Garlic Oil

Garlic oil is obtained from the bulbs of *Allium sativum*. It is cultivated in Central Asia, Southern Europe, the USA, and India. It is a perennial plant cultivated in well-drained moderately clay loamy soil. It needs cool moist climatic conditions during

the growth and dry period during maturity. The cultivation of this plant is done by planting bulbs generally in September to late October. Harvesting takes about four months. It is also taken as an alternate crop with many other vegetables.

Black Pepper Oil

Black pepper oil is obtained from the dried unripe fruits of *Piper nigrum*. It is indigenous to and cultivated in southern India. It is also cultivated in Indonesia, Brazil, Malaysia, and Sri Lanka. It can be cultivated from sea level up to an altitude of 1200 m. Well-distributed annual rainfall of 250 cm is ideal for successful cultivation. Temperature variation of 10–40 °C is tolerated by the plant. It thrives best in virgin soil rich in humus content. Clay is best suited for the plant, but it can be grown on red loams, sandy loams, and even alluvial soil. When grown on sloped land, slopes facing south are to be avoided and plants protected from severe sunlight. The plant is cultivated by sowing the seeds or by propagating cuttings. Plants grown from cuttings start bearing fruit from the second year and survive up to 15 years. Seed-raised plants start fruiting after 7–8 years and can survive up to 60 years. The cuttings are planted in March–April keeping a distance of 3–4 m in either direction. Supports or stands for every vine are also raised at the time of planting the cuttings. Plants climb well on silver oak, coconut, areca nuts, palms, etc. Since it is a perennial vine and may cause depletion of soil fertility suitable fertilizers are applied. Flowering occurs in May/June and berries which are orange or red colored in the spikes are harvested by hand in February/March. The berries are air-dried. They develop wrinkles and also become black in color during drying.

Vetiver Oil

Vetiver oil is obtained from the roots of *Vetivera zizanioides*. The plant is found growing in India, Myanmar, Sri Lanka, and East and West Africa. It is cultivated in Indonesia, Caribbean islands, India, Malaysia, Philippines, and the USA. Cultivation of this plant is done by sowing the seeds or from slips. A well-drained sandy loam is most suitable for cultivation. A temperature range of 25–38 °C and rainfall of 100–200 cm are desired. It thrives best in marshy places and in a humid climate. Planting of slips is done just before the outbreak of monsoon. The distance between two plants and between two rows is approximately 22.5 cm. Proper arrangements of irrigation must be made after rainy season is over. Fertilizers and manures are necessary for proper growth. When the plant is about 15–18 months of age, the roots are collected by uprooting in dry months of the year. If necessary, digging is done for collection of roots. The root is slashed, cut into small pieces and used for extraction of the oil.

Rosemary Oil

Rosemary oil is obtained from fresh flowering tops of the plant *Rosmarinus officinalis*. It is indigenous to Southern Europe and South Asia. It is cultivated in India and the Mediterranean basin. It is cultivated in gardens and on slopes. Its cultivation is successful in light calcareous soil. It is cultivated by sowing the seeds or with slips. After flowering, the plants are cut about 10 cm above the ground and used for extraction of the oil.

Palmarosa Oil

It is obtained from the leaves and tops of *Cymbopogon martinii*. This plant is indigenous to South East Asia, especially India, and is cultivated throughout India. It is a perennial grass and can be cultivated by sowing seeds or propagated by vegetative means. The seedlings are raised in nursery beds and then planted by keeping a distance of 1 m in between two rows. It needs light, well-drained calcareous soil with sunny situation, and rainfall of about 70–80 cm per year. The grass is irrigated when necessary and when the grass flowers the crop is harvested.

Citronella Oil

It is obtained from the fresh leaves of *Cymbopogon nardus*. It is indigenous to Sri Lanka and cultivated in Myanmar, Malaysia, Indonesia, Fiji, Sri Lanka, and India. It is cultivated by vegetative propagation from slips or even by sowing seeds. It needs to be irrigated regularly during winter and summer. The crop is ready for harvesting after eight months of growth. It can be harvested several times with regular intervals.

Clove Oil

Clove oil is obtained from the dried flower buds of *Eugenia caryophyllus*. It is indigenous to Amboynas and Moluccas islands and cultivated in Zanzibar, Pemba, Penang, Madagascar, Caribbean islands, Sri Lanka, and southern India.

Deep rich loamy soil with high humus content is suitable for clove cultivation. It is also found growing favorably in open sandy loam and lateritic soils. Water logging of soil must be avoided. It needs warm humid climate and grows well in the vicinity of sea. An annual rainfall in the range of 150–250 cm is another requirement for cultivation of this plant. It thrives best in regions ranging from the sea level up to 900 m. It is propagated by seed germination and seeds are sown from August to October. Seeds are planted in nursery beds at a distance of 10 cm and germination takes four to five weeks. The seedlings of clove are slender and delicate, with a poor growing rate. After six months they are transplanted to pots where they are allowed to grow for a year. Thereafter they are transferred to the field and are shaded in initial stages of growth. The plant can also be grown with areca nut, coconut, and nutmeg plants. Plants are provided with suitable fertilizers. Generally, fertilizers are given twice: in May/June and in October. The plant starts bearing after 7–8 years and a satisfactory yield is achieved only after 15–20 years of growth. Cloves are handpicked or collected by beating with bamboos. This operation commences when the cloves start changing color from green to slightly pink. When the trees are tall and cloves are beyond reach, platforms ladders are used for collection. The cloves are dried in the sun and freed and freed from foreign material and graded. On drying, cloves become perfectly crimson or brownish black in color.

Ginger Oil

Ginger oil is obtained from the rhizomes of *Zingiber officinale*. It is indigenous to South East Asia and cultivated in Caribbean islands, Africa, Australia, Mauritius, Jamaica, Taiwan, and India. Ginger needs a warm humid climate and is cultivated in areas with heavy rainfall. It is cultivated even at sea level, but still thrives best at an altitude of 1000–1500 m. If sufficient rainfall is not available proper arrangements of irrigation are necessary. Sandy, clay, or red loamy soils are suitable for ginger. It is cultivated by sowing rhizomes in June. Carefully preserved seed rhizomes are cut into small pieces and at least one living bud is allowed in each piece. Ginger is a soil-exhausting crop and being a rhizome needs to be supplemented with good quality suitable fertilizers and manures. The crop is ready for harvesting in about six months, when its leaves become yellow. Harvesting is done by digging the rhizomes. They are washed properly, scraped, and then dried to improve the color and to prevent further growth.

Lemon Grass Oil

Lemon grass oil is the volatile oil obtained from the leaves and aerial parts of *Cymbopogon citrates* and *Cymbopogon flexuosus*. It is indigenous to India and cultivated in India, China, Myanmar, East and West Africa, Guatemala, South America, Java, Madagascar, and many other countries. Both species are tufted perennial grass that grows well in sunny warm, humid conditions, such as the tropics. They need at least 2500–3000 mm annual rainfall. A daytime temperature of 25–30 ° C is considered optimum for maximum oil production with no extremely low night temperatures. The plants can be grown on a range of soils, typically around neutral pH. *Cymbopogon flexuosus* is normally propagated from seeds whereas roots propagate *C. citrates*. For obtaining roots, the usual method is to divide clumps from an existing or exhausted plantation following cutting. The first harvest of both species usually takes place 6–8 months after planting. They are cut three to six times annually, over 4–8 years of plantation. The cutting interval depends on the ecological conditions of the cultivation site.

SUMMARY POINTS

- The odorous volatile principles of plant and animal sources are known as essential oils and have been traditionally used in food and beverages.
- Systematic cultivation strategies of essential oil-bearing plants are necessary to maintain their regular supply for essential oil production.
- There are about 400 species, from 67 plant families which are cultivated commercially for production of essential oils.
- There are various factors influencing cultivation of aromatic plants.
- Illustrative cultivation methods of some important essential oil-bearing plants are presented.

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Chapter 4

Methods for Extracting Essential Oils

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List of Abbreviations

CP Cold expression
MAE Microwave-assisted extraction
MHG Microwave hydrodiffusion and gravity
SD Steam distillation
SFME Solvent-free microwave-assisted extraction
UAE Ultrasonic-assisted extraction

INTRODUCTION

It is known from antiquity that several plant species contain volatile chemical compounds which can be extracted as "essential oils" using an appropriate lipid solvent. Essential oils represent a small fraction of a plant's composition but confer the characteristic properties for which aromatic plants are used in the pharmaceutical, food, and fragrance industries. Nowadays it is clear that essential oils have a complex and variable composition, consisting of numerous constituents, especially hydrocarbons and oxygenated compounds. Their aroma is the result of the combination of the aromas of each one of these compounds. Trace components are also important as they give the oil a characteristic natural odor. Thus, it is important that the natural proportion of the components is maintained during extraction of the essential oils from plants by any technology employed. Different methods are used to separate these oils from the various plant materials. Although it seems relatively simple to isolate such oils, the composition of oil may vary to a large extent depending on the extraction method used (Anitescu et al., 1997; Cassel et al., 2009). Conventional extraction technologies are characterized by several drawbacks and are usually energy-consuming. Increasing energy cost and the more environmentally friendly approach adopted (i.e., reduction of carbon dioxide (CO_2) emissions) made stakeholders look to alternative technologies that are cost-effective, sustainable, and capable of producing products with the same or improved characteristics.

CONVENTIONAL ESSENTIAL OIL EXTRACTION METHODS

Cold Expression

Expression or cold pressing is the oldest extraction method and is used almost exclusively for the production of citrus essential oils. This method refers to any physical process during which the essential oil glands in the peel and cuticles are broken in order for the oil to be released. This process results in the production of a watery emulsion, which is subsequently centrifuged to separate out the essential oil (Bousbia et al., 2009). Until the start of the twentieth century, industrial production of cold pressed citrus oils was carried out manually. Currently, the only systems in use for the industrial production of peel oils are "sfumatric" "speciale sfumatrici" and "Pellatrici" machines, "Food Machinery Corporation whole fruit process," "bergamot oil extractors," and the "brown oil extractors" (Arnodou, 1991; Dugo and Di Giacomo, 2002). The reason for extracting citrus peel essential oils using mechanical methods is the thermal instability of the aldehydes present. Essential oils from noncitrus fruits such as berries are not usually extracted with this method.

Solvent Extraction

Solvent extraction can be used to extract essential oils that are thermally labile (e.g., from blossom). During this method, the plant material is placed into a solvent bath which dissolves it. After the extraction the liquid mixture that contains the

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essential oil (along with other compounds) goes through a filtration process and a subsequent distillation. Solvents that are commonly used for extraction are alcohol, hexane, ethanol, petroleum ether, and methanol. The main advantage of extraction over distillation is that a lower temperature is used during the process, therefore reducing the risk of chemical changes due to high temperatures, which are used during distillation. Solvent extraction is inexpensive and relatively fast and because the diffusion rates are influenced by temperature, it is possible to increase the speed of the process by using hot solvents. The essential oil produced will contain a small quantity of solvent as a residue and therefore its use for food applications is not possible. However, if the solvent used is alcohol, it is safe for consumption and considered "food grade". This method is commonly used by the perfume industry.

The "Enfleurage" Method

Enfleurage is another conventional extraction method that dates back to antiquity. It has been used mainly for the extraction of essential oils from flowers (e.g., jasmine). During this method a purified odorless cold fat is spread on the plant material (e.g., flowers). The odors are given off by the flowers and are consequently dissolved in the fat. New flowers replace the old ones and the process is repeated for very long periods until saturation of the fat is reached. Afterwards the fat is collected and extracted with alcohol. By today's standards it is a time-consuming, labor-intensive, and costly method. It does not appear to have any applications for essential oils used in food industry and it is virtually obsolete nowadays.

Distillation

The most commonly used extraction method is the steam distillation or water distillation method (Figure 1). This extraction process can last between 1 and 10 h. The amount of oil produced depends on length of distillation time, temperature, pressure, and type of plant material (Naves, 1974). During distillation, plant materials are exposed to boiling water or steam in order to release the essential oil within them through evaporation. As the steam and essential oil vapors are being condensed, they are collected and separated in a vessel usually called the ''Florentine flask" (Dugo and Di Giacomo, 2002). Even though extraction of an essential oil by distillation appears to be a straightforward process, it has many drawbacks. Because the essential oils are exposed to boiling water for long time periods, the formation of artifacts is a possible issue due to the high temperature or the acidity of water. This can lead to differences in the composition of the volatile oils being extracted. During distillation the hydrolysis of esters to alcohols and acids can take place which may cause serious implications in the case of oils with high quantities of esters. Moreover, some essential oils require rectification. This process involves the redistillation of the oil so as to eliminate undesirable impurities (e.g., waxes) as well constituents that can impart an unacceptable odor.

Extraction with Supercritical Gases

In general, conventional extraction methods such as steam distillation and solvent extraction have been traditionally used; however, these methods have disadvantages such as low yield, loss of volatile compounds, long extraction times, and toxic solvent residues (Khajeh et al., 2005). This has led to the development of alternative extraction techniques that can overcome these problems. An

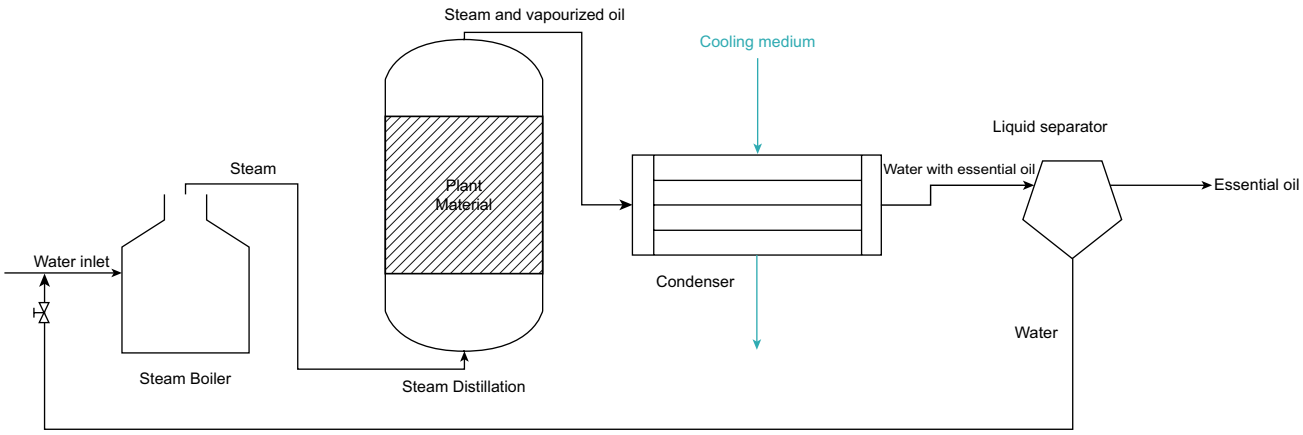

FIGURE 1 Typical industrial steam distillation unit for extraction of essential oils. *Adapted from Masango (2005).*

alternative for these conventional methods is supercritical fluid extraction (SFE) which has been introduced and studied in depth. SFE can be performed in batch, semi batch, and continuous modes. In general, the solid material is put in a vessel in which the supercritical fluid is added under a specific flow rate until the appropriate extraction conditions are reached.

Benefits of Supercritical Fluid Extraction

SFE has several characteristics that make it an efficient alternative technique for extraction of essential oils compared with conventional solvent extraction methods as outlined below (Otterbach and Wenclawiak 1999; Lang et al., 2001).

- It is usually performed at low temperatures, making it a very suitable method for thermally sensitive compounds.
- The solvation power (i.e., interaction of a solute with the solvent) of the fluid in use can be controlled by changing pressure and/or temperature, thus leading to very high selectivity. However, high pressure should be avoided because it can lead to extraction of undesired high molecular weight compounds (e.g., simultaneous exaction of waxes during essential oil isolation).
- Supercritical fluids, because of their lower viscosity and higher diffusivity, can enter porous solid materials more effectively compared with liquid solvents resulting in faster mass transfer and rapid extraction compared with solvent extraction methods.
- It is a more environmental friendly method since it uses nontoxic fluids such as CO_2 or in some cases uses significantly less quantities of organic solvent of just a few millilitres.
- It can be scaled according to application from analytical up to large industrial scale.

Comparison of Supercritical Fluid Extraction to Conventional Methods/Factors Affecting Extraction Conditions

For successful SFE, several factors must be taken into account. These factors include the type of sample, sample preparation, type of fluid, choice of modifiers, method of fluid feeding and extraction conditions including pressure, temperature, flow rate, and extraction time (Lang et al., 2001). In practice, SFE is performed usually using CO_2 due to its low critical temperature, low cost, nontoxicity, lack of odor and taste, and easiness of removing without leaving residues. Moreover, it has good solvent properties for nonpolar and some polar molecules (Modey et al., 1996; Khajeh et al., 2005). By varying process conditions (e.g., temperature and pressure of the CO_2) during extraction, an oil of a desired composition can be selectively extracted. Eikani et al. (1999) compared the extraction of cumin essential oil obtained from *Cuminum cyminum* by supercritical CO_2 with oil obtained by conventional steam distillation. They showed that the physicochemical properties of the oils extracted by supercritical CO_2 and steam distillation were different. The results of the compositional analysis showed that the most obvious difference between the two methods is in the *p*-mentha-1,3-dien-7-al and *p*-mentha-1,4-dien-7-al composition. A comparison of steam distillation, solvent extraction, SFE, and liquid CO_2 used to obtain the volatile oil from Western Australian sandalwood showed that SFE afforded the highest yields of extracts and total volatiles (Piggott et al., 1997). A study by Pourmortazavi and Hajimirsadeghi (2007) also showed that different extraction compositions could be obtained by different extraction methods. They studied SFE of volatile oil from *Juniperus communis* leaves using CO_2 under different conditions of pressure, temperature, modifier content, and dynamic extraction time. The comparison of SFE (200 atm pressure and 45 °C temperature for 15 min static followed by 20 min dynamic) with steam distillation revealed that a total of 22 compounds were present in SFE extracts whereas in the distilled oil only 11 components were identified and quantified (Table 1). According to Khajeh et al. (2005) the SFE of *Ferula assafoetida* offers several advantages over steam distillation. SFE results in shorter extraction times (25 min vs 4 h for steam distillation) and lower energy cost and higher selectivity. The possibility of manipulating the composition of the oil, by changing the parameters of the extraction (pressure, temperature, modifier volume, and dynamic extraction time) is more attainable in SFE. However, although it was shown that compositions of oils obtained by SFE and steam distillation were not qualitatively different, they did differ quantitatively.

NOVEL "GREEN" EXTRACTION METHODS

Microwave-Assisted Extraction

An additional technology that has attracted particular attention is microwave-assisted extraction (MAE) due to its unique heating mechanism (based on friction), reasonable cost, and good performance under atmospheric conditions. Compared with conventional extraction methods (e.g., Soxhlet extraction), MAE leads to higher extraction

TABLE 1 Comparison of the Main Components of Iranian Black Cumin Oils Obtained by Supercritical Fluid Extraction (SFE) and Hydrodistillation (HD)

Constituent	Supercritical Fluid Extraction Yield (%)	Hydrodistillation Yield (%)
α-Pinene	0.8	2.8
β-Pinene	1.5	3.7
Myrcene	0.6	1.0
p-Cymene	–	5.6
o-Cymene	7.8	0.1
Limonene	6.8	10.6
γ-Terpinene	38.0	45.7
Cuminaldehyde	11.5	12.7
Cuminyl alcohol	–	6.4
α-Methyl-benzenemethanol	25.6	3.5

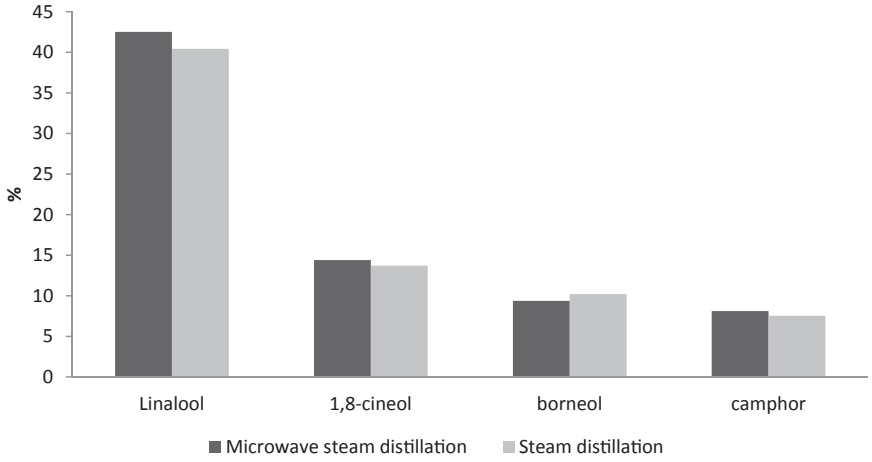

FIGURE 2 Quantities of dominant components of lavender flowers essential oil (*Lavandula angustifolia*) obtained by MSD and SD.

yields, shorter extraction times, and higher selectivity (Chen et al., 2007). Moreover, MAE can also be considered superior to SFE in terms of simplicity and operation cost (Zhang et al., 2011). However, it usually requires a higher quantity of organic solvent which makes it less environmentally friendly than SFE (Huie, 2002). In an attempt to take advantage of microwave heating, researchers have combined microwaves with conventional methods developing new methods such as microwave-assisted solvent extraction, vacuum microwave hydrodistillation, microwave hydrodistillation, compressed air microwave distillation, and microwave-accelerated steam distillation. Sahraoui et al. (2008) developed a new method for the extraction of dry lavender flowers (*Lavandula angustifolia* Mill., Lamiaceae) called microwave steam distillation (MSD) and compared it to conventional steam distillation (SD). Essential oils isolated by MSD and SD were quantitatively as well qualitatively similar (Figure 2), but MSD was better than SD in terms of rapidity (6 min vs 30 min, respectively).

Another attempt was made by Wang et al. (2006), who developed a new extraction method of essential oils from *C. cyminum* L. and *Zanthoxylum bungeanum* which they named microwave-assisted hydrodistillation (MAHD). Using MAHD to extract the essential oil from *Thymus vulgaris* Golmakani and Rezaei (2008) obtained a similar yield at significantly shorter extraction time compared to hydrodistillation. This made the extraction process less energy demanding and more sustainable.

One of the most promising and the most commonly used methods of the modified MAE for essential oils is solvent-free microwave-assisted extraction (SFME). SFME significantly reduces the extraction times compared with conventional

TABLE 2 Production of Different Essential Oils by Microwave-Assisted Extraction (MAE)

Essential Oil	Type of Equipment	Benefits	References
Cuminum cyminum/Zanthoxylum bungeanum	Microwave oven with stirrer, with a microwave absorption medium	Rapid and energy efficient method	Want and Weller (2006)
Eletaria cardamomum	Focused microwave oven	No solvent/reduces extraction time	Lucchesi et al. (2007)
Laurus nobilis	Microwave oven with stirrer, with water as solvent	No solvent, safe, energy efficient	Flamini et al. (2007)
Lavandula angustifolia Mill., Lamiaceae	Microwave steam distillation	Significantly faster than steam distillation⇒reduced costs	Sahraoui et al. (2008)
L. angustifolia Mill., Lamiaceae	Microwave accelerated steam distillation	Superior to steam distillation in terms of energy saving, rapidity (product yield, cleanliness and product quality).	Chemat et al. (2006)
Eureka (*Citrus limon*), Villa Franŋa (*C. limon*), lime (*Citrus aurantifolia*), Marsh seedless (*Citrus paradisi*), Tarocco (*Citrus sinensis*), Valencia late (*C. sinensis*), Washington navel (*C. sinensis*), Tangelo seminole (*C. paradisi*).	Microwave hydrodiffusion and gravity (combination of microwave heating and gravity working at atmospheric pressure)	Green extraction method producing comparable yields in significantly shorter times compared with HD and cold expression and an essential oil of higher purity.	Bousbia et al. (2009)

methods ranging from a few hours to 20–30 min for essential oil extraction. In addition, it is characterized by a medium capital and low operating cost (Chan et al., 2011). The essential oils extracted by SFME from cardamom essential oil (*Eletaria cardamomum* L.) are more valuable compared with those extracted by hydrodistillation (HD) because of the higher amount of oxygenated compounds present consisting of highly aromatic compounds (Lucchesi et al., 2007). All of the above make SFME a superior extraction method to HD. Table 2 presents some examples of different methods based on microwaves for the extraction of essential oils. Although MAE has been successfully used in laboratory scale, the number of industrial-scale uses stays low (Leonelli and Maso, 2010).

Controlled Pressure Drop Process

Another very promising method is the instant controlled pressure drop process, or DIC (Allaf et al., 1998). This method was initially used for texturing and drying various products and was later extended as a benchmark method for extraction of high-quality essential oils. It is based on the thermomechanical processing caused by subjecting the product to a rapid transition from high steam pressure to a vacuum. Processing by DIC enhances global diffusivity and the availability of the liquid in the plant and does not require the use of solvents (Rezzoug et al., 2005). The possibility of using DIC for extraction of essential oils from plants has been proven and compared to conventional methods of extraction. The study of Kristiawan et al. (2008) showed that it is possible to extract the essential oils from Indonesian Kananga in less than 6 min with a yield of 2.8 g/100 g dry matter. A similar yield (2.5 g/100 g dry matter) was achieved with steam distillation but after 16 h of processing. Mellouk et al. (2004) isolated the essential oil from juniper berries using DIC. They reported that two cycles of 150 s at 0.6 MPa yielded 95% of the oil obtained by steam distillation in 12 h. Similar reductions between HD and DIC in terms of extraction time were reported by Allaf et al. (2013) along with differences obtained in terms of the oxygenated and nonoxygenated fractions (Table 3). According to Berka-Zougali et al. (2010), DIC allows for higher extraction yields (10%) of essential oils from Algerian myrtle leaves compared with steam distillation. It is also a faster method (2 min compared with 180 min) and leads to the production a higher quality essential oil as shown by its composition and antioxidant activity. It also requires lower energy levels and water use. Essential oil extraction based on this process is an interesting alternative not only to standard techniques of essential oil extraction, such as extraction with solvents or SD, but even more efficient methods such supercritical fluid extraction (Rezzoug et al., 2005).

TABLE 3 Composition of Obtained by Hydrodistillation (HD) and Instant Controlled Pressure Drop Process (DIC) Essential Oil Composition of Orange Peels (*Citrus sinensis*)

Compound Groups	Controlled Pressure Drop Process	Hydrodistillation
Total oxygenated compounds (mg/g of dry matter)	0.61	0.22
Total nonoxygenated compounds	15.9	1.7
Yield (mg/g of dry matter)	16.57	1.97
Extraction time (in min)	2	240

Ultrasound-Assisted Extraction

Ultrasonic-assisted extraction (UAE) in food processing technology is of interest because it is able to facilitate component extraction (e.g., oils, proteins, polysaccharides). The major advantages of the application of ultrasound are the minimum effects on extractable compounds, reduction/avoidance of organic solvents (as it is effective also with generally recognized as safe solvents), and a reduction in extraction time (Vilkhu et al., 2008). The effects of ultrasound are due to the phenomena of cavitation, that is, production and breakdown of microscopic bubbles. When the bubbles increase in size they collapse violently. This violent collapse induces mechanical forces which lead to cell membrane damage (Cameron et al., 2009), resulting in high yield of extracted materials and fast rate of extraction. There are numerous studies illustrating the advantages of UAE. Shah et al. (2005) and Sharma and Gupta (2006) showed that ultrasonic pretreatment of *Jatropha curcas*, almond, and apricot seeds led to higher yield and a reduction in oil extraction time compared with aqueous enzymatic extraction. The benefits from ultrasound pretreatment before hydrodistillation were also shown by the study of Assami et al. (2012). Ultrasound treatment of *Carum carvi* seeds followed by hydrodistillation resulted in producing higher essential oil recovery in a shorter time (30 and 90 min for treated and untreated samples, respectively) without affecting oil composition. Ultrasound pretreatment has also been combined with newer extraction techniques giving very promising results. Supercritical fluid extraction of oil from almonds after treatment with ultrasound increased the oil yield and decreased processing time by approximately 30% compared with supercritical fluid extraction alone (Riera et al., 2004). Ultrasound, although expensive in capital cost, can enhance extraction process which can lead to increased yield in less time and thus provide a viable alternative to conventional as well as newer commercial oil production processes.

Effects of Extraction Methods on Essential Oil Characteristics

Analysis of essential oils has shown that their chemical profile may differ not only in the quantity of the different constituents but also in the structure of molecules extracted, depending on the method of extraction which influences the characteristics of the essential oil. It has been shown that different extraction methods can produce essential oils with a more natural organoleptic profile. This difference can be attributed to the different composition of oils according to the method used to extract it (solvent distillation vs distillation) (Burt, 2004). Périno-Issartier et al. (2013) conducted a comparative study of the ability of eight different methods (conventional and novel) to extract the essential oils from lavender flowers and showed that a panel of trained assessors could detect differences between the oils. In general, extraction methods based on microwaves gave very similar results whereas oil obtained by turbohydrodistillation had a strong musky odor due to the fact that it was significantly damaged based on results of its composition after the extraction. When solvent extraction (ethanol or hexane), HD, and SFE were used to obtain the essential oil from *Tetraclinis articulata*, their antioxidant activity was different between the essential oil obtained by the different extraction methods. 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid and 1,1-diphenyl-2-picrylhydrazyl assays showed that SFE essential oil showed the most pronounced antioxidant activity, most likely due to reduced thermal degradation of active constituents (Herzi et al., 2013). In addition, a study by Okoh et al. (2010) showed that essential oil produced by SFME was more active against Gram-positive and negative bacteria tested compared with oil obtained by HD, most likely because of the higher content of oxygenated compounds which have a high antifungal and antibacterial action. The composition of essential oil extracted by different methods, even if similar, may differ in terms of relative concentration of identified compounds which can subsequently give different oil properties. Therefore, the type of extraction must be chosen according to the use of the essential oil.

SUMMARY POINTS

- There is a plethora of methods with which essential oils can be extracted aimed at food applications.
- Essential oil composition and yield can differ greatly between extraction methods.
- It is difficult to determine under which conditions the composition of the mixture of volatile compounds extracted reflects the actual composition.
- Conventional methods such as distillation and expression are used most commonly to extract food grade essential oils. However, disadvantages such as long extraction times and degradation of essential oil compounds are a significant concern.
- Super critical fluid extraction with the use of CO_2 appears to be a clean and efficient technology to obtain essential oils and offers many advantages over conventional extraction methods. Moreover, the possibility of obtaining a certain composition by varying the process conditions during SFE is an advantage if a product with certain composition must be obtained. On the downside, it is considered a costly method.
- SFME is another very promising extraction method able to produce high quality essential oils in a minimum amount of time with low environmental impact.
- Ultrasound, although of considerable cost, can significantly enhance conventional and novel/alternative extraction methods in terms of extraction speed, yield, and quality of essential oil.
- The seasonality/collection period of the plant material must be taken into account as it may influence the composition of the extracted essential oil.

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Chapter 5

Biologically Active Essential Oils against Stored Product Pests

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INTRODUCTION

Synthetic pesticides employed in the preservation of the stored products, such as methyl bromide and phosphine, are very toxic compounds and have serious adverse effects to the environment. The use of methyl bromide is avoided because of its contribution to the depletion of the ozone layer. Additionally, insect species are also reported to develop resistance to the phosphine. Other alternative synthetic fumigants such as sulfuryl fluoride, carbonyl sulfide, ethane dinitrile, and ethyl formate also have the previously mentioned adverse effects (Rajendran and Sriranjini, 2008; Shaaya et al., 1997). The growing world population and increasing food demand together with increasing environmental concerns of society dictate alternative methods for preservation of stored products. Essential oils and their components could be a natural, environmentally friendly, and relatively safe alternative to synthetic fumigants (Isman, 2000; Regnault-Roger, 1997).

More than 1000 species of pests are known to cause damage to stored products (Rajendran, 2002). An immense amount of literature exists on the insecticidal activity of the essential oils against these pests. Therefore in this review only essential oils that have been tested for toxicity against the major stored product pest *Sitophilus* sp. (Coleoptera: Curculionidae) are discussed. In the scope of this chapter, essential oil composition and type of activity observed against these pests is summarized as well as potential areas of research and future prospects. The main components of the active essential oils which require further attention is also included. However, reports on the activity of individual volatile natural/synthetic compounds have been excluded.

ACTIVE ESSENTIAL OILS AGAINST *SITOPHILUS GRANARIUS* (L.)

Essential oils from 57 taxa from Apiaceae, Amaranthaceae, Anacardiaceae, Asteraceae, Hypericaceae, Lamiaceae, Rutaceae, and Verbenaceae families have previously been investigated for their activity against *S. granarius* (L.). The activities of the previously investigated essential oils are summarized in Table 1. Essential oils from species of the Asteraceae and Amaranthaceae families seem to be the most active against this pest. *Chenopodium ambrosioides* (L.) Mosyakin and Clemants show very high fumigant toxicity at doses as low as 0.1–0.3 μL oil/petri dish, producing 63–100% mortality (Tapondjou et al., 2002). *Achillea biebersteinii* Afan. chemotype that was reported to contain 1,8 cineole, piperitone, and camphor as main components afforded high contact toxicity at very low concentrations; however, other chemotypes indicated in the same report which contained 1,8 cineole, camphor, and α-thujone produced lower contact activity (Polatoglu et al., 2013). *Artemisia* sp. oils which contain unusual compounds such as chamazulene, nuciferol propionate, and nuciferol butanoate have also been reported to have high fumigant toxicity at very low concentrations (Kordali et al., 2006). Additionally, significant attention should be given to the species of the Lamiaceae family that include *Ocimum* sp., *Origanum* sp., and *Micromeria* sp. that produce relatively high fumigant toxicity at low concentrations (Aslan et al., 2005; Caglar et al., 2007; ObengOfori and Reichmuth, 1997). The main components of the essential oils with previously reported activity against *S. granarius* are given in Table 2.

ACTIVE ESSENTIAL OILS AGAINST *SITOPHILUS ORYZAE* (L.)

Essential oils from 138 taxa from Amarylidaceae, Amaranthaceae, Anacardiaceae, Annonaceae, Apiaceae, Asteraceae, Araceae, Aristolochiaceae, Burceraceae, Cruciferae, Cyperaceae, Cupressaceae, Hypericaceae, Lamiaceae, Lauraceae, Liliaceae, Myristicaceae, Myrtaceae, Piperaceae, Poaceae, Ranunculaceae, Rutaceae, Santalaceae, Valerianaceae, Verbenaceae, and Zingiberaceae families have previously been investigated for their activity against *S. oryzae*. The activities of the

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TABLE 1 Reported Insecticidal Activity of the Essential Oils Obtained from Various Plant Species against *Sitophilus granarius*

Family	Species	Essential Oil Content	Type of Insecticidal Activity	Conditions and Activity of the Oil	Reference
Amaranthaceae	*Chenopodium ambrosioides* (L.) Mosyakin and Clemants	*p*-Cymene 50.0%, α-Terpinen 37.6%	Fumigant activity	At 25 °C, after 24 h, 0.1–0.3 µL oil/petri dish 63–100% mortality.	Tapondjou et al. (2002)
Asteraceae	*Achillea biebersteiini* Afan.	Chemotype 1: 1,8 cineole 30.6%, piperitone 28.9%, camphor 11.7% Chemotype 2: 1,8 cineole 31.1%, camphor 14.4%, α-thujone 12.9%	Contact and fumigant activity	Contact (chemotype 1): at 25 °C, after 24h, 0.1–0.2 µL 10–20% v oil/v acetone 62.33–100% mortality. Contact (chemotype 2): at 25 °C, after 24 h, 0.1–0.2 µL v oil/v acetone 3.33–78.91% mortality. Fumigant (chemotype 1): at 25 °C, after 24 h, 10 µL 10% v oil/v acetone 36.64% mortality. Fumigant (chemotype 2): at 25 °C, after 24 h, 10 µL 10% v oil/v acetone 1.49% mortality.	Polatoglu et al. (2013)
	Achillea teretifolia Willd.	1,8 cineole 15.9%, borneol 8.1%, camphor 7.0%, T-cadinol 5.9%, *trans*-nerolidol 5.1%, terpinen-4-ol 5.0%	Contact and fumigant activity	Contact: At 25 °C, after 24 h, 0.1–0.2 µL v oil/v acetone 43.32–82.35% mortality. Fumigant: at 25 °C, after 24 h, 10 µL 10% v oil/v acetone 4.32% mortality.	
	Artemisia absinthium L.	Chamazulene 17.8%, Nuciferol propionate 5.1%, Nuciferol butanoate 8.2%	Fumigant activity	After 24 h, 3–9 µL oil/L air 46.7–73.3% mortality.	Kordali et al. (2006)
	Artemisia santonicum L.	Camphor 18.2%, 1,8 cineole 7.5%, β-Eudesmol 7.2%	Fumigant activity	After 24 h, 3–9 µL oil/L air 40.0–66.7% mortality.	
	Artemisia spicigera C. Koch	Camphor 34.9%, 1,8 cineole 9.5%, borneol 5.1%	Fumigant activity	After 24 h, 3–9 µL oil/L air 43.3–80.0% mortality.	
Lamiaceae	*Micromeria fruticosa* (L.) DRUCE	–	Fumigant activity	At 25 °C, after 24 h, 0.5–2 µL oil/L air 12–58% mortality.	Aslan et al. (2005)
	Nepata racemosa L.	–	Fumigant activity	At 25 °C, after 24 h, 0.5–2 µL oil/L air 10–52% mortality.	
	Origanum acutidens L.	–	Fumigant activity	At 25 °C, After 24 h, 4–8 µL oil 16.6–50.0% mortality.	Caglar et al. (2007)
	Origanum onites L.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 21.00–29.33% mortality.	Yildirim et al. (2011)
	Origanum rotundifolium Boiss.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 29.00–30.00% mortality.	
	Ocimum suave (Wild.)	Eugenol 59.7%, *Trans*-β-ocimene 14.5%, β-Cubebene	Fumigant, contact, and repellent activity	Fumigant: at 27 °C, after 24 h, 1–10 µL eugenol/petri dish 15–94% mortality. Contact: At 27 °C, after 48 h, 1–10 µL eugenol/1 mL acetone per beetle 40–100% mortality. Repellent: at 27 °C, 1–10 µL eugenol/disc 67–95% repellency	Obeng-Ofori and Reichmuth (1997)
	Origanum vulgare L.	–	Fumigant activity	At 25 °C, after 24 h, 0.5–2 µL oil/L air 16–62% mortality.	Aslan et al. (2005)

Lamiaceae	*Rosmarinus officinalis* L.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 20.33–27.00% mortality.	Yildirim et al. (2011)
	Salvia hydrangea DC. Ex Benth.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 0.67–4.33% mortality.	
	Salvia multicaulis Vahl.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 2.00–1.00% mortality.	
	Salvia nemerosa L.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 0.67–2.00% mortality.	
	Salvia sclarea L.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 0.00–3.00% mortality.	
	Satureja hortensis L.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 31.00–33.00% mortality.	
	Satureja spicigera (C. Koch.) Boiss.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 9.33–27.67% mortality.	
	Thymus fallax Fisch. and Mey.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 0.67–24.00% mortality.	
	Thymus sipyleus Boiss.	–	Fumigant activity	At 25 °C, after 24 h, 1–20 µL oil 2.67–18.67% mortality.	

TABLE 2 The Main Components of the Essential Oils with Reported Activity against Species

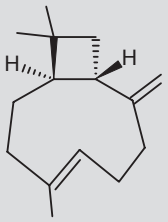
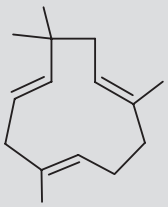
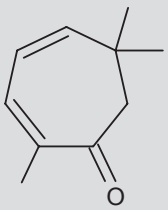
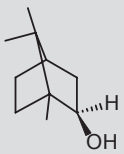
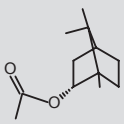
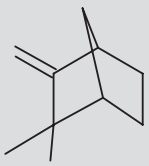
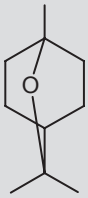
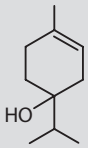
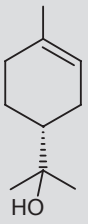
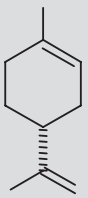
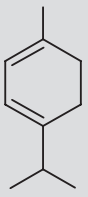
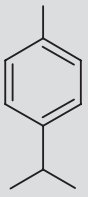
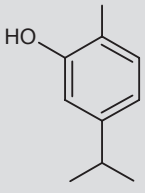
Compound	Structure	Activity Type	Pest Species	Reference
β-Caryophyllene		Repellent, contact	*Sitophilus. zeamais*	Bekele and Hassanali (2001)
Humulene		Fumigant, contact	*Sitophilus oryzae*	Lee et al. (2001)
			S. zeamais	Bekele and Hassanali (2001) and Suthisut et al. (2011)
Eucarvone		Fumigant	*S. oryzae*	Kim and Park (2008)
Borneol		Fumigant	*Sitophilus granarius*	Kordali et al. (2006)
			S. oryzae	Lee et al. (2001)
iso-Borneol		Fumigant, contact	*S. zeamais*	Suthisut et al. (2011)
Bornyl acetate		Fumigant	*S. granarius*	Kordali et al. (2006)
Camphor		Contact, fumigant, repellent	*S. granarius*	Kordali et al. (2006)
			S. oryzae	Lee et al. (2001)
			S. zeamais	Bekele and Hassanali (2001) and Suthisut et al. (2011)
Camphene		Fumigant, contact	*S. oryzae*	Kim and Park (2008)
			S. zeamais	Bekele and Hassanali (2001) and Suthisut et al. (2011)

TABLE 2 The Main Components of the Essential Oils with Reported Activity against Species—cont'd

Compound	Structure	Activity Type	Pest Species	Reference
1,8 Cineole		Fumigant, contact, repellent	*S. granarius*	Kordali et al. (2006)
			S. oryzae	Lee et al. (2001)
			S. zeamais	Bekele and Hassanali (2001) and Suthisut et al. (2011)
Terpinen-4-ol		Fumigant, contact	*S. granarius*	Kordali et al. (2006)
			S. oryzae	Lee et al. (2001)
			S. zeamais	Bekele and Hassanali (2001) and Suthisut et al. (2011)
α-Terpineol		Fumigant	*S. granarius*	Kordali et al. (2006)
			S. oryzae	Lee et al. (2001)
Limonene		Fumigant, contact	*S. oryzae*	Lee et al. (2001)
			S. zeamais	Bekele and Hassanali (2001)
α-Terpinene		Fumigant	*S. oryzae*	Lee et al. (2001)
p-Cymene		Fumigant, repellent	*S. oryzae*	Lee et al. (2001)
Carvacrol		Fumigant	*S. oryzae*	Lee et al. (2001)

Continued

TABLE 2 The Main Components of the Essential Oils with Reported Activity against Species—cont'd

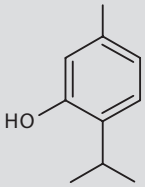
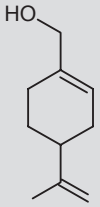
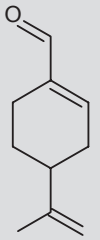
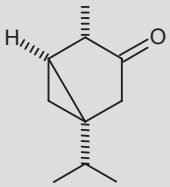
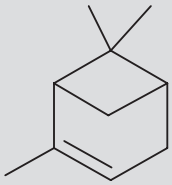
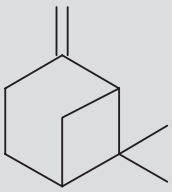
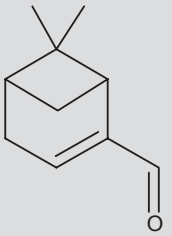
Compound	Structure	Activity Type	Pest Species	Reference
Thymol		Fumigant	*S. oryzae*	Lee et al. (2001)
Perilla alcohol		Fumigant	*S. oryzae*	Lee et al. (2001)
Perilla aldehyde		Fumigant	*S. oryzae*	Lee et al. (2001)
Thujone		Fumigant	*S. oryzae*	Lee et al. (2001)
α-Pinene		Fumigant, contact	*S. zeamais*	Suthisut et al. (2011)
β-Pinene		Fumigant, contact	*S. zeamais*	Suthisut et al. (2011)
Myrtenal		Fumigant, contact	*S. oryzae*	Lee et al. (2001)

TABLE 2 The Main Components of the Essential Oils with Reported Activity against Species—cont'd

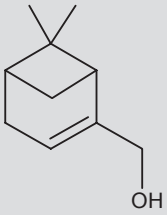
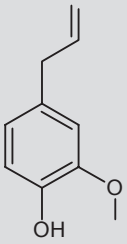
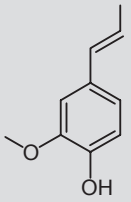
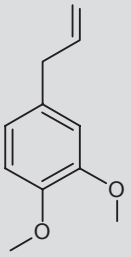
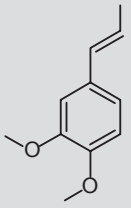
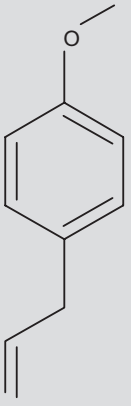
Compound	Structure	Activity Type	Pest Species	Reference
Myrtenol		Fumigant	*S. oryzae*	Lee et al. (2001)
Eugenol		Contact, repellent	*S. granarius*	Obeng-Ofori and Reichmuth (1997)
		Fumigant	*S. oryzae*	Lee et al. (2001)
			S. zeamais	Obeng-Ofori and Reichmuth (1997)
iso-Eugenol		Fumigant, contact	*S. oryzae*	Lee et al. (2001)
			S. zeamais	Bekele and Hassanali (2001)
Methyl eugenol		Fumigant	*S. oryzae*	Lee et al. (2001)
Methyl iso-eugenol		Fumigant	*S. oryzae*	Lee et al. (2001)
Estragole (Methyl chavicol)		Fumigant, contact	*S. zeamais*	Bekele and Hassanali (2001)

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TABLE 2 The Main Components of the Essential Oils with Reported Activity against Species—cont'd

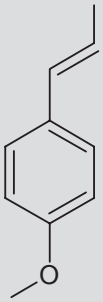
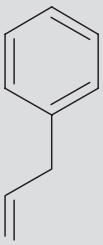
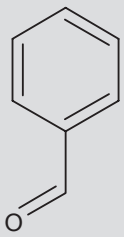
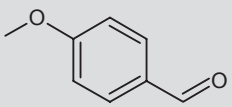
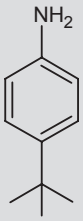
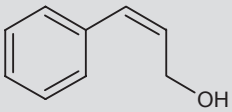
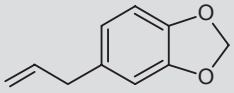
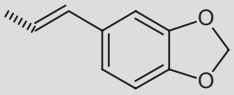
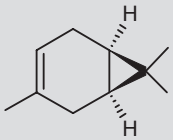
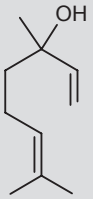
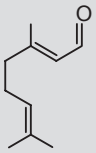
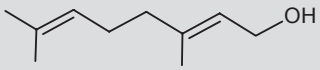
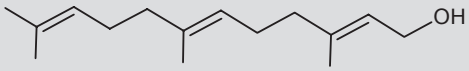
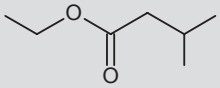
Compound	Structure	Activity Type	Pest Species	Reference
(*E*)-Anethole		Fumigant	*S. oryzae*	Lee et al. (2001)
Allyl benzene		Fumigant	*S. oryzae*	Lee et al. (2001)
Benzaldehyde		Fumigant	*S. oryzae*	Lee et al. (2001)
Anisaldehyde		Fumigant	*S. oryzae*	Lee et al. (2001)
4-t-Butylaniline		Fumigant	*S. oryzae*	Lee et al. (2001)
Cinnamyl alcohol		Fumigant	*S. oryzae*	Lee et al. (2001)
Safrole		Fumigant, contact	*S. oryzae*	Kim and Park (2008)
iso-Safrole		Fumigant	*S. oryzae*	Lee et al. (2001)

TABLE 2 The Main Components of the Essential Oils with Reported Activity against Species—cont'd

Compound	Structure	Activity Type	Pest Species	Reference
δ-3-Carene		Fumigant, contact	*S. oryzae*	Kim and Park (2008)
Linalool		Fumigant, contact	*S. oryzae*	Lee et al. (2001)
Citral		Fumigant	*S. oryzae*	Lee et al. (2001)
Geraniol		Fumigant	*S. oryzae*	Lee et al. (2001)
Farnesol		Fumigant	*S. oryzae*	Lee et al. (2001)
Ethyl isovalerate		Contact	*S. zeamais*	Bekele and Hassanali (2001)

previously investigated essential oils are summarized in Table 3. According to previous reports, significant attention should be given to species of the Myrtaceae, Lamiaceae, Asteraceae, and Zingiberaceae families. Essential oils of *Eucalyptus camaldulensis* Dehnh., *Eucalyptus codonocarpa* Blakely and McKie, *Eucalyptus intertexta* R.T. Baker, and *Eucalyptus sargentii* Maiden species are reported to have very high fumigant activity against *S. oryzae* at low application doses (Lee et al., 2004; Nagebhan and Moharramipour, 2007). However, only the essential oil composition of *E. codoocarpa* was reported. The main components of the oil were *p*-cymene and piperitone (Lee et al., 2004). Additionally, essential oils of *Mentha* sp., *Origanum* sp., *Ocimum gratissimum* L., and *Salvia officinalis* L. of the Lamiaceae family also produced high fumigant activity at low doses (Franz et al., 2011; Gendo et al., 2008; Shaaya et al., 1997). The *Ocimum gratissimum* essential oil was reported to contain the components methyl eugenol and (*Z*)-*β*-ocimene as their main components (Gendo et al., 2008). *Curcuma longa* L. essential oil which contains myrcene, *p*-cymene, and 1,8 cineole also have high fumigant toxicity at low application doses (Tripathi et al., 2002). The essential oils of *Mentha* sp., *Achillea santolina* L., and *Citrus reticulate* Blanco have also been reported to have high contact activity at low doses (Mohamed and Abdelgaleil, 2008; Shaaya et al., 1997). The main components of the essential oils with previously reported activity against *S. oryzae* are given in Table 2.

ACTIVE ESSENTIAL OILS AGAINST *SITOPHILUS ZEAMAIS* MOTSCHULSKY

Essential oils from 112 taxa from Amarylidaceae, Amaranthaceae, Anacardiaceae, Annonaceae, Apiaceae, Asclepiadaceae, Asteraceae, Atherospermataceae, Brassicaceae, Capparaceae, Cleomaceae, Cupressaceae, Dipsacaceae, Ericaceae, Geraniaceae, Gomotegaceae, Lamiaceae, Lauraceae, Monimiaceae, Myristicaceae, Myrtaceae, Piperaceae, Poaceae, Rubiaceae, Rutaceae, Schisandraceae, Verbenaceae, Vitaceae, and Zingiberaceae families were investigated for their activity against *S. zeamais*. The activities of the essential oils are summarized in Table 4. Previous reports indicate that essential oils from species of Asteraceae, Brassicaceae, Ericaceae, Lamiaceae, Lauraceae, Rubiaceae, and Rutaceae families are active against

TABLE 3 Reported Insecticidal Activity of the Essential Oils Obtained from Various Plant Species against *Sitophilus oryzae*

Family	Species	Essential Oil Content	Type of Insecticidal Activity	Conditions and Activity of the Oil	References
Anacardiaceae	*Schinus terebenthifolius* Raddi	γ-Muurolene 45.3%, α-Thujene 15.9%, δ-3-Carene 7.8%, Cadinene 6.8%	Fumigant and contact activity	Fumigant: at 26 °C, after 72 h, LC_{50} 56.5 µL/L. Contact: at 26 °C, after 24 h, LC_{50} 0.42 mg/cm^2.	Mohamed and Abdelgaleil (2008)
Annonaceae	*Cananga odorata* (Lam.) Hook.f. and Thomson	–	Fumigant activity	After 24 h, LD_{50} 73.1 µL oil/L air.	Lee et al. (2001)
Apiaceae	*Angelica dahurica* Bentham et Hooker	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 0% mortality.	Kim and Park (2008)
	Cnidium officinale Makino	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 0% mortality.	
	Foeniculum vulgare Mill.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
	Pimpinella anisum L.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	
		–	Fumigant activity	At 28 °C, after 24 h LC_{50} > 15 µm, µL oil/L air.	Shaaya et al. (1997)
Asteraceae	*Achillea santolina* L.	1,6-Dimethyl-1,5-cyclooctadiene 60.5%, fragranol 10.5%, β-thujone 8.9%, 1,8 Cineole 8.6%, Camphor 5.4%	Fumigant and contact activity	Fumigant: at 26 °C, after 72 h, LC_{50} 79.6 µL/L. Contact: at 26 °C, After 24 h, LC_{50} 0.14 mg/cm^2.	Mohamed and Abdelgaleil (2008)
	Arnica sp.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
	Artemisia judaica L.	Piperitone 49.1%, Camphor 34.5%	Fumigant and contact activity	Fumigant: at 26 °C, After 72 h, LC_{50} > 100 µL/L. Contact: at 26 °C, After 24 h, LC_{50} 0.08 mg/cm^2.	Mohamed and Abdelgaleil (2008)
	Atractylodes japonica Koidzumi	–	Fumigant activity	At 25 °C, After 24 h, 9–15 µg/mL. 0% mortality,	Kim and Park (2008)
	Caesulia axillaris Roxb.	–	Contact activity	At 7–45 °C, After 12 month, 600–1300 mg oil/kg wheat, 100% mortality.	Varma and Dubey (2001)
	Saussurea lappa Clarke	–	Fumigant activity	At 25 °C, After 24 h, 0.9–15 µg/mL, 3.3% mortality.	Kim and Park (2008)
Araceae	*Acorus gramineus* Solander	–	Fumigant activity	At 25 °C, after 24 h, 25 µg/mL 20% mortality.	
Aristolochiaceae	*Asiasarum sieboldi* F. Maekawa	Methyl eugenol 41.7%, eucarvone 17.3%, 3-carene 8.5%	Fumigant activity	At 25 °C, After 24 h, 25 µg/mL, 100% mortality.	
Burceraceae	*Boswellia carterii* Birdwood	–	Fumigant activity	At 25 °C, After 24 h, 0.9–15 µg/mL, 56.6% mortality.	
	Boswellia sacra Flueck.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
	Commiphora sp.	–	Fumigant activity	After 24 h LD_{50} > 150 µL oil/L air.	
Cyperaceae	*Cyperus rotundus* L.	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL, 13.3% mortality.	Kim and Park (2008)
Cupressaceae	*Juniperus* L. sp.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
	Juniperus chinensis L.	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL, 0% mortality.	Kim and Park (2008)
	Juniperus oxycedrus L. subsp *oxycedrus*	Myrcene Germacrene D	–	2.18 mg of essential oil was tested at three doses; 0.125, 0.250, and 0.5 g/kg of wheat, corresponding to 125, 250, and 500 ppm, respectively, and silica gel alone at 0.5 g/kg of wheat corresponding to 500 ppm, at different exposure intervals (24 and 48 h and 7 and 14 d) for *S. oryzae*. For 7 d of exposure, 100% and 98% of *S. oryzae* adults died when they were treated with 500 and 250 ppm of enhanced silica gel, respectively.	Athanassiou et al. (2013)

Hypericaceae	*Hypericum* L. sp.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
Lamiaceae	*Agastache rugosa* (Fischer and C. Meyer) O. Kuntze	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 10% mortality.	Kim and Park (2008)
	Lavandula L. sp.	–	Fumigant activity	At 28 °C, after 24 h, LC_{50} > 15 µL oil/L air.	Shaaya et al. (1997)
		–	Fumigant activity	After 24 h, LD_{50} 54.0 µL oil/L air.	Lee et al. (2001)
	Laurus nobilis L.	–	Fumigant activity	At 28 °C, after 24 h, LC_{50} > 15 µL oil/L air.	Shaaya et al. (1997)
	Majorana hortensis Moench	Terpinene-4-ol 29.9%, β-Terpinene 11.3%, *Trans*-Sabinene hydrate 10.8%, α-Terpinene 6.8%	Fumigant and Contact activity	Fumigant: at 26 °C, after 72 h, LC_{50} > 100 µL/L. Contact: at 26 °C, After 24 h, LC_{50} 0.10 mg/cm^2.	Mohamed and Abdelgaleil (2008)
	Mentha sp.	–	Fumigant activity	At 28 °C, after 24 h, LC_{50} 7.5 µL oil/L air.	Shaaya et al. (1997)
		Menthol 92%	Fumigant and contact activity	Fumigant: after 24 h, 0.25–2 µL oil/µL acetone per 100 mL jar 0–85% mortality. Contact: after 24 h, 0.01–0.09 µL oil/µL acetone in 100 mL jar per insect 10–95% mortality.	Franz et al. (2011)
	Mentha arvensis L.	–	Contact activity	At 7–45 °C, after 12 month, 600–1300 mg oil/kg wheat. 100% mortality.	Varma and Dubey (2001)
	Mentha microphylla C. Koch	Piperitenone oxide 46.7%, piperitone oxide 28.0%, 1,8 cineole 13.3	Fumigant and contact activity	Fumigant: at 26 °C, after 72 h, LC_{50} 0.21 µL/L. Contact: at 26 °C, after 24 h, LC_{50} 0.01 mg/cm^2.	Mohamed and Abdelgaleil (2008)
	Ocimum sp.	–	Fumigant activity	At 28 °C, after 24 h, LC_{50} > 15 µL oil/L air.	Shaaya et al. (1997)
	Ocimum gratissimum L.	Methyl eugenol 64.3%, β-(*Z*)-ocimene 10.4%, *E*-caryophyllene 5.1%	Fumigant and repellent activity	Fumigant: at 30 °C, After 24 h, 1–10 µL oil/L air After 24 h 6–38% mortality. Repellent: At 30 °C, After 24 h, 0.05–0.2% (v/w) oil 85–100% repellence.	Gendo et al. (2008)
	Origanum sp.	–	Fumigant activity	At 28 °C, After 24 h, LC_{50} 15.4 µL oil/L air.	Shaaya et al. (1997)
	Pogostemon cablin (Blanco) Benth.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
	Rosmarinus officinalis L.	–	Fumigant activity	At 28 °C, After 24 h, LC_{50} > 15 µL oil/L air.	Shaaya et al. (1997)
		1,8 Cineole 34.5%, α-pinene 15.5%, β-pinene 10.1%, camphor 8.4%, camphene 5.6%			Lee et al. (2001)
	Salvia fruticosa Mill.	–	Fumigant activity	At 28 °C, after 24 h, LC_{50} > 15 µL oil/L air.	Shaaya et al. (1997)
	Salvia officinalis L.	–	Fumigant activity	At 28 °C, after 24 h, LC_{50} 11.7 µL oil/L air.	
	Thymus vulgaris L.	–	Fumigant activity	After 24 h, LD_{50} 63.9 µL oil/L air.	Lee et al. (2001)
Myristicaceae	*Myrstica fragrans* Houttuyn	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 53.3% mortality.	Kim and Park (2008)

Continued

TABLE 3 Reported Insecticidal Activity of the Essential Oils Obtained from Various Plant Species against *Sitophilus oryzae*—cont'd

Family	Species	Essential Oil Content	Type of Insecticidal Activity	Conditions and Activity of the Oil	References
Myrtaceae	*Angophora floribunda* (Sm.) Sweet	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 55.7 µL oil/L air.	Lee et al. (2004)
	Baeckea virgate (J.R. Forst. and G. Forst.) Andrews	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Callistemon citrinus (Curtis) Skeels	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Callistemon sieberi DC.	1,8 Cineole 58.9%, α-terpineol 14.2%, α-pinene 12.8%	Fumigant activity	At 25 °C, after 24 h, LD_{50} 27.3 µL oil/L air.	
	Callistemon viminalis (Sol. Ex Gaertn.) G. Don	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 46.7 µL oil/L air.	
	Eucalyptus sp.	1,8 Cineole 81.1%, limonene 7.6%			Lee et al. (2001)
	Eucalyptus approximans Maiden	–	Fumigant activity	At 25 °C, After 24 h, LD_{50} 42.5 µL oil/L air.	Lee et al. (2004)
	Eucalyptus bicostata Maiden, Blakely and Simmonds	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 38.8 µL oil/L air.	
	Eucalyptus blakelyi Maiden	1,8 Cineole 56.9%, *p*-cymene 5.5%, crytone 5.0%	Fumigant activity	At 25 °C, After 24 h, LD_{50} 31.2 µL oil/L air.	
	Eucalyptus camaldulensis Dehnh.	1,8 Cineole 45.6%, (–) spathulenol 32.3%, bicyclogermacrene 11.2%	Fumigant and contact activity	Fumigant: at 26 °C, after 72 h, LC_{50} 30.81 µL oil/L air. Contact: at 26 °C, After 24 h, LC_{50} 0.11 mg/cm^2.	Mohamed and Abdelgaleil (2008)
		–	Fumigant activity	At 27 °C, after 24 h, LC_{50} 12.06 µL oil/L air.	Negahban and Moharramipour (2007)
	Eucalyptus cinera Benth.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 42.2 µL oil/L air.	Lee et al. (2004)
		–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Eucalyptus codonocarpa Blakely and McKie	*p*-Cymene 22.8%, piperitone 53.3%,	Fumigant activity	At 25 °C, after 24 h, LD_{50} 19 µL oil/L air.	
	Eucalyptus curtisii Blakely and C.T. White	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Eucalyptus dives Schauer	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Eucalyptus elata Dehnh.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 59.8 µL oil/L air.	
	Eucalyptus eximia Schauer	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 100 µL oil/L air.	
	Eucalyptus intertexta R.T. Baker	–	Fumigant activity	At 27 °C, after 24 h, LC_{50} 6.93 µL oil/L air.	Negahban and Moharramipour (2007)
	Eucalyptus leucoxylon F. Muell.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 54.2 µL oil/L air.	Lee et al. (2004)

Myrtaceae	*Eucalyptus macrorhyncha* F. Muell. Ex Benth.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 100 µL oil/L air.	Lee et al. (2004)
	Eucalyptus maidenii F. Muell.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 49.6 µL oil/L air.	
	Eucalyptus mannifera Mudie	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 33.9 µL oil/L air.	
	Eucalyptus michaeliana Blakely	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 46.3 µL oil/L air.	
	Eucalyptus moorei Maiden and Cambage	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Eucalyptus nicholii Maiden and Blakely	1,8 Cineole 82.2%	Fumigant activity	At 25 °C, after 24 h, LD_{50} 29 µL oil/L air.	
	Eucalyptus nortonii (Blakely) L.A.S. Johnson	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 40.5 µL oil/L air.	
	Eucalyptus ovata Labill.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 66.7 µL oil/L air.	
	Eucalyptus pauciflora subsp. *niphophila* (Maiden and Blakely) L.A.S. Johnson and Blaxell	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 100 µL oil/L air.	
	Eucalyptus polyanthemos Schauer	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Eucalyptus rossii R.T. Baker and H.G. Sm.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 100 µL oil/L air.	
	Eucalyptus sargentii Maiden	–	Fumigant activity	At 27 °C, after 24 h, LC_{50} 12.91 µL oil/L air.	Negahban and Moharramipour (2007)
	Eucalyptus sideroxylon A. Cunn. Ex Woolls	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 35.1 µL oil/L air.	Lee et al. (2004)
	Eucalyptus stellulata Sieber ex DC.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Kunzea sp.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 53.1 µL oil/L air.	
	Kunzea baxteri (Klotzsch) Schauer	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 100 µL oil/L air.	
	Kunzea ericoides (A. Rich.) Joy Thomps.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 100 µL oil/L air.	
	Leptospermum polygalifolium Salisb.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Melaleuca sp.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} > 50 µL oil/L air.	
	Melaleuca alternifolia (Maiden and Betche) Cheel	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
	Melaleuca armillaris (Sol. Ex Gaertn.) Sm.	1,8 Cineole 42.8%, terpinene-4-ol 15.9%, r-terpinene 8.9%	Fumigant activity	At 25 °C, after 24 h, LD_{50} 30.6 µL oil/L air.	Lee et al. (2004)

Continued

TABLE 3 Reported Insecticidal Activity of the Essential Oils Obtained from Various Plant Species against *Sitophilus oryzae*—cont'd

Family	Species	Essential Oil Content	Type of Insecticidal Activity	Conditions and Activity of the Oil	References
Myrtaceae	*Melaleuca decussate* R. Br.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 41.2 µL oil/L air.	Lee et al. (2004)
	Melaleuca ericifolia Sm.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 43.9 µL oil/L air.	
	Melaleuca lanceolata Otto	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 43.6 µL oil/L air.	
	Melaleuca linariifolia Sm.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 38.5 µL oil/L air.	
	Melaleuca thymifolia Sm.	–	Fumigant activity	At 25 °C, after 24 h, LD_{50} 40 µL oil/L air.	
	Syzygium aromaticum (L.) Merrill and Perry	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
Poaceae	*Cymbopogon* sp.	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	
	Cymbopogon citratus Stapf.	Geranial 47.5%, neral 31.5%, myrcene 11.1%	Fumigant and contact activity	Fumigant: after 24 h, 0.25–2 µL oil/µL acetone per 100 mL jar 10–90% mortality. Contact: after 24 h, 0.01–0.05 µL oil/µL acetone in 100 mL jar per insect 10–90% mortality.	Franz et al. (2011)
Rutaceae	*Citrus* × *paradisi* Macfad.	–	Fumigant activity	After 24 h LD_{50} 87.0 µL oil/L air.	Lee et al. (2001)
	Citrus reticulate Blanco	Limonene 83.9%, γ-Terpinene 10.8%	Fumigant and contact activity	Fumigant: at 26 °C, after 72 h, LC_{50} 58.6 µL/L. Contact: at 26 °C, after 24 h, LC_{50} 0.61 µL/L.	Mohamed and Abdelgaleil (2008)
	Citrus × *sinensis* (L.) Osbeck	–	Fumigant activity	After 24 h, LD_{50} > 150 µL oil/L air.	Lee et al. (2001)
	Ponciruc trifoliate Rafinesque	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 0% mortality.	Kim and Park (2008)
Santalaceae	*Santalum album* L.	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 10% mortality.	
Valerianaceae	*Nardostachys chinensis* Batal	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL after 24 h 6.6% mortality.	
	Lantana camara L.	*Trans*-Caryophyllene 42.6%, α-Humulene 13.9%, 1,8 cineole 12.7%, Caryophyllene oxide 7.6%	Fumigant and contact activity	Fumigant: at 26 °C, after 72 h, LC_{50} 29.5 µL/L. Contact: at 26 °C, After 24 h, LC_{50} 0.22 mg/cm^2.	Mohamed and Abdelgaleil (2008)
Zingiberaceae	*Amonum cardamomum* L.	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 43.3% mortality.	Kim and Park (2008)
	Amonum globosum Loureiro	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 6.6% mortality.	
	Curcuma longa L.	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 6.6% mortality.	
		Myrcene 40.2%, *p*-cymene 23.1%, 1,8 cineole 13.2%	Contact and fumigant activity	Contact: at 30 °C, After 24 h, LD_{50} 95.5 µg oil/mg wt. of insect. Fumigant: at 30 °C, after 24 h, LD_{50} 11.4 mg oil/L air.	Tripathi et al. (2002)
	Curcuma zedoaria Roscoe	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 0% mortality.	Kim and Park (2008)
	Kaempferia galangal L.	–	Fumigant activity	At 25 °C, after 24 h, 0.9–15 µg/mL 10% mortality.	
	Zingiber officinale Roscoe	α-Zingiberen 13.3%, geranial 15.9%, α-Sesquiphellandrene 7.1%, neral 9.9%, nabinene 9.6%	Fumigant and contact activity	Fumigant: after 24 h, 0.05–0.5 µL oil/µL acetone per 100 mL jar 10–85% mortality. Contact: after 24 h, 0.05–0.20 µL oil/µL acetone in 100 mL jar per insect 10–95% mortality.	Franz et al. (2011)

TABLE 4 The Reported Insecticidal Activity of the Essential Oils Obtained From Various Plant Species against *Sitophilus zeamais*

Family	Species	Essential Oil Content	Type of Insecticidal Activity	Conditions and Activity of the Oil	Reference
Amaranthaceae	*Chenopodium ambrosioides* L.	*p*-Cymene 50%, *α*-Terpinene 37.6%	Contact activity	At 25 °C, After 24 h, 0.2–0.8 μL/cm^2 5–20% mortality.	Tapondjou et al. (2002)
Anacardiaceae	*Schinus terebinthifolius* Raddi	–	Contact and fumigant activity	Contact: at 27.4 °C, after 12 h, LD_{50} 57.7 μL/40 g; fruit 98.8 μL/40 g. Fumigant: at 27.4 °C, after 12 h, LC_{50} 36.4 μL/40 g, fruit 40.6 μL/40 g.	Braga de Castro Coitinho et al. (2011)
Annonaceae	*Dennettia tripetela* Baker F	–	Contact activity	100% mortality.	Okonkwo and Okoye (1996)
	Mondora myristica (Gaerth) Dunal	–	Contact activity	F1 emergence observed, changed color of the maize.	
	Xylopia aethiopica (Dunal) A. Rich.	*β*-Pinene 27.9%, sabinene 23.9%, *α*-pinene 11.1%, *β*-phellandrene 15.9%, terpinen-4-ol 5.1%	Formulation activity	At 30 °C, after 48 h, 2.5–10% (w oil + clay (1:1)/w maize) 22–100% mortality.	Nguemtchouin et al. (2010)
		–	Contact activity	F1 emergence observed, changed color of the maize.	Okonkwo and Okoye (1996)
		β-Pinene 12.9%, sabinene 5.8%, terpinen-4-ol 8.9%	Contact activity	At 23.2 °C, after 2–6 week, 81.67–88.52% mortality.	Tatsadjieu et al. (2010)
Asteraceae	*Artemisia capillaris* Thunb.	1,8 Cineole 13.8%, germacrene D 10.4%, camphor 8.6%	Contact and fumigant activity	Contact: at 27–29 °C, after 7 days, LD_{50} 105.92 μg/insect. Fumigant: at 27–29 °C, after 7 days, LC_{50} 5.31 mg/L air.	Liu et al. (2010)
	Artemisia giridaldii Pamp.	*β*-Pinene 13.2%, iso-elemicin 10.1%, germacrene D 5.7%, terpinen-4-ol 5.4%, (*Z*)-*β*-ocimene 5.1%, 1,8 cineole 12.3%, *α*-curcumene 10.8%	Contact and fumigant activity	Contact: at 29–30 °C, after 24 h, LD_{50} 40.51 μg/insect. Fumigant: at 29–30 °C, after 24 h, LC_{50} 6.29 mg/L air.	Chu et al. (2012b)
	Artemisia subdigitata Mattf.	*β*-Pinene 7.4%, borneol 6.2%, eugenol 5.9%	Contact and fumigant activity	Contact: at 29–30 °C, after 24 h, LD_{50} 76.34 μg/insect. Fumigant: at 29–30 °C, after 24 h, LC_{50} 17.01 mg/L air.	
	Saussurea nivea Turcz	(+)-Limonene 15.5%, caryophyllene oxide 7.6%, linalool 7.2%,*α*-pinene 6.4%, *β*-pinene 5.7%, spathulenol 5.0%	Contact and fumigant activity	Contact: at 29–30 °C, after 24 h, LD_{50} 10.56 μg/insect. Fumigant: at 27–29 °C, after 24 h, LC_{50} 8.89 mg/L air.	Chu et al. (2012a)
Lamiaceae	*Dracocephalum moldavica* L.	1, 8-Cineol 31.3%, terpinen-4-ol 22.8%	Contact and fumigant activity	Contact: at 29–30 °C, after 7 days, LD_{50} 22.1 μg/insect. Fumigant: at 29–30 °C, after 7 days, LC_{50} 2.65 mg/L air.	Chu et al. (2011a,b)

Continued

TABLE 4 The Reported Insecticidal Activity of the Essential Oils Obtained From Various Plant Species against *Sitophilus zeamais*—cont'd

Family	Species	Essential Oil Content	Type of Insecticidal Activity	Conditions and Activity of the Oil	Reference
	Hyptis spicigera Lam.	–	Contact, fumigant, and repellent activity	Contact: at 30 °C, 50 mg oil 36% toxicity. Fumigant: at 30 °C, after 48 h, 0–10 μL/L air, 0–84% mortality. Repellent: after 12 h, 0–10 v/w oil, 47–57%.	Othira et al. (2009)
	Ocimum gratissimum L.	Thymol 47.7%, γ-terpinene 14.3%, cymene 8.5%,	Contact activity	At 23.2 °C, after 2–6 week, 60.00–74.28% mortality.	Tatsadjieu et al. (2010)
	Ocimum kenyense Ayob. ex A.J.Paton	1,8 cineole 36.9%, β-selinene 23.6%, methyl chavicol 12.9%, iso-eugenol 8.2%	Contact activity	At 26 °C, after 24 h, LC_{50} 0.36 mg/cm^2.	Bekele and Hassanali (2001)
Lamiaceae	*Ocimum kilimandscharicum* Guerke	Camphor 70.4%, 1,8 cineole 7.2%, limonene 6.2%, camphene 5.1%	Contact activity	At 26 °C, after 24 h, LC_{50} 0.76 mg/cm^2.	Bekele and Hassanali (2001)
	Ocimum suave (Wild)	Eugenol 59.7%, *Trans*-β-ocimene 14.5%, β-Cubebene 5.3%	Contact and repellent activity	Contact: At 27 °C, After 24 h, 0–10 μL/disc, 0–95% mortality. Contact topical: at 27 °C, After 24 h, 0–7 μL/insect, 0–100% mortality. Contact mortality on grain: at 27 °C, after 24 h, 0–3 μL/kg, 0–100%. mortality. Repellent: at 27 °C, 0–10 μL/kg, 0–91%.	Obeng-Ofori and Reichmuth (1997)
Lauraceae	*Cinnamomum zeylanicum* Blume.	–	Contact activity	At 27.4 °C, after 12 h, LD_{50} 14.2 μL/40 g.	Braga de Castro Coitinho et al. (2011)
	Litsea salicifolia (Roxburgh ex Nees) Hook. f.	(*E*)-citral 40.9%, (*Z*)-citral 29.1%, methyl heptenone 11.2%, limonene 5.6%	Repellent, contact, fumigant, and antifeedant activity	Repellent: at 29–32 °C, after 5 h, 0.16–0.63 μg oil/cm^2 filter paper, 88–88% repellence. Contact: at 27–28 °C, after 24 h, LD_{50} 0.079 μL oil/insect. Fumigant: at 27 °C, After 24 h, LC_{50} 4.435 μL/L air. Antifeedant: at 29–32 °C, after 24 h, 4–10% (v oil/v ethanol), 6.17–29.63% antifeedant action.	Ko et al. (2010)
Myrtaceae	*Eugenia uniflora* L.	–	Contact activity	At 27.4 °C, after 12 h, LD_{50} 11.6 μL/40 g.	Braga de Castro Coitinho et al. (2011)
	Melaleuca leucadendron L.	–	Contact activity	At 27.4 °C, after 12 h, LD_{50} 75.8 μL/40 g.	

Piperaceae	*Piper aduncum* L.	–	Fumigant activity	At 27.4 °C, after 12 h, LC_{50} 15.1 µL/40 g.	
	Piper guineense Schum. Et Thonn	–	Contact activity	100% mortality.	Okonkwo and Okoye (1996)
	Piper hispidinervum (C. DC.)	–	Contact and fumigant activity	Contact: at 27.4 °C, after 12 h LD_{50} 1.0 µL/40 g. Fumigant: at 27.4 °C, After 12 h LC_{50} 0.53 µL/40 g.	Braga de Castro Coitinho et al. (2011)
	Piper marginatum Jacq.	–	Contact and fumigant activity	Contact: at 27.4 °C, After 12 h, LD_{50} 21.1 µL/40 g. Fumigant: at 27.4 °C, after 12 h, LC_{50} 46.7 µL/40 g.	
Rubiaceae	*Paederia scandens* (Lour) Merrill	*β*-Pinene 24.8%, *α*-pinene 8.2%, 1, 8 cineol 8.0%, *δ*-terpinene 5.3%, (*E*)-*β*-ocimene 5.2%	Contact and fumigant activity	Contact: at 27–29 °C, after 24 h, LD_{50} 47.78 µg/insect. Fumigant: at 29–30 °C, after 24 h, LC_{50} 7.14 mg/L air.	Yang et al. (2012)
Rutaceae	*Evodia rutaecarpa* Hook f. et Thomas	–	Contact, fumigant, repellent, and antifeedant activity	Contact: at 30 °C, After 7 days LD_{50} 0.043 µg/mg insect. Fumigant: at 30 °C, after 24 h, LC_{50} 41.0 µg/L air. Repellent: after 5 h 0.84–2.23 µg/cm^2, 56.8–88.0%. Antifeedant: at 30 °C, after 72 h, 0.75–4.49 mg/disk, 0.5–20.46%.	Liu and Ho (1999)
	Murraya exotica L.	Spathulenol 17.7%, *α*-pinene 13.3%, caryophyllene oxide 8.6%, *α*-caryophyllene 7.3%	Contact and fumigant activity	Contact: at 29–30 °C, after 7 days, LD_{50} 11.41 µg/insect. Fumigant: at 29–30 °C, after 7 days, LC_{50} 8.29 mg/L air.	Li et al. (2010)
	Lippia rugosa L.	Geraniol 51.5%, nerol 18.6%, geranial 10.4%	Contact activity	At 23.2 °C, after 2–6 week, 92.78–84.04% mortality.	Tatsadjieu et al. (2010)
Zingiberaceae	*Alpinia conchigera* Griff.	1,8 Cineole 46.2%, *β*-pinene 18.0%, *α*-pinene 6.2%	Fumigant activity	At 30 °C, after 48 h, LC_{50} egg: >593 µL/L air, Larva: 437 µL/L air, pupa 278 µL/L air, adult 85 µL/L air.	Suthisut et al. (2011)
Zingiberaceae	*Curcuma zedoaria* (Berg.) Roscoe	Camphor 36.3%, *α*-zingiberene 12.5%, camphene 10.0%, *α*-curcumene 9.4%, isoborneol 7.2%	Fumigant activity	At 30 °C, after 48 h, LC_{50} 593 µL/L air caused 6% mortality.	Suthisut et al. (2011)
	Zingiber zerumbet Smitt	Camphene 27.0%, *α*-humulene 13.9%, camphor 11.9%, 1,8 cineole 8.1%, zerumbone 8.1%, *α*-pinene 6.6%	Fumigant activity	At 30 °C, after 48 h, LC_{50} 593 µL/L air caused 4% mortality.	

S. zeamais. Essential oils from *Artemisia capillaris* Thunb., *Artemisia giraldii* Pamp., and *Artemisia mongolica* (Besser) Fisch. ex Nakai species produce a very high fumigant toxicity against *S. zeamais* at low application doses. The essential oils of these species contain 1,8 cineole, α-pinene, β-pinene, limonene, germacrene D, iso-elemicin, and caryophyllene oxide compounds as the main components (Chu et al., 2012b; Liu et al., 2010). Significant fumigant activity was reported for the essential oils of *Dracocephalum moldovica* L. (LC_{50} 2.65 mg/L air), *Hyptis spicigera* Lam. (10 µL/L air—84% mortality), and *Litsea salicifolia* (Roxburgh ex Nees) Hook. f. (LC_{50} 4.35 µL/L air) of Lamiaceae and Lauraceae (Chu et al., 2011a,b; Ko et al., 2010; Othira et al., 2009). *Litsea salicifolia* and *D. moldovica* essential oils contain the main components (*E*)-citral, (*Z*)-citral, and methyl heptenone, and 1,8 cineole and terpinen-4-ol, respectively (Chu et al., 2011a,b; Ko et al., 2010). Attention should also be given to the species *Paederia scandens* (Lour) Merrill, *Murraya exotica* L. and *Saussurea nivea* Turcz that have been reported to have high fumigant toxicity against *S. zeamais* (Chu et al., 2012a; Li et al., 2010; Yang et al., 2012). The main components of the essential oils with previously reported activity against *S. zeamais* are given in Table 2.

ESSENTIAL OILS AS STORED PRODUCT PEST CONTROL AGENTS

The reported activities of the essential oils from various plant species in the literature present an alternative to existing synthetic agents. However, there are several problems to be solved before active essential oils can be employed in pest management of stored products. The crude essential oils usually contain a vast array of natural substances. The activity observed in the essential oil is caused by the action of a mixture of compounds. High activity could be observed in the oil but the pure main components could produce low activity due to synergistic activity of the other compounds (Bekele and Hassanali, 2001). The opposite of this situation is also possible due to the antagonistic activity of the compounds (Don-Pedro, 1996). Essential oils obtained from plant species could show variation in their composition due to many factors, including, seasonal variation, harvesting period during the day, soil/irrigation/climatic conditions, and chemotype variation (Polatoglu et al., 2013). A drastic example of chemotype variation on the observed activity for the pest was previously reported (Polatoglu et al., 2013). The chemotype of a plant species is virtually impossible to detect because most of the time chemotype variation does not manifest itself with morphological variations. The problem could be solved by cultivating the appropriate chemotype of the plant, which is the source of pest control agent, and by applying a standard method for cultivation. Additionally, active constituents of the oil obtained from the cultivated or collected plant could be separated prior to usage if feasible. Many compounds that are observed in the essential oils are also produced synthetically in the perfume industry. Instead of using the natural oils that contain active and nonactive compounds, synthetic oils that contain only the active compound or mixture of compounds that have synergistic activity could be produced according to needs (Isman, 2000). In a previous study, the activity of natural *Curcuma zedoria* and *Zingiber zerumbet* essential oils and their synthetic oils which were obtained by combining the main components of these oils were compared, showing that synthetic oils afforded higher activities (Suthisut et al., 2011). The use of crude essential oil or pure essential oil components in fumigation also carries the risk of spoiling the product by changing its color, taste, olfactory properties, or inhibiting germination. In fumigation studies, 1,8 cineole has been reported to leave residues on wheat after aeration for 6 days (Rajendran and Sriranjini, 2008). In another study, carvacrol was reported to leave a noticeable odor on wheat after fumigation (Liu and Ho, 1999). *Xylopia aethiopica* essential oils were reported to change the color of the grain after application (Okonkwo and Okoye, 1996). Essential oils and pure essential oil components could have phytotoxic properties (Polatoglu et al., 2013). The oils could also inhibit seed germination of the stored seeds (Dudai et al., 1993). Seed germination inhibition properties should also be evaluated during insecticidal screening studies for active fumigants to be used for stored products. Generally, essential oils and their components could be recognized as safe as they have low toxicity to mammals. However, some of the essential oil and pure essential oil components could have serious toxicity or harmful activities. Essential oil components safrole, β-asarone, estragole, apiol, anethole, elemicin, β-myrcene, methyl eugenol, and myrsticin have been reported to be carcinogenic and/or genetoxic (van der Berg et al., 2011). Therefore, essential oils containing these components may not be recognized as safe and their use as fumigants of stored food products should be avoided. Another important concern is the application method of the essential oils. Unlike conventional fumigants, essential oils do not produce high vapor pressure and there is a need for a method to provide appropriate vapor pressure for their use as fumigants in large-scale applications (Rajendran and Sriranjini, 2008). Essential oils could be used as fumigants in large-scale applications with carrier gases such as carbon dioxide to provide even penetration to the stored product (Rajendran and Sriranjini, 2008). The use of essential oils or their components together with gases such as nitrogen, oxygen, carbon dioxide, and ethyl formate was reported to increase the insecticidal activity (Rajendran and Sriranjini, 2008). Other methods of application of essential oils as fumigants have also been reported, which involve formulations with montmorillonite clay, kaolinite clay, diatomaceous earth, silica gel, and encapsulation for preserving the active formulation of the essential oil and its activity for a longer period (Athanassiou et al., 2013; Nguemtchouin et al., 2010, 2013; Moretti et al., 2002; Yang et al., 2010). Essential oil components could be employed together with conventional insecticides as synergistic compounds to achieve higher activity. Carvone was reported to increase the insecticidal activity of carbofuran, carbaryl, parathion, and dichlorodiphenyltrichloroethane significantly (Lichtenstein

et al., 1974). As indicated earlier, insect species are gaining resistance to the synthetic/natural pesticides such as organophosphates, pyrethroids, and phosphine (Collins, 1998). *S. oryzae* and *S. granarius* have been reported to be susceptible to providing resistance toward pyrethroids (Heather, 1986; Klajajic and Peric, 2007). The formulations of monoterpenes and synergistic substances such as piperonyl butoxide, S,S,S-tributyl phosphorotrithioate, and dimethyl maleate which act in the inhibition of the enzymes (microsomal P450 monooxygenase, esterase, and glutathione S-transferase) that metabolize/detoxify the active insecticidal compounds were investigated in order to understand the tolerance mechanism. Esterases were suspected to take part in the tolerance mechanism of *S. oryzae* against active monoterpenes (Lopez, Contreras & Pascual-Villalobos, 2010). Essential oils were also reported to inhibit the growth of food-borne fungi such as *Aspergillus* sp., *Curvularia* sp., *Mucor* sp., and *Rhizopus* sp. as well as food-borne pathogenic bacteria *Bacillus* sp. and *Escherichia* sp. (Polatoglu et al., 2012; Varma and Dubey, 2001; Tatsadjieu et al., 2010). Most essential oils afford antifungal activity against various fungal species; therefore, essential oils that have potential activity for stored product pest control could be used as a multipurpose pest controlling agent.

CONCLUSIONS

There are a vast number of reports regarding the insecticidal activities of essential oils against *Sitophilus* sp., including fumigant, contact, repellent activities, etc. obtained from studies that screened for new potential pest control agents from natural origins. The results obtained from this research provide a strong basis for the potential of essential oils to be used in stored product pest management. The use of essential oils as pest control agents of stored products seems very promising. However, there are still many areas of study that should be addressed and many questions to be answered before their active use in large-scale applications. These include:

1. Impact on health/environment:
   - **a.** The toxicity of the potent essential oils/pure essential oil components against mammalians should be evaluated.
   - **b.** Risks/hazards associated with the use of potent essential oils/pure essential oil components should be evaluated.
   - **c.** The toxicity of the potent essential oils/pure essential oil components against nontarget species should be evaluated.
   - **d.** The half-life of the potent essential oils/pure essential oil components in water/soil/air should be evaluated.
2. Effects on the product:
   - **a.** Residual studies should be done on potential essential oils/pure essential oil components on stored food products.
   - **b.** The effects of potential essential oils/pure essential oil components on the appearance, olfactory, and taste properties of the stored food product should be evaluated.
   - **c.** Phytotoxicity (seed germination inhibition, growth inhibition against stored seeds), antifungal (against stored food-borne fungus), and antibacterial (against stored food borne pathogens) activities of the potential essential oils/pure essential oil components should also be evaluated.
3. Problems of the application:
   - **a.** New methods for the application of the potential essential oils/pure essential oil components in large-scale applications should be evaluated.
   - **b.** Pilot tests of the potential essential oils/pure essential oil components on stored products should be evaluated and compared with the laboratory-scale experimental results in order to provide a basis for spotting potential active oils that could be employed in large-scale applications from the laboratory-scale experimental results.
   - **c.** New methods for improving the vapor pressure/even distribution of the potential essential oils/pure essential oil components in large-scale applications should be evaluated.
   - **d.** New methods/formulations for controlled release of the potent essential oils/pure essential oil components should be evaluated.
   - **e.** Stability of the potent essential oils/pure essential oil components during storage and application should be evaluated.
   - **f.** The resistance of the pests against potent essential oils/pure essential oil components and the methods to inhibit this resistance should be evaluated.
4. Plant species that should be screened primarily for insecticidal activity against *Sitophilus* sp.
   - **a.** Edible plant species are generally recognized as safe; therefore initial efforts should be still concentrated on plant species that are found useful as food, herbal tea, spices, and flavor.
   - **b.** Medicinal plant species could also be screened; however, the safety of the essential oils obtained from the medicinal plants should also be evaluated.
   - **c.** Until now essential oils of the species from 38 family have been investigated for their insecticidal activity against *Sitophilus* species. The most active plant species reported in the literature are from Asteraceae, Amaranthaceae, Brassicaceae, Ericaceae, Lamiaceae, Lauraceae, Myrtaceae, Rutaceae, Rubiaceae, and Zingiberaceae. Species of these families could show potential activity against *Sitophilus* species.

d. The essential oils that were reported to inhibit acetylcholinesterase, butyrylcholinesterase enzymes should also be evaluated for their insecticidal activities against *Sitophilus* species (Lopez and Pascual-Villalobos, 2010).
e. Plants reported to be used as insecticides, insect repellents, etc. in ethnobotanical research reports should be evaluated.

5. Additional aspects of reporting the studies on screening essential oils as pest management agents
a. The availability of the plant material for large-scale production of oil should be considered when selecting the plant species for screening. If the plant material is not available for mass scale production of the oil, a synthetic replica of the oil should also be screened for the insecticidal activity.
b. The essential oil composition of the investigated oil in insecticidal activity should always be reported.
c. Information regarding the plant material (collection location, collection altitude, herbarium number, and binomial name of the plant) should always be reported.
d. If possible for the essential oil of a plant species more than one location should be studied since chemotype variation observed in the plant species could drastically affect the chemical composition and the insecticidal activity of the oil.
e. A unified methodology should be applied for reporting the units of the insecticidal activity of the essential oils. This will enable other researchers to compare the results of individual reports. The "LC_{50}–mg oil/L air", "LD_{50}–μg/insect", "repellency index" for fumigant, and contact and repellent activities seem to be appropriate units for comparison of the results of individual reports as these units were employed in most of the previous reports.

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Chapter 6

Essential Oils for Arthropod Pest Management in Agricultural Production Systems

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List of Abbreviations

EIL Economic injury level
GABA Gamma aminobutyric acid
IPM Integrated pest management

INTRODUCTION

Essential oils are secondary metabolites of plants. In general, they are volatile compounds that have a tendency to be readily broken down by abiotic environmental factors (Isman, 2000). Essential oils can be extracted mostly from leaves, but also can be draw from other nonwoody parts of plants (Batish et al., 2008). Approximately 17,500 species of plants are known to possess essential oils and most plants belong to the families of Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae (Regnault-Roger et al., 2012). Extracted essential oils retain scent, of which the major components are monoterpenes, sesquiterpenes, phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones (Batish et al., 2008). These compounds can affect arthropod pests and benefitial insects in production agriculture.

This chapter is mainly focused on arthropod pest management in crop production and production agriculture using essential oils. Target organisms of this chapter include insects and mites, but exclude stored product insects and medically important arthropod pests. In addition, nontarget effects of essential oils as insecticides or acaricides on crop pollinators and natural enemies of arthropod pests in crop production are discussed in this chapter. More detailed information about stored product pests can be found in Chapter 5. Technology for delivery of essential oils as pesticides can be found in Chapter 12.

ESSENTIAL OILS AS PESTICIDES

Essential oils are by-products of plant metabolism and plants use them for protection from various plant-feeding arthropods (e.g., insects and mites) and plant pathogens. For example, volatile monoterpenes in plants can deter or repel herbivorous insect pests and pathogenic fungi (Langenheim, 1994). Some plants use their essential oils as a chemical for communication with different species of plants or animals (i.e., allelochemical). Volatiles of essential oils acting as semiochemicals can benefit plants (i.e., allomone—sender benefits), insects, or mites (i.e., kairomone—receiver benefits) or both the plants and insects (i.e., synomone—both sender and receiver benefit). Example usages of essential oils as semiochemicals are presented in Figure 1. These essential oils are thought to be important chemicals triggering coevolution between plants and insects.

Essential oils can be extracted from plants by using various methods such as hydrodistillation, steam distillation, dry distillation, or cold pressing (Regnault-Roger et al., 2012); hydrodistillation is the oldest way to extract essential oils, dating back to 5000 years ago (Brud, 2010). Quantity and quality of chemical components in an essential oil can be dramatically changed depending on extraction methods (Chiasson et al., 2001) (Figure 2). Extracted essential oils can be widely used for various purposes including medicinal and pharmaceutical treatment, food flavor, and perfumery. Essential oils have

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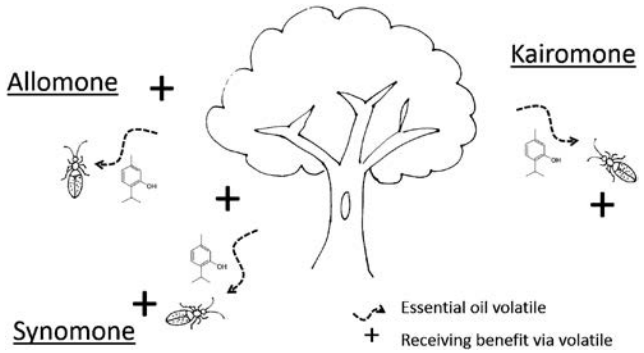

FIGURE 1 An example of use of essential oils as allelochemicals. Volatiles of essential oils produced by plants (senders) may affect arthropod pests (receivers) as an allomone (benefits the sender), kairomone (benefits the receiver), or synomone (benefits both the sender and the receiver).

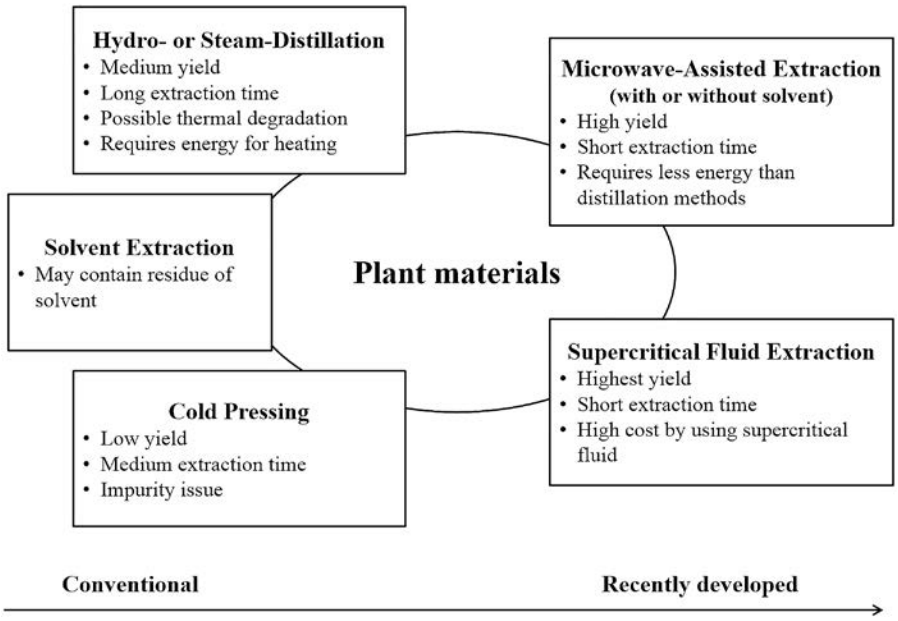

FIGURE 2 Overview of essential oils extraction methods.

received much attention from researchers and pest management practitioners because of their capability to kill pests including insects, plant pathogens, and weeds. Indeed, essential oils have been categorized as botanical pesticides since the 1980s (Regnault-Roger, 1997). Because essential oils are low-risk and naturally driven pesticides, they are can be good control measures, specifically for organic farming.

Plant species that contain essential oils with pesticide properties are found in the families of Amaryllidaceae, Apiaceae, Asteraceae, Atherospermataceae, Burseraceae, Cupressaceae, Fabaceae, Lamiaceae, Lauraceae, Myrtaceae, Phytolaccaceae, Piperaceae, Poaceae, Rutaceae, and Zingiberaceae. Essential oils from these plants can be used as contact poison, stomach poison, repellent, and growth regulator (Regnault-Roger et al., 2012). In the past, many studies have tested the effectiveness of essential oils as pesticides against insect pests in cropping fields, medically important arthropod pests (e.g., mosquitoes, flies, ticks, and mites), and stored product insect pests (e.g., been weevils, flour beetles, and Indian meal moths).

MODES OF ACTION OF ESSENTIAL OILS

Understanding pesticide mode of action is fundamental to the selection and application of pesticides. Pesticide mode of action explains how a pesticide kills target pests. The mode of action involves anatomical, physiological, and biochemical interactions and responses of organisms to pesticides. In other words, mode of action is the way in which a pesticide causes physiological disruption of pests at its target site inside organisms. Essential oils have various modes of action as pesticides. First, monoterpenes in essential oils are neurotoxic to insect pests by blocking neurotransmitters such as acetylcholinesterase (Regnault-Roger, 1997). In addition to monoterpenes, thymol is associated with gamma aminobutyric acid

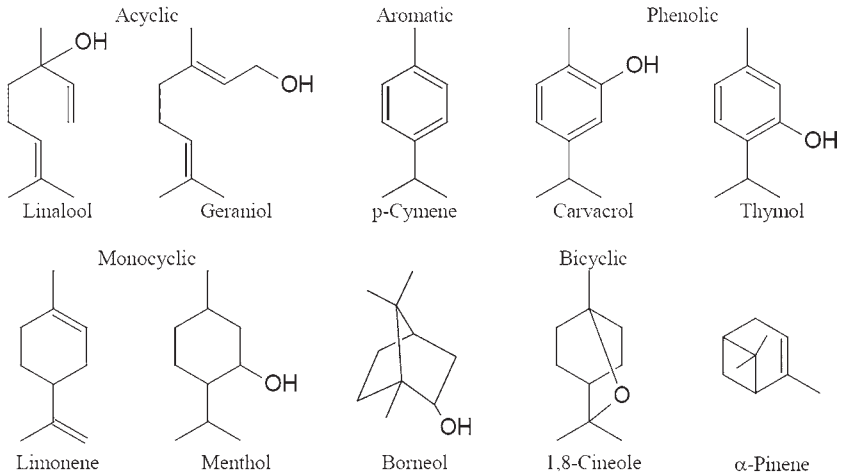

FIGURE 3 Chemical structure of major terpenes that have pesticidal effects on arthropod pests.

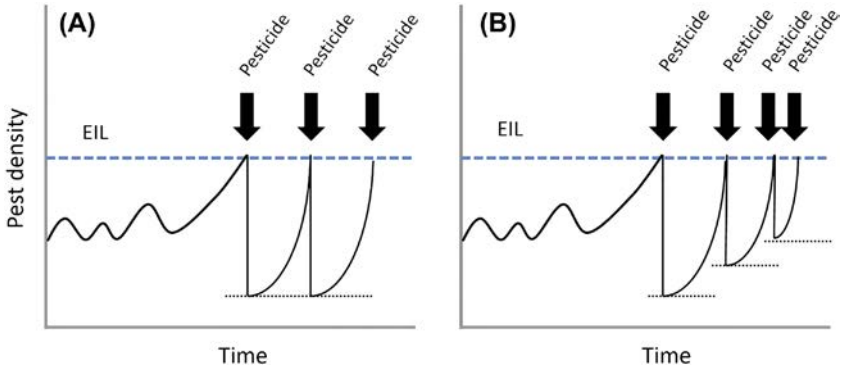

FIGURE 4 Pest management concept of integrated pest management (IPM). (A) Pesticides are used to keep pest density under economic injury level (EIL) and essential oils can be used as a control measure. (B) Many conventional pesticides cause pesticide resistance when the same or similar pesticides are used over and over again. So far, there have been no reports on pest resistance to essential oils.

receptors and chloride channels, and eugenol activates octopanime receptors (Enan, 2001). The rapid toxic effects of essential oils on insect pests are a good indication of a neurotoxic effect (Isman and Machial, 2006). Major terpenes that have pesticidal properties are shown in Figure 3. Second, essential oils can be used as insect growth regulators. Some essential oils are known to block growth hormones (Tarelli et al., 2009), hence insect growth at various life stages can be disrupted (Regnault-Roger et al., 2012). Third, soft-bodied insects are killed by lipophilic plant oils because the oils penetrate waxy layers of the cuticle, causing death by excessive water loss in the insect body (Gillilan, 2012). Fourth, P450 cytochromes responsible for exterminating foreign organisms inside an insect body can be inhibited by essential oils from dill and *Piper* spp. (Regnault-Roger et al., 2012). Lastly, essential oils can block digestive enzymes (Tarelli et al., 2009).

To deliver essential oils to the target site of the insect body, two applications are generally used: fumigation and topical application. Fumigation is possible because essential oils are highly volatile. Such delivery of essential oils can affect pests as antifeedant, repellent, ovicide, or insect growth regulator (Isman, 2000; Regnault-Roger, 1997). Active ingredients of essential oil pesticides include, but are not limited to, monoterpenes, sesquiterpenes carvacrol, cymene, and thymol.

ESSENTIAL OILS FOR ARTHROPOD PEST MANAGEMENT

Modern pest management is based on the concept of bioeconomics and integrated pest management (IPM), that is, using multiple pest control methods together to reduce pest density under economic injury level (EIL). EIL is the lowest number of insects that will cause economic damage (Pedigo, 2002) (Figure 4). This concept was coined originally to reduce the use and amount of synthetic pesticides in cropping fields by utilizing nonchemical methods, such as employing natural enemies (i.e., biological control), modifying environment to render it unfavorable to insects (i.e., ecological control), and utilizing mechanical means (i.e., mechanical control). In addition, the use of natural chemicals extracted from plants (i.e., botanicals) can substitute or supplement the use of synthetic pesticides.

Currently, essential oils are used in IPM strategies as broad-spectrum pesticides, organic pesticides, and low-risk pesticides where high-risk pesticides cannot be generally applied such as greenhouses, schools, and homes. Because essential oils have various modes of action to kill arthropod pests, they can play an important role in IPM, specifically when low residual activity of pesticides is necessary. For example, low-residue essential oils allow short reentry intervals after application or treatment close to harvest. In addition to being used in IPM, essential oils can be an important control measure against arthropod pests in organic farming systems because they are natural products. Essential oils are generally low-risk pesticides and relatively less harmful to humans, so they can be used for greenhouse pest management (Regnault-Roger et al., 2012). Also, the mixture of active ingredients in essential oils can reduce development of insecticide resistance. However, some essential oils still can affect nontarget organisms, so it is important to select essential oils that can kill target pest arthropods while being harmless to beneficial organisms and safe for humans and the environment.

Essential oils are known as broad-spectrum pesticides, meaning that they can harm multiple species of arthropod pests. This is possible because one essential oil can possess multiple active ingredients with pesticide properties. Multiple active ingredients of essential oils can also be antifeedants, molting inhibitors, respiratory inhibitors, oviposition inhibitors, ovicides, deterrents, attractants, or repellents. Table 1 shows a list of arthropod pests and source plant species with essential oils containing pesticidal

TABLE 1 List of Arthropod Pests in Agricultural Production Systems and List of Plant Species Containing Essential Oils Showing Pesticidal Properties

Arthropod Species (Common Name)	Application Method	Plant Species (Common Name)	Key References
Meligethes aeneus (pollen beetle)	Contact	*Carum carvi* (caraway seed)	Pavela (2011)
		Thymus vulgaris (thyme)	
Anomala orientalis (oriental beetle)	Topical	*Thymus vulgaris* (thyme)	Ranger et al. (2013)
Cyclocephala borealis (northern masked chafer)			
Popillia japonica (Japanese beetle)			
Rhizotrogus majalis (European chafer)			
Camptomyia corticalis (mushroom fly)	Fumigant	*Carum carvi* (caraway seed)	Kim et al. (2012)
		Artemisia vulgaris (Armoise)	
		Salvia sclarea (sage, clary)	
		Origanum vulgare (oregano)	
Ceratitis capitata (Mediterranean fruit fly)	Topical/fumigant	*Lavandula angustifolia* (lavender)	Benelli et al. (2012)
		Rosmarinus officinalis (rosemary)	
Bemisia tabaci (silverleaf whitefly)	Fumigant	*Lippia sidoides* (pepper-rosmarin)	Cavalcanti et al. (2010)
	Contact	*Thymus vulgaris* (thyme)	Yang et al. (2010)
	Contact/fumigant	*Allium sativum* (garlic)	Kim et al. (2011)
Trialeurodes vaporariorum (greenhouse whitefly)	Fumigant	*Citrus limon* (lemon)	Delkhoon et al. (2013)
Lipaphis erysimi (turnip aphid)	Topical	*Piper nigrum* (black pepper)	Monika et al. (2013)
		Psoralea corylifolia (babchi)	
Aphis fabae (black bean aphid)	Fumigant	*Mentha pulegium* (pennyroyal)	Kimbaris et al. (2010)
Aphis gossypii (cotton aphid)	Contact	*Artemisia dracunculus* (tarragon)	Mousavi and Valizadegan (in press)
Macrosiphoniella sanborni (chrysanthemum aphid)	Fumigant	*Mentha pulegium* (pennyroyal)	Kimbaris et al. (2010)
Myzus persicae (green peach aphid)			

TABLE 1 List of Arthropod Pests in Agricultural Production Systems and List of Plant Species Containing Essential Oils Showing Pesticidal Properties—cont'd

Arthropod Species (Common Name)	Application Method	Plant Species (Common Name)	Key References
Acyrthosiphon pisum (pea aphid)	Fumigant	*Mentha pulegium* (pennyroyal)	Kimbaris et al. (2010)
		Laurelia sempervirens (Peruvian nutmeg)	Zapata et al. (2010)
Nezara viridula (southern green stink bug)	Contact/fumigant	*Origanum vulgare* (oregano)	González et al. (2011)
		Thymus vulgaris (thyme)	
Diaphorina citri (Asian citrus psyllid)	Topical	*Allium tuberosum* (garlic chive)	Mann et al. (2012)
		Lavandula angustifolia (lavender)	
Cacopsylla chinensis (pear psyllid)		*Allium sativum* (garlic)	Zhao et al. (2013)
Glyphodes pyloalis (lesser mulberry pyralid)	Contact	*Lavandula angustifolia* (lavender)	Yazdani et al. (2013)
Agrotis ipsilon (black cutworm)		*Gaultheria procumbens* (American wintergreen)	Jeyasankar (2012)
Pseudaletia unipuncta (armyworm)	Contact/fumigant	*Anethum graveolens* (dill)	Sousa et al. (2013)
		Cuminum cyminum (cumin)	
	Fumigant	*Foeniculum vulgare* (fennel)	
	Contact	*Petroselinum crispum* (parsley)	
		Laurus azorica (Azores laurel)	Rosa et al. (2010)
Spodoptera littoralis (African cotton leaf worm)	Antifeedant	*Artemisia absinthium* (wormwood)	Bailena et al. (2013)
	Fumigant	*Salvia officinalis* (sage)	Souguir et al. (2013)
	Topical	*Pimenta racemosa* (ciliment)	Pavela (2012)
		Thymus vulgaris (thyme)	
		Origanum vulgare (oregano)	
Trichoplusia ni (cabbage looper)	Topical/contact	*Syzygium aromaticum* (cloves)	Jiang et al. (2012)
		Thymus vulgaris (thyme)	
	Topical	*Allium sativum* (garlic)	Machial et al. (2010)
		Cymbopogon citratus (lemongrass)	
		Thymus vulgaris (thyme)	
Plutella xylostella (diamondback moth)	Fumigant	Mentha pulegium (pennyroyal)	Yi et al. (2007)
		Rosmarinus officinalis (rosemary)	
		Salvia officinalis (sage)	
Choristoneura rosaceana (oblique-banded leafroller)	Topical	*Allium sativum* (garlic)	Machial et al. (2010)
		Cymbopogon citratus (lemongrass)	
		Thymus vulgaris (thyme)	
Chaetodactylus krombeini (Krombine's hairy-footed mite)	Fumigant	*Gaultheria* spp. (wintergreen)	White et al. (2009)
		Mentha spicata (spearmint)	
		Cymbopogon citratus (lemongrass)	
Varroa destructor (varroa mite)	Contact	*Laurus nobilis* (laurel)	Damiani et al. (2009)
		Thymus vulgaris (thyme)	
		Lavandula officinalis (lavender)	
	Fumigant	*Thymus vulgaris* (thyme)	El-Wahab et al. (2012)
		Pimpinella anisum (anise)	
		Cinnamomum zeylanicum (cinnamon)	
		Cymbopogon flexuosus (lemongrass)	

Continued

TABLE 1 List of Arthropod Pests in Agricultural Production Systems and List of Plant Species Containing Essential Oils Showing Pesticidal Properties—cont'd

Arthropod Species (Common Name)	Application Method	Plant Species (Common Name)	Key References
Tetranychus cinnabarinus (carmine spider mite)	Fumigant	*Origanum onites* (oregano)	Sertkaya et al. (2010)
		Thymus vulgaris (thyme)	
Tetranychus urticae (two-spotted spider mite)	Contact	*Deverra scoparia*	Attia et al. (2012)
		Haplophyllum tuberculatum	
		Mentha pulegium (pennyroyal)	
		Chrysanthemum coronarium (garland chrysanthemum)	
		Hertia cheirifolia	
	Fumigation	*Protium bahianum*	Pontes et al. (2007)
	Contact	*Rosmarinus officinalis* (rosemary)	Miresmailli and Isman (2006)
	Fumigation	*Petiveria alliacea* (Guinea henweed)	Neves et al. (2011)
		Mentha spicata (spearmint)	Omar et al. (2009)

properties. Essential oils tend to be more effective on soft-bodied insects such as aphids, whiteflies, and thrips than hard-bodied insects (Isman, 2000). In addition, essential oils can be mixed with conventional pesticides as synergists (Isman et al., 2011).

ADVANTAGES AND DISADVANTAGES OF ESSENTIAL OILS AS PESTICIDES

Ecotoxicology of Essential Oils

In general, essential oils are considered as safe pesticides. There has been no report of biomagnification of essential oils through the food chain (Regnault-Roger et al., 2012). Although allergic reaction of essential oils in humans is reported with clinical and pharmaceutical studies, they show relatively low mammalian toxicity; thus essential oils have been widely used for medicinal and clinical purposes. In addition, essential oils show low toxicity to other vertebrates including fishes and birds because they are readily broken down by light or other abiotic environmental factors and do not persist in soil and water (Isman, 2000). Therefore, essential oils can play an important role in pest management specifically where environmentally friendly control measures are needed such as organic farming, public places, and homes. Essential oils are also known to be used as environmentally friendly herbicides (Batish et al., 2007) and fungicides (Isman, 2000).

Nontarget Effects of Essential Oils in Pest Management

Although essential oils are considered as safe pesticides, there have been several reports about their nontarget effects (see Table 2 for a list of essential oils that affect beneficial insects and mites). Bostanian et al. (2005) showed that four days after topical spray, a *Chenopodium*-based insecticide can affect natural enemies including minute pirate bugs and a parasitoid wasp (*Aphidius colemani*) that are commonly used for controlling aphids and thrips. In contrast, a study by Chiasson et al. (2004) revealed that essential oils were able to control aphids, thrips, and whiteflies successfully, but showed no effect on natural enemies of whitefly. Such nontarget effects of essential oils were further studied with pollinator insects. Amrine (2006) showed that essential oils killed varroa mites affecting honey bees in bee hives. He applied wintergreen oil as an ingredient to grease patties, on which the honey bees feed, while volatiles were slowly emitted to kill mites. Honey bees feed on grease patties and as they do, the wintergreen oil acts as an irritant to the varroa mite causing the mite to fall off the bee through the frames of the hive to the sticky bottom board (Amrine, 2006). White et al. (2009) showed that Krombein's hairy-footed mites parasitizing Japanese horn-faced bees (i.e., a solitary pollinator bee) can be controlled effectively with

TABLE 2 List of Beneficial Arthropods (Nontarget Organisms) in Agricultural Production Systems and List of Plant Species Containing Essential Oils that Can Harm the Beneficial Arthropods

Arthropod Species (Common Name)	Application Method	Plant Species (Common Name)	Key References
Adalia bipunctata (two-spotted ladybird)	Fumigant	*Mentha pulegium* (pennyroyal)	Kimbaris et al. (2010)
Coccinella septempunctata (seven-spotted lady beetle)			
Orius sp. (minute pirate bug)	Contact	*Allium sativum* (garlic)	Mousa et al. (2013)
Orius insidiosus (insidious flower bug)		*Chenopodium ambrosioides* (epazote)	Bostanian et al. (2005)
Apis mellifera (honeybee)		*Thymus vulgaris* (thyme)	Damiani et al. (2009)
Cotesia glomerata (white butterfly parasite)	Fumigant	*Artemisia vulgaris* (armoise)	Yi et al. (2007)
		Thuja occidentalis (white cedar)	
		Eucalyptus globulus (eucalyptus)	
		Myrtus communis (myrtle)	
		Melaleuca viridiflora (broad-leaved paperbark)	
Aphidius colemani	Contact	*Chenopodium ambrosioides* (epazote)	Bostanian et al. (2005)
Osmia cornifrons (Japanese horn-faced bee)	Topical	*Gaultheria* spp. (wintergreen)	White et al. (2009)
		Cymbopogon citratus (lemongrass)	
		Mentha spicata (spearmint)	
Dinarmus basalis	Contact	*Jatropha curcas* (physic nut)	Boateng and Kusi (2008)
Trissolcus basalis	Contact/fumigant	*Schinus molle* var. *areira* (American pepper)	González et al. (2013)
	Contact	*Ocimum basilicum* (sweet basil)	Momen and Amer (2003)
Neoseiulus cucumeris		*Ocimum basilicum* (sweet basil)	Momen et al. (2006)
Phytoseiulus persimilis (Chilean predatory mite)	Topical	*Mentha piperita* (peppermint)	Choi et al. (2004)
	Contact	*Allium sativum* (garlic)	El-Deen and Abdallah (2013)

wintergreen oils applied as a fumigant. When the bee nests were fumigated with wintergreen oils the parasitic mites were killed in less than 1.8 h, but the Japanese horn-faced bee was not affected. However, direct contact of wintergreen oil on the Japanese horn-faced bee caused a high mortality. Contrarily, some essential oils can attract pollinator bees, for example, euglossine bees are attracted by plant emitting volatiles of cineole, a major component of eucalyptus oil (Dressler, 1982).

Major Disadvantages of Using Essential Oils in Pest Management

Although essential oils have many advantages as environmentally friendly pesticides in many agricultural production systems, there are three major disadvantages. First, although effects of individual active ingredients have been studied intensively, very little is known about effects of a mixture of active ingredients of essential oils due to difficulties in their evaluation for effectiveness (Regnault-Roger et al., 2012). Second, while the use of essential oils for pest control has been in practice for many years, there are few commercialized agricultural essential oil-based products available on the market. Third, although some essential oils are highly effective as pesticides, it would be less popular in conventional farming because they are more expensive and less available. However, as stated previously, essential oils can play an important role in organic farming or places where worker and environmental safety is of primary concern (Isman, 2000).

SUMMARY POINTS

- Essential oils with pesticidal properties are found in plants belonging to the Amaryllidaceae, Apiaceae, Asteraceae, Atherospermataceae, Burseraceae, Cupressaceae, Fabaceae, Lamiaceae, Lauraceae, Myrtaceae, Phytolaccaceae, Piperaceae, Poaceae, Rutaceae, and Zingiberaceae families.
- Because essential oils have various modes of action for killing arthropod pests, they can play an important role in IPM and organic farming.
- Currently, essential oils are used as a part of IPM strategies: as broad-spectrum pesticides, organic pesticides, and low-risk pesticides.
- Known modes of action of essential oils include neurotoxicity, insect growth regulation, degradation of waxy layers of insect cuticle, blockage of digestive enzymes, and inhibition of P450 cytochromes.
- Essential oils are readily broken down in the environment and do not persist in soil and water, and there has been no report of biomagnification of essential oils through the food chain.
- Essential oils have fewer nontarget effects on natural enemies, but direct contact of essential oils on beneficial insects (e.g., pollinator bees) can cause mortality.
- There are a few commercialized agricultural and garden essential oil-based products available on the market although some essential oils have shown their effectiveness as pesticides.

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Chapter 7

Use of Essential Oils in Food Preservation

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List of Abbreviations

EOs Essential oils
SC Sodium caseinate

INTRODUCTION

Definition

There is an increased interest in the use of new methods of making food safe that have a natural or "green" image. One such possibility is the use of essential oils (EOs) as food preservatives (Burt, 2004). Preservation of food to extend its shelf-life, while ensuring its safety and quality, is a central preoccupation of the food industry and government agencies. Shelf-life has been defined as the period of time during which the food product will remain safe; be certain to retain desired sensory, chemical, physical, microbiological, and functional characteristics; and comply with any label declaration of nutritional data when stored under the recommended conditions. Therefore, it can be said that any additive that can extend or maintain the shelf-life of a food product can be described as a preservative, and EO is such additive.

EOs can be defined as naturally occurring complex mixtures of volatile lipophilic secondary metabolites that give plants and spices their characteristic essence and color (Benchaar and Greathead, 2011; Benchaar et al., 2008). They can be extracted from different plants and traditionally their extractions are by boiling water and steam distillation. Solvent extraction, supercritical CO_2 extraction, and expression extraction are among other methods that can be used for extraction of EOs (Benchaar and Greathead, 2011). They have been reported to find various applications in medicine, food, and fragrance industries (Noorizadeh et al., 2011).

PRESERVATIVE EFFECTS OF ESSENTIAL OILS

EOs are usually characterized by a strong odor and these mixtures consist of more than 200 constituents which can be grouped basically into two fractions of volatile and nonvolatile fractions (Jalali-Heravi et al., 2010). About 90–95% of the whole EO is made up of the volatile fraction and this consists of monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives, along with aliphatic aldehydes, alcohols, and esters. The nonvolatile residue, however, constitutes about 5–10% of the whole oil, which mainly contains hydrocarbons, fatty acids, sterols, carotenoids, waxes, cumarines, and flavonoids (Basile et al., 1998; Luque de Castro et al., 1999).

The phenolic components according to Burt (2004) are majorly responsible for the preservative effects of EOs in terms of antibacterial properties. These phenolic compounds, defined as part of the important group of secondary metabolites that are produced by plants, are characterized by at least one aromatic ring (C_6) that bears one or more hydroxyl groups (Michalak, 2006). Biosynthesis of phenolic compounds in plants through aromatic amino acids commences via the shikimate pathway. The significance of this pathway is demonstrated by the fact that, under normal growth conditions, 20% of the carbon fixed by plants flows through this route. The aromatic amino acids, phenylalanine, tyrosine, and tryptophan, are formed via this pathway and are later utilized for protein synthesis or transformed via phenylpropanoid metabolism to secondary metabolites such as phenolic compounds (Diaz et al., 2001). Phenolic compounds are divided into several groups and are based on the number of constitutive carbon atoms in conjunction with the structure of the basic phenolic skeleton (Michalak, 2006). The structural formulae of a number of preservative components of some EOs as compiled by Burt (2004) are presented in Figure 1.

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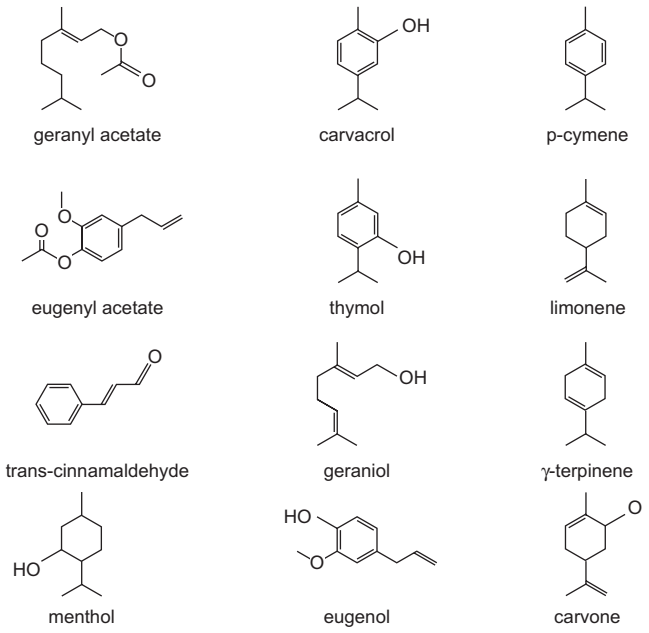

FIGURE 1 Structural formulae of selected components of essential oils. *Adapted from Burt (2004).*

The preservative effects of EOs as antimicrobial agents have been documented (Burt, 2004). The antimicrobial activities of thyme EOs for instance, have been reported to be mostly mediated by thymol and carvacrol, which are the phenolic components of the oil. Thymol, which is structurally very similar to carvacrol, has the hydroxyl group at a different location on the phenolic ring. Both substances appear to make the cell membrane permeable (Lambert et al., 2001). Carvacrol and thymol are able to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to adenosine triphosphate. The presence of magnesium chloride was shown to have no influence on this action, which suggested a mechanism other than chelation of cations in the outer membrane (Helander et al., 1998).

Evidence shows that minor components have a critical part to play in biological activities of EOs, possibly by producing a synergistic effect between other components. After several studies, phenols have been recognized to play an outstanding role on the antimicrobial activity of the EOs. These terpene phenols are joined to the amine and hydroxylamine groups of the proteins of the bacterial membrane, thereby altering their permeability and resulting in the death of the bacteria (Juven et al., 1994).

Olmedo et al. (2013) evaluated the effect of oregano and rosemary EOs on the oxidative and fermentative stabilities of flavored cheese prepared with cream cheese base. Their study shows that, oregano and rosemary EOs demonstrated a protective effect against lipid oxidation and fermentation in flavored cheese prepared with cream cheese base.

Goñi et al. (2009) demonstrated the antimicrobial activity of the vapor generated by a combination of cinnamon and clove EOs against the growth of four Gram-negative (*Escherichia coli, Yersinia enterocolitica, Pseudomonas aeruoginosa*, and *Salmonella choleraesuis*) and four Gram-positive bacteria (*Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus*, and *Enterococcus faecalis*). This study shows for the first time that a combination of EOs in the vapor phase has a preservative effect against microorganism proliferation.

Bounatirou et al. (2007) reported the chemical composition, antioxidant ,and antibacterial activities of EOs isolated by hydrodistillation from the aerial parts of Tunisian *Thymus capitatus* Hoff. et Link during the different phases of the plant development, and from different locations. After comparing the antibacterial properties of these EOs with synthetic antibiotics, higher antibacterial activity was observed with the flowering and the postflowering phase EOs.

ESSENTIAL OIL AS A REPELLENT FOR FOOD PRESERVATION

Food products such as stored grains, fruits, and other cellulosic materials vulnerable to infestation by various pests, mostly arthropods, may be preserved when the deleterious activity of these insects and anthropods is inhibited. Currently, the use of specific synthetic chemicals to control selected insects and arthropods is common practise and it raises several concerns related to environment and human health. Other alternatives being explored is the use of natural products that possess good

efficacy and are environmentally friendly. Among these chemicals, EOs from plants belonging to several species have been extensively tested to assess their repellent properties as a valuable natural resource and have been found to be highly potent.

In relation to human health issues, the United States Environmental Protection Agency has confirmed the efficacy of some EOs and consequently registered citronella, lemon, and eucalyptus oils as insect repellent ingredients for application on the skin. The natural products are being frequently used because they have been ascertained to have low toxicity, produce comparable efficacy in relation to other EOs and synthetic chemicals, and are preferred by customers (Katz et al., 2008). However, Trumble (2002) reported that, in general, natural products used in pest control are not always safer than synthetic products because of inappropriate and/or lack of exclusively comprehensive assays to test effectiveness and safety. A survey of plant EOs that showed significant repellence to arthropod species consisting of the animal species used as repellent reference, the order, various scientific names, the plant source including family plant used, and the resident country was documented by Nerio et al. (2010) and is presented in Table 1. The extensive study shows that EOs from plant and their individual metabolites have indicated optimal potential for repellent activity against several species, not only insects, but other kinds of arthropods. The, natural products have therefore been found with considerable potential as commercial repellent products when they are mixed with fixative materials because the inherent high volatility they naturally possess decreases their effective times of protection.

ESSENTIAL OILS AS CONSTITUENTS OF ANTIMICROBIAL PACKAGING

EOs have been confirmed to be of great importance in the control of microbial populations. Usually, they are conditioned to target specific microorganisms in order to provide higher safety and quality products in the food industry in relation to the established minimum durability of each food item. In particular, EOs have been confirmed as desirable active ingredients in the development of active packaging materials for food products (Day, 1989). The demand for the use of antimicrobial packaging as an active packaging is on the increase and, in essence, the demand for EOs as active ingredients due to continued consumer preference for minimally processed food products (Sadaka et al., 2013). In this regard, EOs and more specifically their bioactive molecules are being comprehensively analyzed for their antimicrobial (Burt, 2004; Kordali et al., 2005; Sökmen et al., 2004), antifungal (Kordali et al., 2005), antiviral (Sökmen et al., 2004), insecticidal (Regnault-Roger et al., 1993), and antioxidant (Kordali et al., 2005; Sökmen et al., 2004) attributes. Several other oils are also used in aromatherapy (Buchbauer and Jirovetz, 1994; Herrmann, 2009; Wood, 2003), cosmetic (Motoyoshi et al., 1978), fragrance industries, or for cancer treatment (Carnesecchi et al., 2001). However, the mechanism of action of the EOs is paramount in food systems regarding their applications. Sadaka et al. (2013) reported that, considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but results from multiple damage in the cells (Figure 2).

The antimicrobial impact of several essential oils in culture medium, or when included into edible films, on some strains of lactic acid-producing bacteria have also been reported. Table 2 summarizes the antimicrobial packaging containing a range of common natural agents including essential oils that have been incorporated into or coated onto synthetic packaging films (Sadaka et al., 2013).

ESSENTIAL OILS AS CONSTITUENT OF EDIBLE PACKAGING

Edible coatings of food materials are among innovations in the food industry. Usually, edible packaging is known to comprise proteins, polysaccharides, and lipids. Although protein and polysaccharide films show good mechanical properties, they are highly permeable to water because of their hydrophilic nature. The hydrophilic tendencies may be improved through the addition of sodium caseinate (SC). SC is a commercially available water-soluble polymer that is obtained by the acid precipitation of casein, the main protein in cow's milk (Audic and Chaufer, 2005). Casein-based edible films are highly nutritional and are therefore attractively appropriate for food applications. They also have excellent inherent sensory properties and commendable potential to provide food products with adequate protection from their surrounding macro climate. Furthermore, the water barrier properties are known to be "inferior" (Han and Gennadios, 2005). In this regard, Fabra et al. (2008) successfully improved the suitability of water vapor permeability of the SC films for food products by adding oleic acid and beeswax mixtures. Inclusion of EOs such as cinnamon oil and ginger oil is another alternative for improving the SC-based edible packaging.

Atarés et al. (2010) characterized SC-based edible films incorporated with cinnamon or ginger EO and determined that cinnamon oil remained homogeneously integrated in the SC matrix, giving rise to films with regular surfaces, but considerably affected the optical properties of SC films. Due to the aggregation of lipids during drying, ginger oil caused an increase in surface roughness and a loss of gloss. All the films produced with the selected EOs effectively protected sunflower oil against oxidation. This is likely to be associated with their low permeability to oxygen at the low relative humidity of the surrounding atmosphere where the study was carried out.

TABLE 1 Plant Essential Oils that Have Shown High Repellency to Arthropod Species

Animal Species Used in Repellent Studies		Essential Oil Used as Repellent				
Order	**Scientific Name**	**Plant Source**	**Family Plant**	**Part Used**	**Country**	**Reference**
Diptera	*Anopheles culicifacies*	*Mentha piperita*	Lamiaceae	Fresh leaves	India	Ansari et al. (2000)
Diptera	*Culex quinquefasciatus*	*Mentha piperita*	Lamiaceae	Fresh leaves	India	Ansari et al. (2000)
Diptera	*Aedes aegypti*	*Zanthoxylum piperitum*	Rutaceae	Dried Fruits	Thailand	Choochote et al. (2007)
Diptera	*Culex pipiens*	*Pimpinella anisum*	Umbelliferae	Seed	Turkey	Erler et al. (2006)
Diptera	*C. pipiens*	*Ocimum basilicum*	Lamiaceae	Dried foliage	Turkey	Erler et al. (2006)
Diptera	*C. pipiens*	*Eucalyptus camaldulensis*	Mirtaceae	Dried fruits	Turkey	Erler et al. (2006)
Diptera	*A. aegypti*	*Baccharis spartioides*	Compositae	N.I.	Argentina	Gillij et al. (2008)
Diptera	*A. aegypti*	*Aloysia citriodora*	Verbenaceae	N.I.	Argentina	Gillij et al. (2008)
Diptera	*Mansonia*	*Eucalyptus maculata, Eucalyptus citriodon*	Mirtaceae	Leaves	Ethiopia	Hadis et al. (2003)
Diptera	*Anopheles gambiae*	*Croton pseudopulchellus*	Annonaceae	N.I.	Kenya	Odalo et al. (2005)
Diptera	*A. gambiae*	*Mkilua fragrans*	Annonaceae	N.I.	Kenya	Odalo et al. (2005)
Diptera	*A. gambiae*	*Endostemon tereticaulis*	Labiateae	N.I.	Kenya	Odalo et al. (2005)
Diptera	*A. gambiae*	*Ocimum forskolei*	Labiateae	N.I.	Kenya	Odalo et al. (2005)
Diptera	*A. gambiae*	*Ocimum fischeri*	Labiateae	N.I	Kenya	Odalo et al. (2005)
Diptera	*A. gambiae*	*Plectranthus longipes*	Labiateae	N.I.	Kenya	Odalo et al. (2005)
Diptera	*A. gambiae*	*Conyza newii*	Compositae	N.I.	Kenya	Omolo et al. (2004)
Diptera	*A. gambiae*	*Tarchonanthus camphoratus*	Compositae	N.I.	Kenya	Omolo et al. (2004)
Diptera	*A. gambiae*	*Tetradenia riparia*	Labiatae	N.I.	Kenya	Omolo et al. (2004)
Diptera	*A. gambiae*	*Lippia javanica*	Verbenaceae	N.I.	Kenya	Omolo et al. (2004)
Diptera	*A. gambiae*	*Lippia ukambensis*	Verbenaceae	N.I	Kenya	Omolo et al. (2004)
Diptera	*A. gambiae*	*Plectranthus marrubioides*	Labiatae	N.I.	Kenya	Omolo et al. (2004)
Diptera	*A. aegypti*	*Cymbopogon citratus*	Poaceae	Fresh aerial parts	Nigeria	Oyedele et al. (2002)

Diptera	*Aspergillus braziliensis*	*Ocimum selloi*	Lamiaceae	Leaves	Brazil	Padilha de Paula et al. (2003)
Diptera	*Anopheles stephensi*	*Ocimum basilicum*	Lamiaceae	Leaves	India	Prajapati et al. (2005)
Diptera	*A. aegypti*	*Ocimum basilicum*	Lamiaceae	Leaves	India	Prajapati et al. (2005)
Diptera	*C. quinquefasciatus*	*Ocimum basilicum*	Lamiaceae	Leaves	India	Prajapati et al. (2005)
Diptera	*A. stephensi*	*Rosmarinus officinalis*	Lamiaceae	Shoot	India	Prajapati et al. (2005)
Diptera	*A. aegypti*	*Rosmarinus officinalis*	Lamiaceae	Shoot	India	Prajapati et al. (2005)
Diptera	*C. quinquefasciatus*	*Rosmarinus officinalis*	Lamiaceae	Shoot	India	Prajapati et al. (2005)
Diptera	*A. stephensi*	*Cinnamomum zeylanicum*	Lauraceae	Bark	India	Prajapati et al. (2005)
Diptera	*A. aegypti*	*Cinnamomum zeylanicum*	Lauraceae	Bark	India	Prajapati et al. (2005)
Diptera	*C. quinquefasciatus*	*Cinnamomum zeylanicum*	Lauraceae	Bark	India	Prajapati et al. (2005)
Diptera	*C. quinquefasciatus*	*Cymbopogon citratus*	Graminae	N.I	India	Pushpanathan et al. (2006)
Diptera	*C. quinquefasciatus*	*Zingiber officinalis*	Zingiberaceae	Rhizomes	India	Pushpanathan et al. (2008)
Diptera	*C. quinquefasciatus*	*Moschosma polystachyum*	Lamiaceae	Fresh leaves	India	Rajkumar and Jebanesan (2005)
Diptera	*C. quinquefasciatus*	*Solanum xanthocarpum*	Solanaceae	Fresh Leaves	India	Rajkumar and Jebanesan (2005)
Diptera	*Anopheles dirus*	*Curcuma longa* L.	Zingiberaceae	Rhizomes	Thailand	Tawatsin et al. (2001)
Diptera	*C. quinquefasciatus*	*Cymbopogon winterianus*	Poaceae	Leaves	Thailand	Tawatsin et al. (2001)

Source: Nerio et al. (2010). N.I., Information not available.

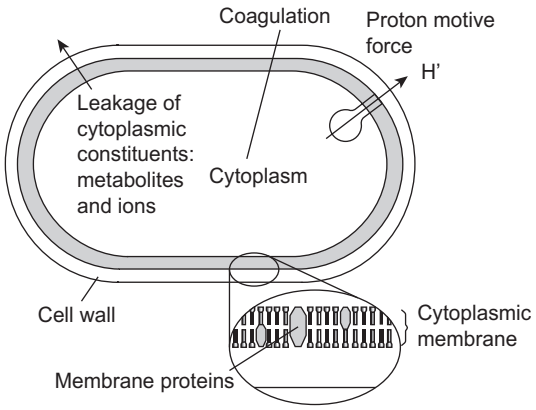

FIGURE 2 Action of essential oils and their components on the bacterial cell. *Source: Sadaka et al. (2013).*

TABLE 2 Overview of Studies Testing Essential Oils or Their Components in Packaging Materials

Antimicrobial Agent	Amount Used	Packaging Material	Process	Packaged Food	References
Anise oil	1, 2, 3, and 4% (w/w)	Chitosan	Direct incorporation by dissolution	Bologna slices	Zivanovic et al. (2005)
Basil oil	1, 2, 3, and 4% (w/w)	Chitosan	Direct incorporation by dissolution	Bologna slices	Zivanovic et al. (2005)
Bergamot oil	0.5, 1, 2, and 3 (w/w)	Chitosan	Direct incorporation by dissolution	Fruit	Sanchez-Gonzalez et al. (2010)
Bergamot oil	5, 10, 15, 20, and 25% (w/w)	Gelatin film	Direct incorporation by dissolution	Unicorn leather-jacket	Ahmad et al. (2012)
Carvacrol	10, 30 and 60% (w/w)	Soy protein isolate and octenyl succinate-based paper	Direct incorporation by dissolution		Ben Arfa et al. (2006,2007)
Carvacrol	0.5, 1 and 1.5% (w/w)	Apple-based film	Incorporation		Du et al. (2008)
Carvacrol	10% (w/w)	Nanocomposite films based on LDPE	Incorporation		Persico et al. (2009)
Carvacrol	1.2% (w/w)	HDPE	Incorporation		Peltzer et al. (2009)
Carvacrol	0.4, 0.6, and 1% (w/v)	*Gelidium corneum*	Direct incorporation by dissolution	Ham	Lim et al. (2010)
Carvacrol (into soy protein)	0, 30 and 60 (w/w)	Paper	Coating		Chalier et al. (2010)
Carvacrol	4% (w/w)	LDPE/EVA copolymer	Incorporation	Products such as cheese and aqueous foods	Cran et al. (2010)
Carvacrol	15% (w/w)	Wheat gluten coated papers	Direct incorporation by dissolution		Mascheroni et al. (2011)

TABLE 2 Overview of Studies Testing Essential Oils or Their Components in Packaging Materials—cont'd

Antimicrobial Agent	Amount Used	Packaging Material	Process	Packaged Food	References
Carvacrol	0.5 and 0.75% (w/w)	Apple and tomato-based films	Direct incorporation by dissolution	Baked chicken wrapped	Du et al. (2012)
Carvacrol	5% (w/w)	EVOH/PP	Direct incorporation by dissolution		Cerisuelo et al. (2012)
Carvacrol	1–10% (w/v)	Biaxially oriented PP	Incorporation of microencapsulated active agents	Refrigerated fresh fish and meat	Guarda et al. (2011,2012)
Carvacrol	4, 6 and 8% (w/w)	PP	Incorporation by melt blending		Ramos et al. (2012)
Carvacrol	1.5 and 3.5% (w/w)	Corn-zein-laminated LLDPE	Coating		
Cinnamaldehyde	0.5 and 1% (w/w)	Chitosan	Direct incorporation by dissolution	Meat	Ouattara et al. (2000)
Cinnamaldehyde	0.5–50 ppm	Paper	Impregnation	Rambutan	Sivakumar et al. (2002)
Cinnamaldehyde	10, 30 and 60%	Paper based on a soy protein isolate and octenyl succinate	Direct incorporation by dissolution		Ben Arfa et al. (2006,2007)
Cinnamaldehyde	10% (w/w)	LDPE/PA	Direct incorporation by dissolution then coating on LDPE		Han et al. (2008)
Cinnamaldehyde	1–4% (w/w)	PP and PE/PVA	Incorporation		Gutierrez et al. (2010)
Cinnamaldehyde	0.5 and 0.75% (w/w)	Apple-based films	Direct incorporation by dissolution	Baked chicken wrapped	Du et al. (2012)
Cinnamaldehyde	6% (w/w)	Soy protein isolate based film	Direct incorporation by dissolution	Olive oil	Hu et al. (2012)
Cinnamaldehyde	1% (w/w)	Cellulose-based packaging	Direct incorporation by dissolution		Sanla-Ead et al. (2012)
Cinnamon essential oil, cinnamon fortified with cinnamaldehyde	14% (w/w)	PP and PE–EVA copolymer	Coating	Bakery products	Lopez et al. (2007)
Cinnamon	4 and 6% (w/w)	Paper	Coating of paraffin formulation containing cinnamon essential oil		Rodriguez et al. (2008)
Cinnamon	2 and 4% (w/w)	PP	Incorporation	Bakery products	Gutierrez et al. (2009)
Bark cinnamon or leaf cinnamon	3 and 6% (w/w)	Active paraffin-based paper	Incorporation to the coating	Cherry tomatoes	Rodriguez-Lafuente et al. (2010)
Cinnamon	2 and 4% (w/v)	PP	Incorporation	Bakery product	Gutierrez et al. (2010)

Continued

TABLE 2 Overview of Studies Testing Essential Oils or Their Components in Packaging Materials—cont'd

Antimicrobial Agent	Amount Used	Packaging Material	Process	Packaged Food	References
Cinnamon essential oil	2 and 2.5 g/m^2	PP wrapped with PE/EVA	Coating on a shelf adhesive active label stuck inside the PE wrap	Bakery products	Gutierrez et al. (2011)
Cinnamon and cinnamaldehyde-enriched cinnamon	1–8% (w/w)	Paper packaging	Coating	Strawberries	Rodriguez et al. (2007)
Cinnamon essential oil	0.062–6.54 g/m^2	PET PP	Coating	Calanda peaches	Montero-Prado et al. (2011)
Cinnamon essential oil	20%, (w/w)	Cellulosic films and paper	Coating of cellulosic emulsion with cinnamon		Espitia et al. (2011)
Clove extract	20% (w/w)	LDPE	Incorporation		Hong et al. (2000)
Clove oil	1–8% (w/w)	Paper packaging	Coating		Rodriguez et al. (2007)
Clove oil	0.5, 1.5, 2% w/w	Chitosan	Direct incorporation by dissolution		Hosseini et al. (2008)
Clove oil	0.75 mL/g	Fish skin gelatin	Direct incorporation by dissolution	Fish	Gomez-Estaca et al. (2009)
Clove oil	3 and 6% (w/w)	Active paraffin-based paper	Incorporation to the coating	Cherry tomatoes	Rodriguez-Lafuente et al. (2010)
Coriander	1, 2, 3 and 4% (w/w)	Chitosan	Direct incorporation by dissolution	Bologna slices	Zivanovic et al. (2005)
Eugenol	l% (w/w)	Cellulose-based packaging	Direct incorporation by dissolution		Sanla-Ead et al. (2012)
Garlic essential oils		Whey protein films	Direct incorporation by dissolution		Seydim and Sarikus (2006)
Grapefruit seed extract	0.1 and 1.0% (w/w)	LDPE	Incorporation	Curled lettuce and soybean	Lee et al. (1998)
Grapefruit seed extract	0.5, 1% (w/w)	Multilayered PH film	Incorporation or coating	Ground beef	Ha et al. (2001)
Lemongrass essential oil	20% (w/w)	Cellulosic films and paper	Coating of cellulosic emulsion with lemongrass		Espitia et al. (2011)
Lemongrass essential oil	5, 10, 15, 20, and 25% (w/w)	Gelatin film	Direct incorporation by dissolution	Unicorn leatherjacket	Ahmad et al. (2012)
Oregano essential oil	1, 2, 3, and 4% (w/w)	Chitosan	Direct incorporation by dissolution	Bologna slices	Zivanovic et al. (2005)
Oregano essential oil		Whey protein films	Direct incorporation by dissolution		Seydim et al. (2006)
Oregano	1–8 % (w/w)	Paper packaging	Coating		Rodriguez et al. (2007)
Oregano essential oil	1–4% (w/w)	PP and PE/EVA copolymer	Coating	Meat	López et al. (2007)

TABLE 2 Overview of Studies Testing Essential Oils or Their Components in Packaging Materials—cont'd

Antimicrobial Agent	Amount Used	Packaging Material	Process	Packaged Food	References
Oregano	1, 2, 3, 4, and 5% (v/v)	Soy protein edible films	Direct incorporation by dissolution	Fresh ground beef patties	Emiroglu et al. (2010)
Oregano essential oil	0.2% (w/w)	Modified chitosan	Coating	Strawberries	Vu et al. (2011)
Oregano essential oil	3 and 6% (w/w)	Active paraffin-based paper	Incorporation to the coating	Cherry tomatoes	Rodriguez-Lafuente et al. (2010)
Oregano essential oil	20% (w/w)	Cellulosic films and paper	Coating of cellulosic emulsion with oregano		Espitia et al. (2011)
Oregano essential oil	1–4% (w/w)	LDPE	Ionizing treatment and incorporation		Valderrama Solano and de Rojas Gante (2011)
Garlic oil		Chitosan	Coating		Pranoto et al. (2005)
Lemon extract	7, 10, and 15% (w/w)	PCL, PLA, LDPE	Incorporation		Del Nobile et al. (2009)
Limonene	0.2% (w/w)	Modified chitosan	Coating	Strawberries	Vu et al. (2011)
Linalool	0.34% (w/w)	EVA–LDPE	Incorporation	Cheddar cheese	Suppakul et al. (2008)
Linalool	1% (w/w)	LLDPE, HDPE, and LDPE–EVA	Incorporation	Hard cheeses	Suppakul et al. (2011)
Linalool	2% (w/w)	LDPE/EVA copolymer	Incorporation	Products such as cheese and aqueous foods	Cran et al. (2010)
Methylchavicol	0.34% (w/w)	EVA–LDPE	Incorporation	Cheddar cheese	Suppakul et al. (2008)
Methylchavicol	l% (w/w)	LLDPE and LDPE–EVA copolymer	Incorporation	Hard cheeses	Suppakul et al. (2011)
Peppermint	0.2% (w/w)	Modified chitosan	Coating	Strawberries	Vu et al. (2011)
Pimento		Milk protein-based	Direct incorporation by dissolution	Beef muscle	Oussalah et al. (2004)
Rosemary essential oil		Whey protein films	Direct incorporation by, dissolution		Seydim et al. (2006)
Rosemary extract	0, 1 and 8% (w/w)	Coextruded bionented PP film	Incorporation		Tovar et al. (2005)
Rosemary essential oil	0.5, 1, 1.5% (v/v)	Chitosan	Direct incorporation by dissolution		Abdollahi et al. (2012)
Red thyme essential oil	0.2% (w/w)	Modified chitosan	Coating	Strawberries	Vu et al. (2011)
Thyme oil	1, 2, 3, 4, and 5% (v/v)	Soy protein edible films	Direct incorporation by dissolution	Fresh ground beef patties	Emiroglu et al. (2010)
Thyme oil	1–4% (w/w)	LDPE	Ionizing treatment and incorporation		Valderrama Solano and de Rojas Gante (2011)

Continued

TABLE 2 Overview of Studies Testing Essential Oils or Their Components in Packaging Materials—cont'd

Antimicrobial Agent	Amount Used	Packaging Material	Process	Packaged Food	References
Thyme oil	0.5, 1.3, and 2% (w/w)	Chitosan	Direct incorporation by dissolution		Hosseini et al. (2008)
Thymol	10, 20 and 35%	Zein film	Direct incorporation by dissolution		Del Nobile et al. (2008)
Thymol	7, 10 and 15% (w/w)	PCL, PLA, LDPE	Incorporation		Del Nobile et al. (2009)
Thymol	1–4 % (w/w)	PP and PE–PVA	Incorporation		Gutierrez et al. (2010)
Thymol	4% (w/w)	LDPE/EVA copolymer	Incorporation	Products such as cheese to aqueous foods.	Cran et al. (2010)
Thymol	1–10% (w/v)	Plastics films	Incorporation of microencapsulated actif agents	refrigerated fresh fish and meat	Guarda et al. (2011,2012)
Thymol	4, 6 and 8% (w/w)	PP	Incorporation by melt blending		Ramos et al. (2012)
Thymol		Biaxially oriented PP	Coating of microcapsules containing active agents		Guarda et al. (2011)
Thymol	6% (w/w)	Soy protein isolate based film	Direct incorporation by dissolution	Olive oil	Hu et al. (2012)
Cinnamon, clove, and clary sage essential oils		PP	Coating		Lee et al. (2011)
Hydrocinnamaldehyde, oregano essential oil, cinnamaldehyde, thymol, and carvacrol		PP	Incorporation		
Oregano essential oil	5, 10% (w/w)	PP/EVOH	Coating	Fresh salad	Muriel-Galet et al. (2012)
Vanillin	6% (w/w)	Soy protein isolate based film	Direct incorporation by dissolution	Olive oil	Hu et al. (2012)
Oregano and pimento		Milk protein-based	Direct incorporation by dissolution	Beef muscle	Oussalah et al. (2004)
Carvacrol and thymol (50:50)	4.6 and 8% (w/w)	PP	Incorporation by melt blending		Ramos et al. (2012)
Carvacrol and thymol		Biaxially oriented PP	Coating of microcapsules containing active agents		Guarda et al. (2011)

Source: Sadaka et al. (2013). EVA, Ethylene vinyl acetate; EVOH, Ethylene vinyl alcohol; HDPE, High density polyethylene; LDPE, Low density polyethylene; LLDPE, Linear low density polyethylene; PA, Polyamide; PCL, Poly caprolactone; PE, Polyethylene; PET, Polyethylene terephthalate; PLA, Polyacetic acid; PP, Polypropylene; PVA, Polyvinyl alcohol.

SUMMARY POINTS

- Essential oils are natural secondary metabolites produced by plants which, when extracted and concentrated, find application in pharmaceutical, sanitary, cosmetic, agricultural, and food industries.
- Essential oils have been identified as natural food additives which can find useful applications in food preservation.
- Essential oils are functional alternatives to synthetic chemicals being explored in food preservation that possess good efficacy and are environmentally friendly.
- Plant essential oils are known to show significant repellent effects and consequently find useful application in pest control.
- Essential oils can be incorporated into or coated onto synthetic packaging films, having a antimicrobial effect on food products to enhance shelf-life.

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Chapter 8

Use of Essential Oils as a Preservative of Meat

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List of Abbreviations

ATP Adenosine triphosphate
BHA Butylhydroxyanisole
BHT Butylated hydroxytoluene
DNA Deoxyribonucleic acid
EO Essential oil
RNA Ribonucleic acid
H^+ Hydrogen ions

INTRODUCTION

Meat, the flesh of animals used as food (Zhou et al., 2010), is highly susceptible to perishing due to its composition (Yadav and Singh, 2004). Endogenous enzyme activity, storage temperature, moisture, atmospheric oxygen, light, and microorganisms influence the freshness and shelf-life of fresh meat (Zhou et al., 2010), as outlined in Figure 1. Lipid peroxidation is the main cause of loss of quality in meat and meat products. The preservation of meat aims to prevent lipid peroxidation, increase the shelf-life and freshness of meat, conserve nutrients, and safeguard consumers. Meat preservation also seeks to develop in the preserved meat physico-chemical characteristics that are consistent with consumer expectations as illustrated in Figure 2.

METHODS AND PRINCIPLES OF MEAT PRESERVATION

Salting, refrigeration, radiation, and chemico-biopreservation are the major conventional methods used to preserve fresh meat. Salt limits the water activity of the meat itself and the water available to spoilage microorganisms both on the surface and deeper layers of the meat (Fennema, 1996). Refrigeration technologies (chilling, superchilling, and freezing) reduce the surface temperature of the meat, facilitate "drying", and reduce endogenous and spoilage-organism-derived enzyme activity (Ockerman and Basu, 2004). At freezing temperature, damaging redox reactions are reduced to a minimum, thus minimising qualitative changes in the frozen meat (Hansen et al., 2004). Despite being highly efficient at inactivating bacteria and its ability to be used to treat packaged meat, irradiation causes color changes due to the susceptibility of myoglobin to energy input (Lawrie and Ledward, 2006). Chemicals such as butylhydroxyanisole (BHA), butylated hydroxytoluene (BHT), sodium nitrites, and nitrates are used alongside radiation (Umemura and Kodama, 2006) as antioxidants in the preservation of meat. In processing meat the aim is to retain its natural, organoleptic, and aesthetic properties.

MEAT PRESERVATION: AN HISTORICAL REVIEW

Drying, curing, and pickling stand out as the major methods used before the more recent preservation technologies (Jones et al., 2001). In ancient times, sun-drying in open air was used to preserve meat. The meat, cut into lean strips, was exposed to the sun. At times the lean strips of meat were salted before exposure to the sun. Smoke-drying whereby strips

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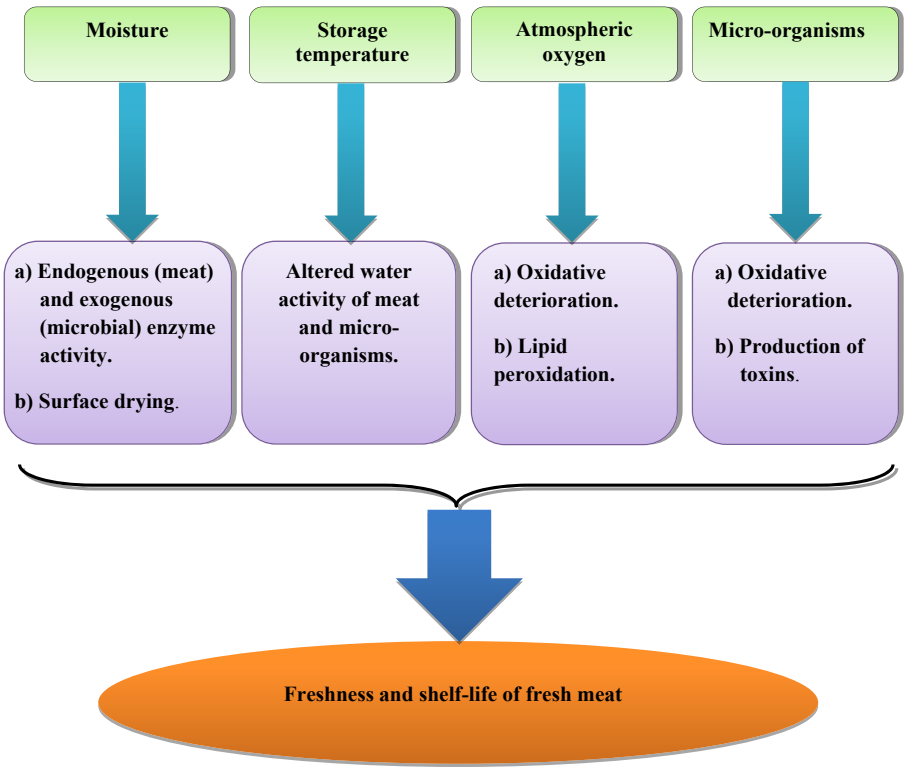

FIGURE 1 Schematic representation of factors affecting the shelf-life of fresh meat. Exogenous and endogenous enzyme catalyzed reactions result in oxidative deterioration of meat and the production of metabolites (toxins) in the meat. A reduction of moisture and water activity increases shelf-life. *Adapted from Zhou et al. (2010).*

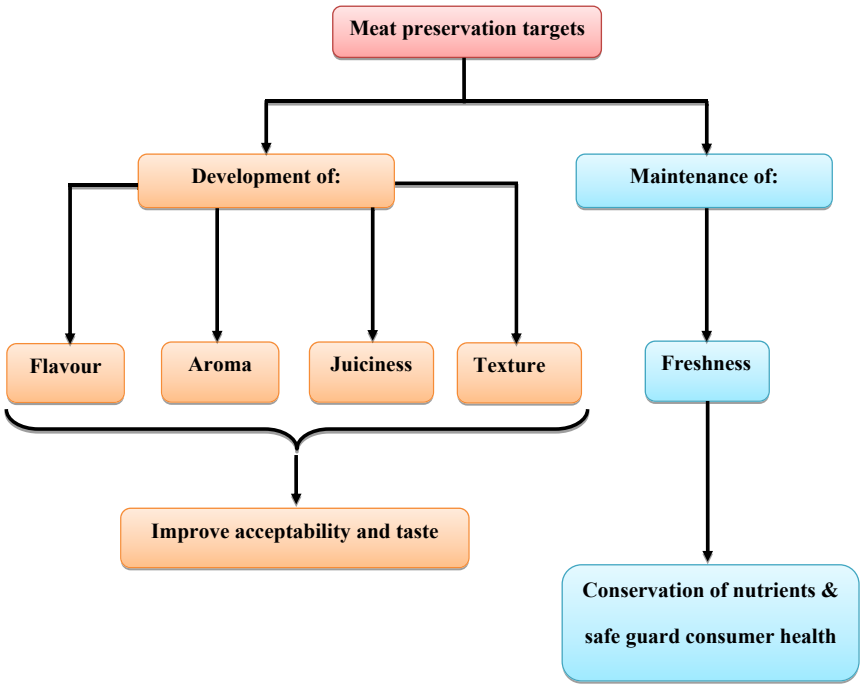

FIGURE 2 Schematic illustration of the physicochemical targets of meat preservation. Meat preservation ultimately results in the long-term maintenance of organoleptic properties and ensures that a wholesome and healthy meat product is delivered to consumers.

of meat or eviscerated fish are exposed to smoke and heat from various wood species was also employed. Sun-drying and smoke-drying have limitations. They lack standardization to the extent that critical parameters (duration, air humidity, and temperature) are not precisely controlled, negatively impacting on the nutritional value, microbial load, and safety of the preserved meat (Ali et al., 2011).

Early curing of meat was salt-based. The process involved use of salt to dehydrate the meat. Different types of salt (rock salt, sea salt, and spiced salts) were used. Most of the salts used to cure meat gave it an unappetizing gray color; however,

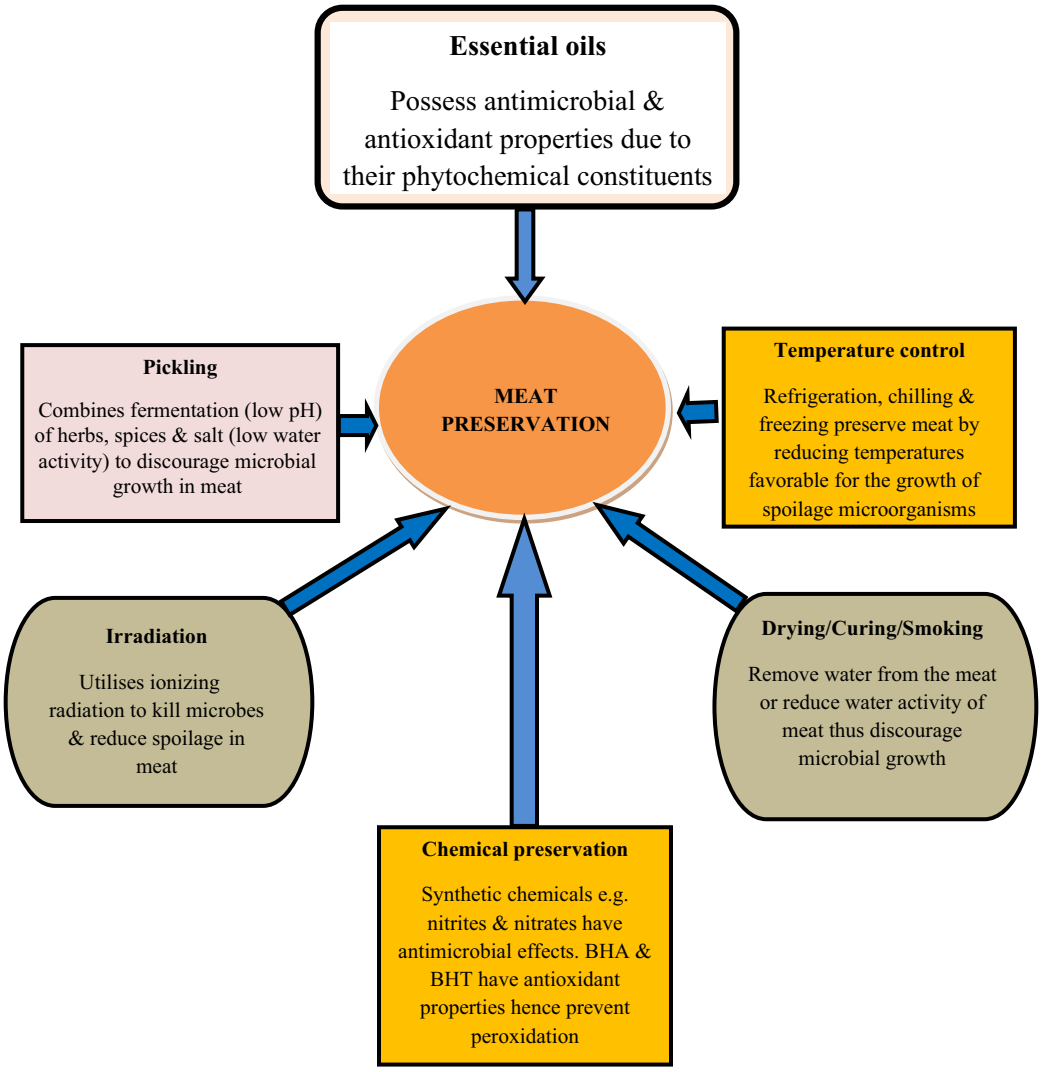

FIGURE 3 Schematic representation of some meat preservation methods.

the discovery and subsequent use of a mixture of salts that imparted a red color in the cured meat made the cured meat more acceptable (Nummer, 2002). It was later discovered that the mixture of salts that imparted the red color to cured meat contained nitrites that inhibited *Clostridium botulinum* (Nummer, 2002).

Pickling began about 4000 years ago in India. It involves preserving food in vinegar or an organic acid and or wine. The low pH of the organic acid or wine helps preserve the food/meat. In the Scandinavian countries it is common place to get pickled herring, salmon, and pork. Pickling is credited with improving the taste and flavor of the preserved meat. Due to the degree of fermentation that occurs during pickling, the process contributes to vitamins and healthy bacteria. However, pickling permanently alters the taste of meat such that pickled meat cannot be used as a substitute for fresh meat in a recipe. Figure 3 shows a schematic illustration of some of the meat preservation methods.

USE OF ESSENTIALS OILS IN THE PRESERVATION OF MEAT

Benefits of the Use of Essential Oils in Meat Preservation

Other than refrigeration, one of the major current technologies for the preservation of meat is the use of synthetic chemical preservatives (Gutierrez et al., 2009). Increased health consciousness by consumers has fueled negative perceptions about synthetic food additives. Some synthetic chemical meat preservatives are extremely toxic and are known to promote the development of cancer (Kahl and Kappus, 1993; Willis and Saidman, 2013). Preservation of meat with synthetic chemicals

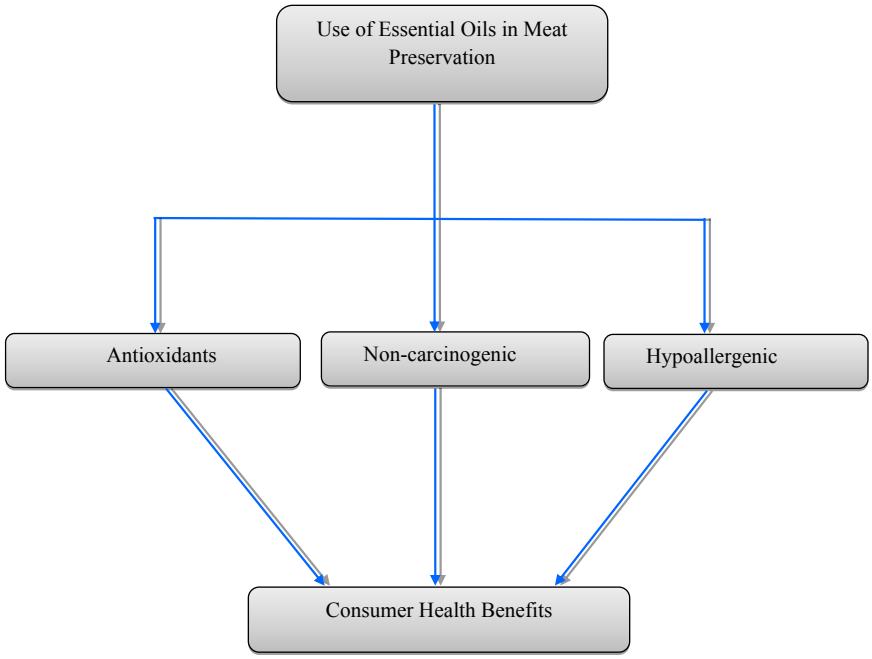

FIGURE 4 Schematic illustration of the health benefits of using essential oils to preserve meat. By virtue of their phytochemical constituents essential oils add extra nutritional value and potential health benefits.

is faced with an increasing consumer boycott due to the detrimental residual effects of the chemicals on consumer health and the environment (Yadav and Singh, 2004). These negative perceptions tend to arise from the widespread (sometimes conflicting) reports on the adverse effects of the additives. For example, both carcinogenic and anticarcinogenic effects of the commonly used synthetic antioxidants BHA and BHT have been reported (Botterweck et al., 2000). Nitrites and nitrates which are added to cured and processed meats as antioxidants, flavourants, and to inhibit microbial growth (Hord et al., 2009) have also been shown to have inconclusive effects on the health of humans.

The outcry by consumers against the use of synthetic chemicals as preservatives of meat has led research to focus on safer and eco-friendly natural products as candidate preservatives. The pursuit of natural products as preservatives of meat has currently focused largely on plant-derived products particularly essential oils (EOs) (Yadav and Singh, 2004). Several benefits of using EOs as preservatives have been identified: hypoallergenicity (Bleasel et al., 2002), the improvement of aroma, taste, and health benefits to consumers (e.g., anticancer, antioxidant) that the EOs may confer by virtue of their constituents (Bhat et al., 2011). Figure 4 highlights some of the health benefits associated with the use of EOs in meat preservation.

Specific Examples: Essentials Oils Currently Used in the Preservation of Meat

Although there is a lot of literature exploring the antimicrobial properties of EOs derived from numerous plants, for most EOs the *in vitro* results do not easily translate into viable use as meat preservatives. Studies with rosemary, cinnamon, bay, sage, garlic, oregano, and ginger have shown positive results for their capacity to act as meat preservatives (Irkin et al., 2011; Skandamis and Nyachas, 2002; Tsigarida et al., 2000).

EOs are generally more effective when they come in direct contact with organisms (Skandamis and Nyachas, 2002), hence mechanisms have to be put in place to ensure that the EOs are at concentrations that are effective to exert antimicrobial actions. The direct contact with meat can, however, affect the organoleptic properties of the meat through structural and chemical reactions/interactions. Essentials oils tend to have an intense aroma even at low concentrations and could overwhelm consumers. It would be very difficult and costly on a large scale to directly apply EOs to meat so as to ensure that controlled and adequate amounts of EOs come into contact with the meat to effectively preserve it. The use of active packaging wherein the preservatives may either be incorporated into the packaging materials and then diffuse or partition into the meat, or be released through evaporation in the headspace which is between the packaging and the meat (Skandamis and Nyachas, 2002) is seen as a possible solution. Studies with *Rosmarinus officinalis* incorporated at 4% (w/w) of active packaging inhibited the increase of products of putrefaction such as cadavarine and the bacteria that produce them including Enterobacteriaceae and *Brocothrix thermospacta* (Sirocchi et al., 2013). Sachets that provide slow release of EOs can also be incorporated into packaged meat and used to preserve the meat (Sánchez-González et al., 2011).

MECHANISMS OF ACTION OF ESSENTIAL OILS IN THE PRESERVATION OF MEAT

Preservation against Microbial Spoilage

Bacteria, mold, and yeast species of the genera *Pseudomonas*, *Acinetobacter-Moraxella*, *Enterobacteriaceae, Fusarium*, *Mucor, Candida, and Torulopsis* cause spoilage of fresh meat. EOs possess antibacterial, antifungal, antiparasitic, and antiviral properties (Oussalah et al., 2007; Silva et al., 2011; Schnitzler et al., 2011) and are made up of a complex mixture of phytochemicals. Some key phytochemicals in EOs include monoterpenes, sesquiterpenes, terpenoids, alcohols, aldehydes, ketones, phenolics, esters, and other complex aromatic and aliphatic compounds (Bertoli et al., 2010).

The composition, concentration, and proportions of chemical constituents determine the biological effects of EOs. EOs containing higher concentrations of tepernoids exhibit higher antifungal activity compared with EOs rich in monoterpenes and sesquiterpenes (Kordali, 2005). EOs rich in thymol and carvacrol (phenolics) have higher membrane damaging activities in bacteria than those less rich in phenolics.

While the antimicrobial properties of EOs are mediated via several mechanisms, they largely disrupt cell membranes leading to necrosis of prokaryotic and eukaryotic microbes. The bacterial cell wall and cell membrane are important barriers that regulate the movement of substances between the cell and its environment. The lipophilic moieties present in most EOs freely pass through the cell wall and accumulate in the cytoplasmic membrane leading to marked changes in membrane stability, hydrophobicity, fluidity, and fatty acid composition (Sikkema et al., 1995). Thus, EOs disrupt the permeability barrier in cells and induce various morphological and physiological changes. In bacteria, the disintegration of the plasma membrane results in the loss of RNA, proteins, and ions, and disrupts the delicate ionic gradient that is crucial for cellular survival (Ballal et al., 2007). Potassium, present in high concentrations in prokaryotic and eukaryotic cells, is a critical cation in bacteria (Gram-negative and Gram-positive) that is obligatory for maintaining osmotic balance, turgor pressure (Epstein, 1986), pH (Booth, 1985), glucose metabolism, oxygen consumption, and activation of cellular enzymes (Suelter, 1970). Loss of potassium, mediated via the disruption of cell membranes by EOs, ultimately leads to cell death. EOs also destabilize cell membrane architecture, disrupting proton pump function, which then causes uncontrolled flux of H^+ ions resulting in the disruption of the intracellular pH and inhibition of H^+ ion-dependent movement of solutes across the membrane.

Besides regulating movement of substances across the cell, the bacterial cell membrane is also involved in energy transduction. Adenosine triphosphate (ATP) production and hydrolysis are dependent on the membrane potential and the proton motive force and these are reduced by lipophilic constituents of EOs. Bacterial cells treated with EOs have been shown to dramatically lose ATP, are forced into a bioenergetic compromise, and ultimately lose viability (Bakkali et al., 2008). The decrease in ATP in damaged cells is not necessarily due to leakage to the outside environment but due a disruption in the synthesis and hydrolysis of ATP on the damaged membranes (Bakkali et al., 2008).

Structural and compositional changes of the lipid bilayer following exposure to EOs results in disruption of the structure and function of ATPases, transferases, oxidoreductases, channel proteins, and other proteins involved in respiration and metabolism. EOs also coagulate cellular contents and inhibit enzymes important for metabolism in bacteria, for example, the inhibition of amino acid decarboxylase by EOs rich in cinnamaldehyde. The efficacy of the EOs in preventing the growth of microorganisms is dependent on the stage of growth of the target cell with dividing cells being more susceptible to destruction by EOs due to their increased permeability.

Preservation against Lipid Oxidation

The presence of saturated fats in meat makes it liable to oxidative degradation when exposed to air resulting in the development of off-flavors. Oxidative deterioration in meat is aggravated by the presence of prooxidants such as free iron but is reduced by pretreating meat with oregano and sage EOs before storage (Fasseas et al., 2008). The presence of phenolic compounds in EOs imparts in them antioxidant properties that makes EOs useful in preserving meat products against spoilage from lipid peroxidation.

However, some EOs are believed to exhibit prooxidant cytotoxic properties in eukaryotic cells by damaging mitochondrial membranes. EO phenols, like other antioxidants (retinol, tocopherol), switch from an antioxidant to a prooxidant state if used in high concentrations. At high concentrations, EOs damage the mitochondrial membrane leading to the release of superoxide ions and other potent reactive species that damage DNA and also oxidize the EO phenolic compounds to phenoxyl radicals that will further damage protein and DNA.

CURRENT CHALLENGES: EOs IN MEAT PRESERVATION

Despite the demonstrated potential of using EOs in meat preservation, there are a number of challenges that need to be overcome before the wholesale use of EOs as meat preservatives. Although *in vitro* studies showed the potential of EOs and their

TABLE 1 A Summary of Some of the Advantages and Disadvantages of Using Essential Oils as Meat Preservatives

Advantages	Disadvantages
Some EOs may potentially reduce oxidative spoilage of meat better compared with commonly used synthetic antioxidants	Possible interaction of EO phytochemicals with the meat constituents may reduce the efficacy of the EOs in preventing meat spoilage
Improvement of organoleptic characteristics of meat and meat products	Strong aromas and flavors associated with some of the EOs may cause negative organoleptic changes that will affect the consumer acceptability of the meat
Antimicrobial, antioxidant, and potential anticancer properties which confer health benefits to consumers	EOs may eliminate a certain target bacterial population but in turn produce favorable microenvironments that promote growth and virulence of undesirable microorganisms

phytochemical constituents in meat preservation, it is difficult to extrapolate results from *in vitro* tests to whole meat. Due to the possible interaction of EOs with meat constituents, generally a greater concentration of EOs is required to achieve the same effect in meat compared with *in vitro* assays (Burt, 2004). Use of high concentrations of the EOs to achieve significant antimicrobial activity causes changes in quality, taste, and aroma of the meat (Gutierrez et al., 2009; Sultanbawa, 2011). The strong aroma and flavors associated with some EOs are such that even if low concentrations of the EOs were used to preserve meat, there would be negative organoleptic changes that would affect the acceptability of the preserved meat. Although hypoallergenicty is one of the advantages of using EOs, it is notable that there are several case reports of contact allergies to EOs (Bleasel et al., 2002) and with widespread use there could be an even greater incidence of reports of allergies. Despite several EOs having been approved as food flavourings, there have been reports indicating skin irritation and toxicity in some people who use them frequently. More safety studies need to be conducted before widespread use of EOs in meat preservation.

Incorporation of EOs as antimicrobial agents in meat may eliminate a certain target bacterial population but in turn produce favorable conditions that promote growth and virulence of other undesirable microorganisms due to changes in microbial ecology (Davidson and Branen, 2005). As individual components, the constituents of EOs are not potent enough to inactive meat spoilage organisms. Gutierrez et al. (2009) demonstrated that a combination of *Origanum majorana* and *Thymus vulgaris* EOs had an additive effect against meat spoilage organisms than when either EO was used individually. It is critical to understand the effect of EOs and optimize their combinations for use in meat preservation in order to better exploit their synergistic effects against both spoilage and pathogenic organisms.

The application of EOs as meat preservatives depends on maintaining the stability and bioactivity of their constituent phytochemicals. The physical stability of most phytochemicals in EOs is decreased when they interact with the constituents of meat (Sultanbawa, 2011) possibly due to high binding capacity of proteins and fats in meat to volatile compounds in the EOs (Sultanbawa, 2011). One of the solutions suggested to increase stability of EOs is to encapsulate them rather than applying them directly onto meat and meat products.

FUTURE PROSPECTS OF USING EOs IN MEAT PRESERVATION

Although research has demonstrated the potential of EOs to preserve meat, for example, the better capacity of sage and rosemary EOs to reduce oxidative spoilage of meat compared with BHA and BHT, the commonly used synthetic antioxidants, the practical widespread use of EOs demands that a cautious approach be taken until the issues surrounding allergic reactions, application concentrations, and negative effects on meat acceptability have been thoroughly researched (Table 1).

SUMMARY POINTS

- Fresh meat is highly perishable.
- There is growing concern surrounding the use of synthetic chemicals to preserve meat.
- Research is exploring the potential of EOs as meat preservatives.
- EOs have antioxidant activity—they prevent lipid peroxidation in meat.
- EOs have antibacterial and antifungal activity.
- EOs have greater health benefits compared with synthetic preservatives.
- EOs add gustatory and aroma value.
- EOs are environmentally friendly.
- High doses of EOs are generally required for preserving meat.
- More research is needed to ascertain the use of EOs in meat preservation.

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Chapter 9

Essential Oil-Based Nanoemulsion Formation by Low- and High-Energy Methods and Their Application in Food Preservation against Food Spoilage Microorganisms

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INTRODUCTION

Preservation of food quality and ensuring consumer safety are two of the most significant concerns in the food industry. Food products, especially those that are perishable, can get easily contaminated by spoilage-causing microorganisms during their preparation, storage, and distribution and must be protected to avoid sensory deterioration and to extend shelf life (Rasooli, 2007). However, the most serious concern is that of illness-causing food-borne pathogens. Food preservation and pathogen control strategies including heating, refrigeration, and addition of chemical antimicrobial compounds are being employed to make foods safe for consumption. One such approach involves the use of natural food grade antimicrobial compounds such as essential oils (EOs). Plant EOs are hydrophobic natural aromatic substances obtained from different plant parts by hydrodistillation, steam distillation, expression, or solvent extraction techniques (Burt, 2004). EOs are highly volatile, hydrophobic liquids usually extracted from the bark, leaves, or stems of herbs and spices with excellent antioxidant and antimicrobial properties against a broad spectrum of bacteria and fungi (Weiss et al., 2009).

Food antimicrobials can be synthetically derived or occur naturally in plant and microbial sources. In the food industry, EOs are used as natural antimicrobial compounds and are the best replacement for synthetic chemical preservatives. Due to their bioactive compounds, they tend to be biodegradable and biocompatible in nature. The antimicrobial activities of different EOs and their components have already been reported against different species of microorganisms (Di Pasqua et al., 2006); nevertheless, direct incorporation of EOs in food produces organoleptic properties. There are also technological limitations, such as hydrophobicity, reactivity, and volatility of the bioactive molecules constituting the EOs. This problem may be overcome by formulating EOs into nanoemulsions that are transparent and can be used in food and beverage products, thereby decreasing the amount of the EOs required (Qian et al., 2012). Emulsions with extremely small droplets (20–200 nm) are called nanoemulsions. The method of preparation determines the stability of the formulated nanoemulsion. Formulation of nanoemulsion can be done by two methods: low- and high-energy emulsification. In the low-energy emulsification method, the chemical properties of the emulsion system are exploited thereby converting a microemulsion to a nanoemulsion. High-pressure homogenization and ultrasonication are the two methods employed in high-energy emulsification. The method that is most widely used is ultrasonication because it is economical and easy to use.

Encapsulation of EOs with the help of a nanoscale food-grade emulsifier represents a feasible and efficient approach in increasing the physical and chemical stability of the bioactive compounds, protecting them from interaction with the food components and, because of the nano sized droplets, increasing their bioactivity through the activation of passive mechanisms of cell absorption (Weiss et al., 2009). There has been growing interest in utilizing nanoemulsions to encapsulate bioactive components for applications in food and beverage products (McClements, 2011).

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METHODS OF NANOEMULSION FORMATION

When two immiscible liquids are dispersed as droplets in a continuous phase the resultant mixture is referred to as an emulsion. The process by which an emulsion is formed is called emulsification. A number of different approaches for forming colloidal dispersion use low-energy, high-energy, and combined emulsification methods (Figure 1). The high-energy emulsification methods induced by the action of devices such as high shear stirring, ultrasonic emulsification, high-pressure homogenization, and membrane emulsification. The most widely used low-energy methods include the phase inversion temperature (PIT) method, the emulsion inversion point (EIP) method, and the spontaneous emulsification method.

Low-Energy Emulsification Methods

The formation of a nanoemulsion using low-energy emulsification relies on the spontaneous formation of oil droplets when either their composition or their environment is altered (Shinoda and Saito, 1968). A number of methods for preparing nanoemulsion are based on this principle, including spontaneous emulsification, PIT, and phase inversion composition (PIC) or EIP.

Low-energy emulsification is highly preferred than high-energy methods due to their soft nature and nondestructive phases, but they are often more limited in the types of oils and emulsifiers that can be used. It requires high concentrations of surfactants to form nanoemulsions by these approaches, which may limit their use for many food-related applications.

Spontaneous Emulsification

Spontaneous emulsification is a cost-effective and energy efficient alternative method that takes advantage of the chemical energy stored in the system (Bilbao-Sáinz et al., 2010). Based on these systems are prepared in pharmaceutical industries are referred to as either self-emulsifying drug-delivery systems or self-nanoemulsifying drug delivery systems. There are three principal mechanisms of this process:

1. the Marangoni effect (interphase instability) induced by the surface tension in dispersion results in individual droplets,
2. when the interfacial tension reaches almost zero, dispersion occurs accompanied by a spontaneous increase in the surface area of the interface,
3. when two phases mix together a new phase condenses in the supersaturation areas.

In particular, this mechanism occurs when two phases (an organic phase and an aqueous phase) are mixed together spontaneously. For example, an organic phase consisting of nonpolar oil and a hydrophilic surfactant and/or water-soluble organic solvent may be slowly added to water. Alternatively, water may be added to an organic phase containing nonpolar oil, water-miscible organic solvent, and surfactant.

A number of physicochemical mechanisms occur when two phases mixed together. For example, when two phases are brought together upon mixing, as the component moves from dispersed phase to the continuous phase, it will cause an

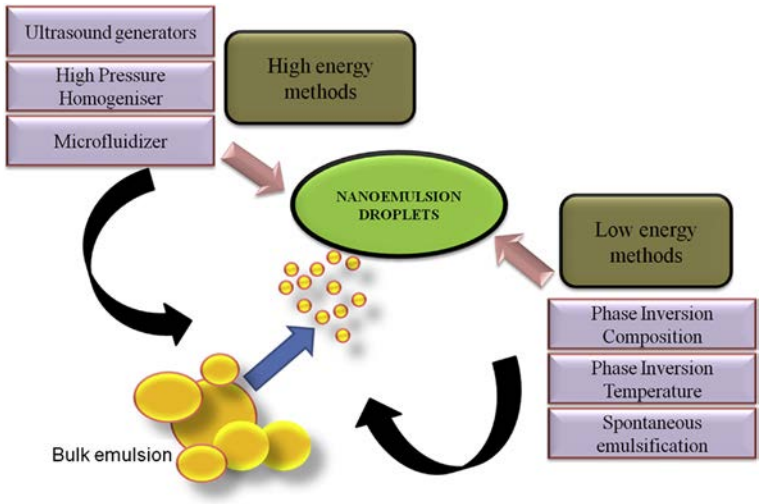

FIGURE 1 Preparation of nanoemulsion by various methods such low- and high-energy emulsification methods.

increase in the oil–water interfacial area, large turbulent force, and spontaneous formation of droplets. This type of application is widely used in the food and beverage industries when a product becomes cloudy when water is added to the flavored systems, such as anise flavored spirits, for example, ouzo from Greece (Horn and Rieger, 2001). This helps in the formation of small droplets when water is added to ethanol containing anise oil which scatters light. The main disadvantage of this approach is a requirement of a high surfactant concentration which is undesirable in food-related applications due to regularity, organoleptic properties, and cost of the surfactant.

Phase Inversion Temperature (PIT)

Many applications prefer the use of PIT emulsification which is the most widely used low-energy methods. This is based on the changes in solubility of surfactants in the continuous phase with temperature. This method relies on the specific ability of surfactants (nonionic) to alter their affinity to water and oil as a function of temperature at a fixed composition (Shinoda and Saito, 1968). The surfactant is hydrophilic at low temperatures but becomes lipophilic with increasing temperature. This is due to dehydration of the polyoxyethylene chains present in the nonionic surfactants. This method renders a change from an oil in water (O/W) to a water in oil (W/O) type emulsion. The exact mechanism underlying this principle is that at low temperatures the surfactant monolayer becomes positive, which may coexist with an excess dispersed (oil) phase (O/W). While, at high temperature it becomes negative, coexisting with an excess aqueous phase (W/O). At an optimum curvature, the hydrophilic–lipophilic balance (HLB) temperature, the spontaneous curvature, is zero and a bicontinuous phase containing equivalent amounts of water and oil phases coexists with both the phases. This method has the advantage of extremely low interfacial tensions at the HLB temperature to promote emulsion formation. The main drawback of this system is the rapid formation of coalescence and instability of emulsions even though emulsification is spontaneous at the HLB temperature. By rapidly cooling or heating the emulsion system prepared at the particular HLB temperature, kinetically stable emulsions (O/W or W/O) can be produced with a fine droplet size and narrow size distribution or by diluting with water or oil. The nanoemulsions immediately formed are considered to be kinetically stable and the process is irreversible.

Phase Inversion Composition (PIC)

PIC is a method similar to PIT but the optimum curvature of the surfactant is changed by altering the composition of the system, rather than the temperature (the solvent quality is changed by mixing two partially miscible phases together). For example, phase inversion can be achieved by adding a salt which is stabilized by an ionic surfactant in an O/W system and can be made to phase invert to a W/O emulsion. Alternatively, a W/O emulsion containing a high salt concentration can be converted into an O/W emulsion by diluting it in water so as to reduce the ionic strength below a critical level.

Emulsion Inversion Point (EIP)

In this method, the emulsion phase inversion occurs from one type of emulsion system to another (O/W to W/O or vice versa) and is through a catastrophic phase inversion (CPI) rather than a transitional phase inversion, as with the PIC or PIT methods. This is formed by increasing the volume of the dispersed phase which has high affinity toward surfactant. Submicron droplets may only form if a CPI of unstable to stable emulsion occurs in the vicinity of reduced interfacial tension (Jahanzad et al., 2010). Also, the addition of a second surfactant to the formulation could provide more stable nanoemulsions of minimum size than a single surfactant. The size of the droplets formed depends on the process variables, such as stirring speed and the rate of water addition. It has been shown that the emulsion inversion point (CPI) method can be used to produce nanoemulsions ($r < 100$ nm) from food-grade ingredients (Bilbao-Sáinz et al., 2010).

High-Energy Methods

High-energy methods involve the use of intense disruptive forces to break up larger droplets into smaller droplets and typically involve the use of mechanical energy known as microfluidization, high-pressure homogenization, or ultrasonication. These devices are capable of producing the tiny droplets required to form nanoemulsions. The smallest size of the droplets that can be produced using a high-energy approach depends on the homogenizer type, operating conditions (e.g., energy input, time, and temperature), sample composition (e.g., oil type, emulsifier type, relative concentrations), and the physicochemical properties of the constituent phases (e.g., interfacial tension and viscosity) (Wooster et al., 2008). High-energy methods are effective in reducing larger droplet sizes into fine ones, but may not be suitable for some unstable molecules, such as proteins or peptides. These methods are highly applicable for producing food-grade nanoemulsions.

High-Pressure Homogenizers

Initially, high-pressure homogenizers were widely used for nanoemulsion preparation in the food industry. A coarse emulsion was prepared and then this mixture subjected to very high pressures and pumped through a restrictive valve. This is very effective in reducing droplet size from preexisting coarse emulsions to submicron emulsions (Quintanilla-Carvajal et al., 2010). The exact mechanism involves a pump that pulls the coarse emulsion into a chamber on its backstroke and then forces it through a narrow orifice at the end of the chamber on its forward stroke. Due to the intense disruptive forces (turbulence, shear, and cavitations) produced during collision, the coarse emulsion breaks down from larger droplets into smaller ones. Minimal droplet size is based on the homogenization pressure or number of passes increases; this leads to a decrease in interfacial tension and the emulsifier adsorption on the droplet surface increases to prevent recoalescence (Jafari et al., 2008). Also, when the two phase (disperse-to-continuous) viscosity ratio falls within a certain range ($0.05 < \eta D/\eta C < 5$) (Walstra, 1993). A number of studies have used high-pressure homogenization to produce nanoemulsions that could potentially be used in the food industry.

Microfluidizer

In microfluidizer, emulsification occurs through collision of the flow streams of two immiscible liquids moving in microchannels through a narrow orifice. This is similar in design to a high-pressure homogenizer but works on the principle of dividing an emulsion flowing through a channel into two streams, passing each stream through a separate fine channel, and then directing the two streams at each other in an interaction chamber. Therefore, intense disruptive forces generated within this chamber when two fast-moving streams of liquid phases impinge upon each other, leading to formation of highly reduced size of droplets. These methods of emulsion preparation have traditionally been used in the pharmaceutical and beverage industries. Studies have shown that small droplets can be produced provided that conditions are optimized to facilitate droplet disruption and inhibit droplet coalescence. The droplet size reduction is similar to high-pressure homogenizers, where conditions include increasing homogenization pressure, number of passes, emulsifier concentration and, moreover, when the disperse-to-continuous phase viscosity ratio is within a limited range (Wooster et al., 2008). A number of studies have examined the major factors that determine the size of the droplets produced by this mechanism in the formation of food-grade nanoemulsions (Leong et al., 2009).

Ultrasonic Generators

Nanoemulsion droplets are formed under sonication due to cavitation bubble collapse and release of a substantial amount of energy. Nanoemulsion preparation by sonication is shown in Figure 2. Use of ultrasonication formulation of food-grade nanoemulsion against food-borne bacterial pathogens has been studied (Sugumar et al., 2013a; Ghosh et al., 2013c). Sonication utilizes high intensity sonic waves and produces intense disruptive force to break up oil and water phases into fine droplets (Leong et al., 2009). The energy input is provided by the probes that contain piezoelectric crystals that expand

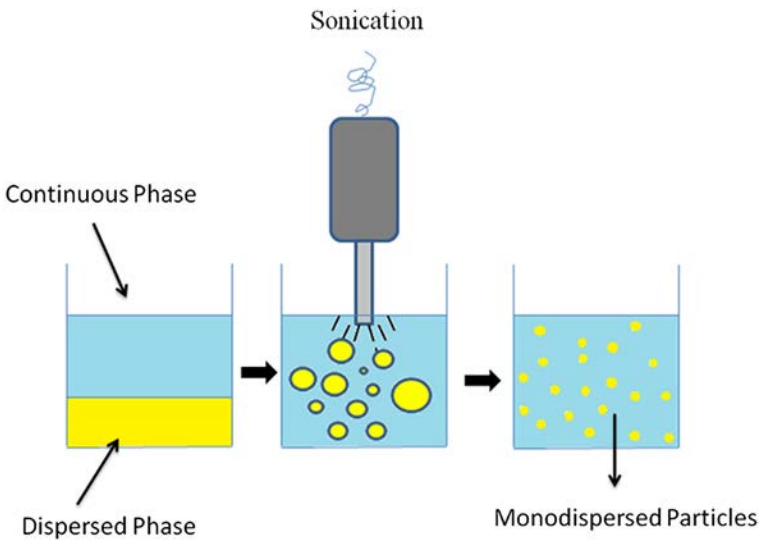

FIGURE 2 General preparation of nanoemulsion by sonication technique.

and contract in response to an alternating electrical voltage. The tip of the sonicator probe is made of titanium alloy placed within the liquids being homogenized, where it generates intense mechanical vibrations that lead to cavitation, turbulence, and interfacial waves. To achieve these remarkably small sizes it is necessary to optimize the design of the ultrasonic chamber, operating conditions, and composition (e.g., surfactant concentration and type, and oil type).

All of these parameters must therefore be optimized to produce nanoemulsions containing fine droplets (Sugumar et al., 2013b). Benchtop sonicators are widely used in research laboratories to produce small batches of nanoemulsions. Also, large-scale continuous flow homogenizers based on sonication are also available for commercial production of nanoemulsions in industries. In these devices, the sample to be homogenized is made to flow through a channel containing an element capable of generating intense ultrasonic waves.

ANTIMICROBIAL ACTIVITY OF NANOEMULSION USING ESSENTIAL OILS IN FOOD PRESERVATION

EOs are natural concentrated aromatic and volatile liquids extracted from different parts of plant material, such as roots, flowers, leaves, bark, wood, seeds, fruits, peel, and whole plant. EOs have been used traditionally in medicine, perfumery, and cosmetics and have been added to foods as a part of spices or herbs. Almost 3000 different varieties of EO are known, and 300 are used commercially in the flavor and fragrances industries (Burt, 2004). These are considered to be secondary metabolites, and they often possess biological and antimicrobial properties (Tajkarimi et al., 2010). It was proposed that the antibacterial action is mainly due to membrane disruption by the lipophilic compounds. However, after being formulated into an emulsion, the antimicrobial activity of EOs is affected by the composition of the whole system.

Nanoemulsion is colloidal dispersion of oil in water system which can be prepared either by low- or high-energy methods. Nanoemulsions are optically transparent or translucent, and they are kinetically stable. In preparation methods, nanoemulsion requires a smaller amount of surfactant, or sometimes no surfactant, and it can also be produced which differs from conventional counterparts. Emulsions can be divided into three types based on the size of liquid droplets: (1) macroemulsion; (2) microemulsion; and (3) nanoemulsion. The visual appearance of these emulsions is shown in Figure 3 and general properties of emulsions are given in Table 1.

FIGURE 3 Visual appearance of emulsion: (A) emulsion, (B) microemulsion, and (C) nanoemulsion.

TABLE 1 Properties of Different Emulsion Types (McClements, 2011)

Emulsion Type	Optical Properties	Preparation Methods	Surfactant Load	Diameter	Stability
Macroemulsion	Milky white	Homogenization	Moderately low to medium	0.1–100 μm	Kinetically stable
Microemulsion	Transparent	Low-energy emulsification	Moderately high (>10–20%)	5–50 nm	Thermodynamically stable
Nanoemulsion	Transparent to translucent	High-energy emulsification (pressure/sonication)	Low to medium (<10%)	10–100 nm	Kinetically stable

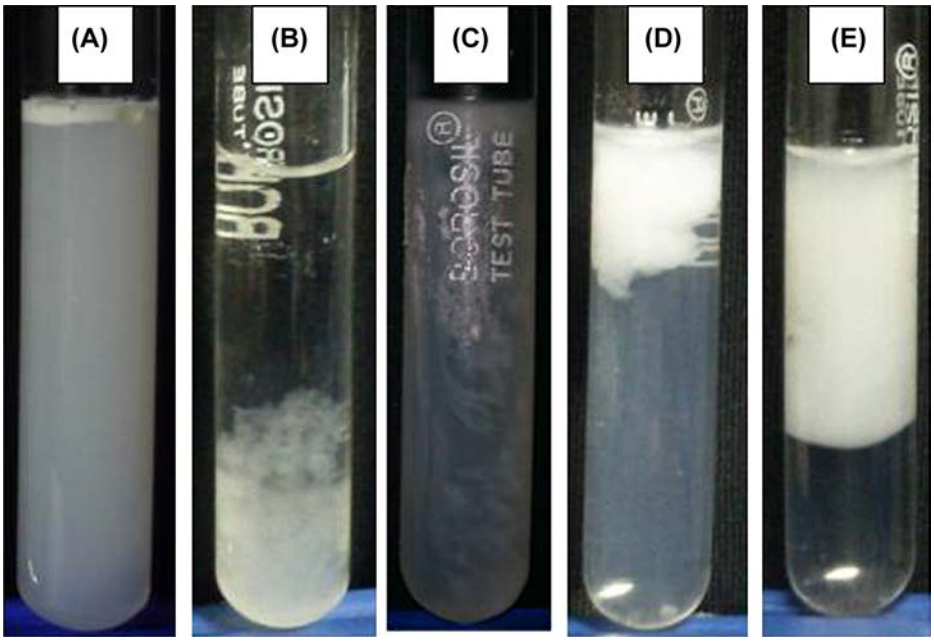

FIGURE 4 Emulsion instability mechanism, (A) creaming, (B) sedimentation, (C) flocculation, (D) coalescence, and (E) phase separation.

Nanoemulsions are more stable to phenomena such as gravitational separation, flocculation, and coalescence, thus providing longer physical stability to the emulsion system. This is due to the size of nanodroplets, which is of relevance to food applications for extended shelf-life during storage. Also, due to a lower surfactant concentration and dilutable emulsion with reduced droplet diameter, this is widely applicable in the food and beverage industries. Emulsion systems will undergo decay when they become unstable, as shown in Figure 4. Droplets larger than 1 μm in the emulsion system may preferentially settle to the top (creaming) or bottom (sedimentation) due to gravitational force. Phase separation occurs when the density difference is more between the two phases. Flocculation occurs when droplets become clumped together when it is unstable. This may lead to film rupture between two droplets and leading to particle growth (coalescence).

Preliminarily, emulsion particle size can be analyzed using either static or dynamic light scattering analysis and morphology can be studied through electron microscopic technique. Figure 5 shows a eucalyptus oil nanoemulsion transmission electron micrograph, which interprets the emulsion droplets as spherical. Physical stability can be studied with different environmental stress conditions (pH, salt, and different temperature) and chemical stability of the emulsion system (bioactive compound) can be studied using high-performance liquid chromatography or spectroscopic analysis upon accelerated aging conditions.

Use of nanoemulsions as antimicrobial agents has been reported by many researchers due to its size-based activity. The antimicrobial activity of a nanoemulsion can be evaluated by time–kill assay using plate count methods, growth kinetics by broth dilution, mechanistic study by membrane integrity, and morphological changes by microscopic analysis such as electron microscopy and fluorescence microscopy. The exact mechanism attributed to antibacterial action is the cell membrane penetration by active compounds present in the nano sized droplets (Weiss et al., 2009), releasing the contents

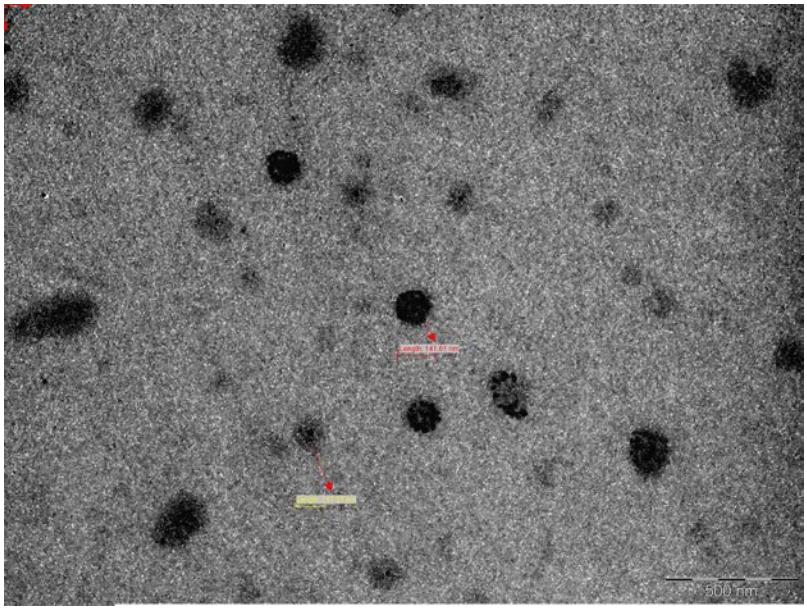

FIGURE 5 Transmission electron microscopy image of eucalyptus oil nanoemulsion.

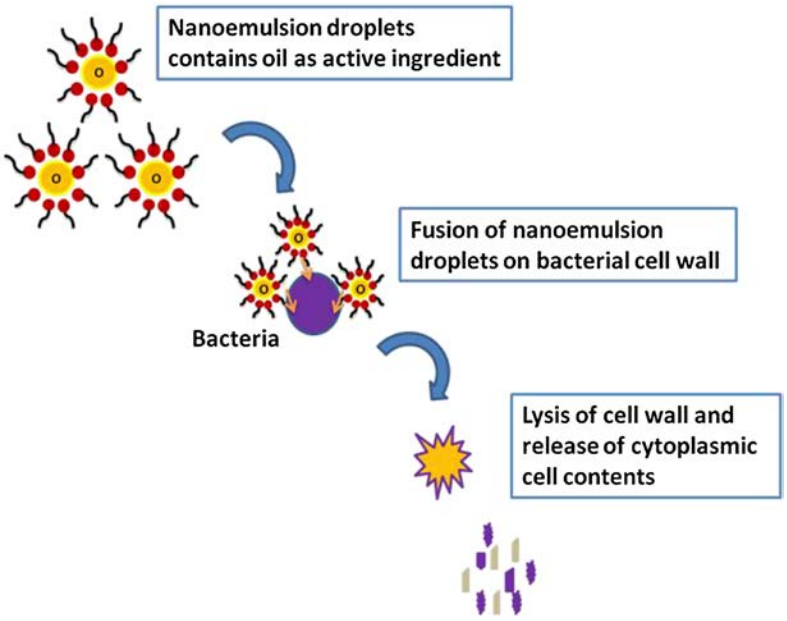

FIGURE 6 Possible antibacterial mechanism of nanoemulsion droplets against bacteria.

of the cell cytoplasm as illustrated in Figure 6. Peppermint oil nanoemulsion was studied against the food-borne bacterial pathogens *Listeria monocytogenes* and *Staphylococcus aureus* (Liang et al., 2012). Similarly, EO-based nanoemulsions (thyme oil, orange oil) have been reported against food-borne bacteria and yeast (Ziani et al., 2011; Sugumar et al., 2015). Cinnamon oil nanoemulsion was studied against *Bacillus cereus*, a spore forming bacteria. Its antibacterial activity is mainly due to the presence of eugenol in the emulsion system, as confirmed by gas chromatography–mass spectrometry analysis (Ghosh et al., 2013a). The same group also reported eugenol-loaded antimicrobial nanoemulsion against bacteria in real food system (orange juice) (Ghosh et al., 2013b). EOs that possess antimicrobial activity due to the presence of secondary metabolites present making a safer alternative to synthetic and semisynthetic compounds. Nanoemulsion formation using EOs ensures the physical and chemical stability of the food in which it is applied. From this study, it can be concluded that nanoemulsion of EOs can be used as a commercially feasible antimicrobial agent that can be used for the preservation of food and beverages.

SUMMARY POINTS

- Nanoemulsion is beginning to have a noticeable impact on food technologies which are good tools for producing high-bioavailability products.
- Nanoemulsion can be prepared by low- or high-energy emulsification method.
- Nanoemulsions are optically transparent systems and they are kinetically stable.
- Due to organoleptic properties usage of oil components is major problem in food industries. To overcome this, nanoemulsion can be used as an alternative method in preservation of foods.
- Compared with microemulsion, nanoemulsion requires less surfactant and a dilutable formulation for application studies, especially in the food and beverage industries.
- Nanoemulsion provides a greener approach due to plant/herb-based oil systems and more biocompatibility with the environment.
- Cost-effective, eco-friendly, and uses nontoxic formulations.
- More effective antimicrobial activity with low oil concentration due to nanoscale droplet diameter.
- Ease of preparation using either low- or high-energy methodologies.
- Easier penetration of lipid membrane of bacterial cells due to nanodroplets in the formulations that provide fast killing of bacteria within a minute of interaction.

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Chapter 10

Use of Essential Oils in Poultry Production

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INTRODUCTION

The global demand for animal protein will continue to grow as the world population and the average income increase. So far, the poultry industry has been successful and coped well with this increase in demand by continuous improvements in genetics, nutrition, and health. However, genetic selection for fast growing strains has altered the immune system of modern poultry and increased the challenges the nutritionists face to balance fast growth with maintaining optimum gut health in poultry. In addition, concerns over bacterial resistance to antibiotics administered to food-animals, and general consumer demand for more natural and safe meat caused the ban on the use of antibiotics as growth promoters (AGPs) in European Union countries; more countries are expected to follow. This has encouraged animal nutritionists to explore more natural feed additives and alternative feed management strategies to improve the intestinal health and the digestive efficiency of poultry. Consequently, the interest in plant extracts and essential oils (EOs) as a natural replacement of AGPs has increased rapidly in recent years. The composition of EOs is variable and may include several mixtures of compounds that have been extracted from different plants or parts of plants. EOs have several properties to support their use in poultry feed, namely feed intake stimulants, increased digestive enzyme secretion, improved immune response to diseases, antioxidant, antiseptic, and insect repellent. This chapter will discuss how these properties of EOs can influence poultry production.

ESSENTIAL OILS DEFINITION AND MECHANISMS

EO are aromatic, oily extracts obtained from different parts of a plant, for example, flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, or roots. The major plant families from which the term "essential oil" is thought to have been derived are from *Quinta essential*; the name coined in the sixteenth century by Paracelsus von Hohenheim for the effective component of a drug (Burt, 2004).

EOs are formed in plants from isopentenyl pyrophosphate units which are polymerized yielding a large variety of components. EOs can be divided into four groups according to their chemical structure: terpenes, terpenoids, phenylpropenes, and "others". Their function in plants has been hypothesized to be antibacterial, antifungal, insecticidal, and antiviral defense. Furthermore, their strong flavor makes plants less palatable for herbivores (Langeveld et al., 2014).

Steam distillation is the most common method of EO production. Supercritical extraction with carbon dioxide has been exploited and yields a cleaner product with a more natural aroma, but it is more expensive. Solvents, such as hexane, can also be used for the extraction of EOs (Burt, 2004).

Extraction of EOs from natural sources has a potential disadvantage in that the purity and relative composition of the product may vary depending on the variety of the plant used, season-to-season variation, and geographical differences (Dadalioglu and Evrendilek, 2004). Furthermore, the extraction technique used also influences the composition of the extract obtained. EOs can also be produced synthetically, which provides a product with higher purity at lower cost. However, the complex composition of EOs in natural extracts is responsible for the characteristic aroma and may also have benefits for the antimicrobial activity of the extract. Combinations of EO may exhibit synergistic antimicrobial effects (Bassole and Juliani, 2012).

Antimicrobial Mechanisms

EOs are chemically diverse, therefore, there are likely to be various mechanisms by which they exert their antimicrobial activity. However, EOs are generally hydrophobic and, therefore, are likely to enter into cell membranes of microbes and membranes of cell organelles in eukaryotes (Burt, 2004). The hydrophobic character of EOs may also explain why they tend to be less effective against Gram-negative organisms as the outer membrane, which is made up of lipopolysaccharide,

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also provides a hydrophilic, negatively charged barrier. EOs with a positive charge may therefore be more effective against Gram-negative organisms (Trombetta et al., 2005). The cytoplasmic membrane appears to be the main target site for EOs, causing disruption or permeabilization of the membrane. This in turn leads to loss of pH gradient and subsequent adenosine triphosphate production. It may also lead to leakage and denaturation of the cytoplasmic content and ultimately cell lysis. Metabolic activities may also be affected. The presence of the hydrophobic components in the membrane may also lead to denaturation of membrane proteins and enzymes. The effects that EOs exert on the cell are indicated by an upregulation of general stress responders such as GroEL and DnaK (Hyldgaard et al., 2012). Different EOs are likely to have different molecular targets. This may explain why combinations of EO may be more effective than a single EO component (Bassole and Juliani, 2012) or in combination with other antimicrobial components (Langeveld et al., 2014).

Antioxidant Properties

The common chemical structure of EOs contains double bonds, conjugated double bonds, and phenolic structures. These structural characteristics allow for double bond rearrangements to function as antioxidants (Miguel, 2010). Thymol, carvacrol, and eugenol appear to be the most powerful antioxidants. In addition to the direct potential health benefits of the antioxidant activity of EO, there is also the preservative aspect of protecting foods from lipid oxidation (see section below on carcass quality). The antioxidant activity of EO also explains their reported antiinflammatory properties, by suppressing the arachidonic acid metabolism (Miguel, 2010). The antiinflammatory properties of EO may have a positive effect on feed efficiency as inflammation requires substantial energy from the host.

EFFECT OF ESSENTIAL OILS ON POULTRY PERFORMANCE

The number of studies examining the use of EOs in poultry production has increased rapidly in response to market demand to use more natural products in the food chain. However, most of these studies have focused on broiler chickens with only few studies on other poultry species. Therefore, the focus of this section will be on the effects of EOs on broiler chickens with reference to other poultry species where appropriate.

The performance response to EO supplementation and the composition of the EO used in broiler diets from some publications is presented in Table 1. The results of these trials have been inconsistent. While some studies have reported beneficial effects of EOs on weight gain (Ertas et al., 2005; Tiihonen et al., 2010; Amerah et al., 2011), others reported no effect (Botsoglou et al., 2002; Jang et al., 2007). For feed intake, it appears that EOs reduce feed intake in a majority of the animal trials which resulted in improvement in feed conversion ratio (Brenes and Roura, 2010). Interestingly, EOs are regulated in the European Union and United States as a sensory additive to improve feed intake or change the organoleptic properties of the feed. However, most research suggests a number of additional modes of action. Jamroz et al. (2005) reported no effect on body weight, but feed conversion improved by 4.2% on a maize-based diet and 2.0% on a wheat and barley diet when the diet was supplemented with plant extract consisting of capsaicin, cinnamaldehyde, and carvacrol. Similarly, Cabuk et al. (2006) reported a significant reduction in feed intake and improvement in feed conversion ratio (FCR) with no effect on weight gain in broilers fed EO derived from selected herbs (oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil, and citrus peel oil).

In the limited studies comparing AGP and EO in broiler diets, the performance response was also variable. Hernández et al. (2004) reported that an EO blend, containing oregano, cinnamon, and pepper and labiatae extract from sage, thyme, and rosemary extracts, fed to broilers gave performance levels similar to those fed avilamycin. In contrast, others reported better performance of EOs compared with AGPs in broiler chickens (Ertas et al., 2005; Mountzouris et al., 2011).

The variable response to EOs between the different studies have been attributed to the type of EO used and the inclusion level (Ertas et al., 2005; Jamroz et al., 2005; Cross et al., 2007). For example, comparing different types of herbs (thyme, oregano, marjoram, rosemary, or yarrow), dietary thyme oil or yarrow herb inclusion had the most positive effects on broiler performance, while oregano herb and yarrow oil were the poorest (Cross et al., 2007). Other factors may include basal diet (Jamroz et al., 2005), feed form (Amerah et al., 2011), and health status of the animals. Furthermore, the purity and relative composition of the product may vary depending on the variety of the plant used, the harvesting season, and the geographical differences (Dadalioglu and Evrendilek, 2004). It should be noted, however, that some of the current commercial products use pure nature identical EO, therefore, they will not be influenced by these factors.

Dietary supplementation of EO to poultry feed has been reported in several studies to stimulate digestive enzymes secretion. Total and specific activities of pancreatic trypsin, chymotrypsin, lipase, α-amylase, and intestinal maltase were found to increase in birds fed EOs (Jang et al., 2007; Jamroz et al., 2005). This stimulation of digestive enzymes may explain the improvement in nutrient digestibility with EO supplementation (Table 2; Jamroz et al., 2005; Amad et al., 2011; Amerah et al., 2011).

TABLE 1 Effect of Essential Oils on the Performance of Broiler Chickens (% Difference from Control)

Additive Composition	Diet Type	Feed Form	Duration (Day)	Inclusion (Tonne of Feed)	Weight Gain	Feed Intake	Feed Conversion Ration	References
EO blend (oregano, clove and anise)	Maize	–	1–35	100 g	3.4	0.32	–3.7[a]	Ertas et al. (2005)
				200 g	16.3[a]	2.10	–12.4[a]	
				400 g	–0.21	3.10	3.10	
EO blend (capsaicin, cinnamaldehyde and carvacrol)	Maize	–	1–41	100 g	0	–	–4.2[a]	Jamroz et al. (2005)
	Wheat and barley			100 g	–3.2	–	–2.0	
EO blend (oregano, laurel leaf, sage leaf, myrtle leaf, fennel seed, citrus peel)	Maize	Mash	1–42	24 g	0	–3.8[a]	–5.6[a]	Cabuk et al. (2006)
				48 g	0	–5.0[a]	–3.7[a]	
Thymol	Maize	Mash	3–35	25 g	4.7	4.3	–1.2	Jang et al. (2007)
				50 g	2.7	4.3	1.2	
EO blend (clove, cinnamon ceylanensis and cinnamon camphora)	Mixed diet (wheat, barley, maize)	Pellet		100 g	–1.5	–	0.5	Isabel and Santos (2009)
EO blend (thymol and cinnamaldehyde)	Wheat	Mash	1–42	100 g	4.7[a]	3.9	–2.0	Tiihonen et al. (2010)
EO blend (thyme and star anise)	Maize and wheat	Mash	1–42	150 g	1.70	–2.07	–3.27[a]	Amad et al. (2011)
				750 g	2.31	–4.15	–5.88[a]	
				1500 g	3.05	–4.81	–7.19[a]	
EO blend (thymol and cinnamaldehyde)	Wheat	Pellet	1–35	100 g	3.1[a]	1.3	–1.3	Amerah et al. (2011)
EO from thyme	Wheat and barley	Mash	0–7	1000 g	–8.9[a]	0.6	9.3	Cross et al. (2011)
			8–21		–3.6	–4.6	–1.6	
			22–42		–3.12	–3.36	0	
EO blend (oregano, anise, and citrus)	Maize	Mash	1–42	80 g	–5.2	–6.3	0	Mountzouris et al. (2011)
				125 g	2.6	–4.4	–7.4[a]	
				250 g	1.6	–4.8	–7.4[a]	

Continued

TABLE 1 Effect of Essential Oils on the Performance of Broiler Chickens (% Difference from Control)—cont'd

Additive Composition	Diet Type	Feed Form	Duration (Day)	Inclusion (Tonne of Feed)	Weight Gain	Feed Intake	Feed Conversion Ration	References
Clove oil (85% eugenol)	Maize	Pellet	1–42	100 g	3.5	0	−5.2	Agostini et al. (2012)
				200 g	3.5	−1.8	−4.2	
				1000 g	3.1	−0.9	−3.6	
				2500 g	1.8	0	−2.1	
EO blend from copaiba	Maize	–	1–40	150 mL	−0.73	1.18	1.89	Aguilar et al. (2013)
				300 mL	−2.32	0.29	2.71	
				450 mL	−2.18	0.19	2.42	
				600 mL	−3.09[a]	−0.06	3.12	

[a]*The response is significantly different from the control (P<0.05).*

TABLE 2 Effect of Essential Oils on Nutrient Digestibility With EO Supplementation in Broiler Chickens (% Difference From Control)

Additive Composition	Diet Type	Day	Inclusion (Tonne of Feed)	Fat Digestibility	Protein Digestibility	Amino Acids Digestibility	Phosphorus Digestibility	References
EO blend (capsaicin, cinnamaldehyde, and carvacrol)	Wheat and barley	48	150 g	5.6	0.2	–	–	Jamroz et al. (2003)
		48	300 g	15[a]	2.66[a]	–	–	
EO blend (capsaicin, cinnamaldehyde, and carvacrol)	Maize	21	100 g	0	1.1	2.6[a]	–	Jamroz et al. (2005)
	Wheat and barley		100 g	−1.26	2.09	1.2[a]	–	
	Maize	41	100 g	1.81	2.84	0	–	
	Wheat and barley		100 g	2.36	−1.44	2.3	–	
EO blend (thyme and star anise)	Maize and wheat	21	150 g	2.6[a]	5.2[a]	–	7.0[a]	Amad et al. (2011)
			750 g	3.5[a]	5.4[a]	–	11.7[a]	
			1500 g	4.0[a]	6.7[a]	–	16.4[a]	
		42	150 g	1.1[a]	6.5[a]	–	6.5[a]	
			750 g	1.8[a]	7.6[a]	–	11.2[a]	
			1500 g	3.0[a]	9.0[a]	–	15.9[a]	
EO blend (thymol and cinnamaldehyde)	Wheat	35	100 g	–	1.5[a]	–	–	Amerah et al. (2011)
EO from thyme	Wheat and barley	21	1000 g	−10.8		–	–	Cross et al. (2011)
EO blend (oregano, anise, and citrus)	Maize	28	80 g	3.6	6.8	–	–	Mountzouris et al. (2011)
			125 g	12.7	5.5	–	–	
			250 g	−1.8	4.1	–	–	

[a]*The response is significantly different from the control ($P<0.05$).*

Amerah et al. (2011) attributed the improved weight gain in birds given EOs to the improvements in the ileal nitrogen digestibility observed with EO supplementation. The positive effect of EOs on amino acid digestibility, protein digestibility as well as dry matter digestibility has been observed in other studies (Jamroz et al., 2003, 2005; Hernández et al., 2004). Jamroz et al. (2005) reported higher apparent ileal digestibility of several amino acids both in maize or wheat-barley based diets supplemented with EO. Amad et al. (2011) showed a linear increase in the apparent ileal digestibility of crude ash, crude protein, crude fat, calcium, and phosphorus with the increase in the dose of a product consisting of a mixture of EO with thymol and anethole as lead active components, and herbs and spices.

Only limited studies have examined the effects of EOs on poultry species other than broiler chickens. Bolukbasi et al. (2010) reported that dietary supplementation of bergamot oil reduced feed intake and *n*-6 to *n*-3 ratio in egg yolk, increased egg shell strength and the *n*-3 fatty acids (eicosapentaenoic acid, docosahexaenoic acid). Under hot climate conditions EO mixture was found to improve egg production, FCR, and reduce cracked-broken egg rate compared with the control (Cabuk et al., 2006). Similar improvement on eggshell characteristics under moderate or hot climatic conditions was reported by Bozkurt et al. (2012) with no effect on performance or humoral immune response in laying hens fed EO mixture. In broiler breeders, supplementing diets with EO mixture improved fertility, the hatchability of total eggs set, total settable eggs, total chicks, and the chick weight (Bozkurt et al., 2009). Similarly, in adult quails, thymol and isoeugenol supplemented groups showed increased hatchability, by 18.8 and 11.8%, respectively, compared with their control counterparts (Luna et al., 2012).

EFFECT OF ESSENTIAL OILS ON GUT HEALTH IN POULTRY

The antimicrobial properties of EO are well documented in the literature. These properties can be selective against some pathogenic bacteria such as *Escherichia coli* and *Clostridium perfringens* and stimulate the growth of other beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (Tiihonen et al., 2010; Mountzouris et al., 2011). *Clostridium perfringens* is the main causative agent of necrotic enteritis (NE) in poultry. NE is a very costly disease, and costs the worldwide poultry industry USD 2 billion annually (Cooper and Songer, 2009). The subclinical form of this disease may cause a reduction in performance by 12% in body weight and increase FCR by 10.9% without noticeable mortality (Skinner et al., 2010). The ban of AGPs from animal diets in some countries has increased the incidence of this disease in these countries. Therefore, the interest in studies on the use of EOs to combat NE has increased. Si et al. (2009) examined the effect of seven EOs on the antimicrobial activity toward *C. perfringens* and lactobacilli obtained from the chicken intestine. These researchers reported that most of the studied EO (2-*tert*-butyl-6-methylphenol, carvacrol, chamomile roman oil, citral oil, citronellal, geraniol, and *trans*-cinnamaldehyde) showed inhibition of *C. perfringens*, but had little effect on the total number of lactobacilli and anaerobic bacteria in the digesta. Ouwehand et al. (2010) reported that under anaerobic conditions, several *C. perfringens* strains were found to be sensitive to carvacrol, cinnamaldehyde, citral, limonene, and thymol. Similarly, Timbermont et al. (2010) reported that the growth of *C. perfringens* strain 56 was inhibited in vitro by low concentrations of thymol and cinnamaldehyde. These results were confirmed in animal trials which showed that specific blends of EO can control *C. perfringens* colonization and proliferation in the gut and therefore may be of help to reduce incidences of NE (Mitsch et al., 2004).

Subclinical coccidiosis can also be an important contributing factor in the development of NE in broiler chickens. An in vitro study has shown that some EOs (artemisia, thyme, clove, or tea tree) are very efficient in destroying *Eimeria* oocyst at very low concentrations (Remmal et al., 2011). A positive effect of EOs on coccidiosis has been previously reported in vivo as well (Giannenas et al., 2003). Studies have shown that cinnamaldehyde supplementation decreased gross lesion scores on the intestinal mucosa, reduced *Eimeria acervulina* oocyst shedding between 5 and 9 d postinfection and increased *Eimeria tenella*-stimulated parasite antibody responses at 9 d postinfection compared with controls in broilers challenged with coccidia (Orengo et al., 2012). Giannenas et al. (2003) reported that birds infected with *E. acervulina* and fed diet supplemented with dietary oregano oil resulted in body weight gains and feed conversion ratios similar to the noninfected group.

EOs may reduce stress during critical situations and relieve the host animal from a strong immune defense, therefore helping animals to grow better (Windisch et al., 2008). A study with broilers demonstrated that EO can increase immunoglobulin A levels, leading to improved intestinal immunocompetence and performance of young chicks (Kettunen et al., 2006). It should be noted that the effects of EOs on the immune system were not consistent in all studies. This variation in response between animal trials may be related to the health status of the animals in each trial. However, using genomics technology on the intestinal tissues in chickens supports the immune-enhancing properties of EOs (Lillehoj et al., 2011).

EFFECTS OF ESSENTIAL OILS ON CARCASS QUALITY AND TASTE

Poultry meat has high contents of polyunsaturated fatty acids that can cause off-flavors, off-odors, and reduce meat shelf life. Consumer demand to reduce synthetic preservatives increased the interest in the antioxidant and antimicrobial properties of EOs (Botsoglou et al., 2002, 2003; Luna et al., 2010). Dietary supplementation of oregano EO has been found to exert antioxidant property in chicken or turkey meat during refrigerated or frozen storage (Botsoglou et al., 2002, 2003). Luna et al. (2010) found that thymol and carvacrol feed supplementation can delay lipid oxidation in thigh, but not in breast muscle, after its storage. It should be noted, however, that thigh meat is more susceptible to oxidation compared with breast meat (Luna et al., 2010). The antioxidant properties of EOs on meat suggest the retention of these EOs in the meat which may influence flavor. Jamroz et al. (2003) reported that dietary supplementation of EO mixture (capsaicin, carvacrol, and cinnamaldehyde) improved meat smell, tenderness, and juiciness compared with control treatment. Cross et al. (2011) compared cooked chicken meat from birds fed rosemary, thyme EO, yarrow, or garlic. They reported small differences in flavor intensity, but significant differences in abnormal and garlic flavors were due to the garlic supplementation. These data suggest no adverse effects of EO on meat flavor apart from garlic supplementation. The data also suggest the presence of EO residues in meat.

The effect of EO on the changes in carcass percentage, breast meat yield, and abdominal fat is summarized in Table 3. The effect of EOs on carcass dressing percentage is minimal. Interestingly, several studies have shown positive effects of EO on breast meat yield percentage and reduction in abdominal fat when birds fed diets supplemented with EO (Jamroz et al., 2003; Isabel and Santos, 2009). The exact mechanism of these effects on carcass quality is unknown, but may be related to the positive effects of EOs on dietary amino acids digestibility, suggesting more energy is utilized for protein deposition rather than fat deposition.

EFFECT OF ESSENTIAL OILS ON POULTRY RED MITE (*DERMANYSSUS GALLINAE*)

The poultry red mite (*Dermanyssus gallinae*) is a growing epidemiological problem for the poultry industry that causes losses in the performance of laying hens and consequently major economical losses. It has been suggested that adding EO to laying hen diets may be an efficient and natural alternative to synthetic repellents and acaricides for poultry red mite. Although some EOs are effective repellents, their effectiveness is usually short-lived which is likely related to their volatile nature. The exact mechanism of the acaricidal effect of EO is not yet clear, but it is thought that some EO could inhibit insect cytochrome P450 and thus reduce detoxification of xenobiotics. However, most insect repellent activity appears to come from neurotoxic effects, blocking γ-aminobutyric acid neurons and reducing the ability of cells in the nervous system to form cyclic adenosine 3′,5′-monophosphate (Regnault-Roger et al., 2012). Interestingly, most studies were conducted in vitro and there is lack of information on the effect of EOs on laying hens performance under the challenge of red mites.

REGULATORY PERSPECTIVE

Many EOs, oleoresins, and natural extractives, as well as synthetic flavoring substances and adjuvants used in animal feed are generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA) as a source of flavor or flavor enhancer (21 CFR §582.20–582.60), and are also listed in the Official Publication of the Association of American Feed Control Officials (AAFCO). However, the GRAS status of these EOs or synthetic flavors is contingent upon their use at the minimum concentration required to achieve the intended effect of imparting or enhancing flavor. Therefore, EOs and synthetic flavors cannot be applied in animal feed at just any concentration to achieve other objectives. Similarly, in the European Union, EOs are categorized under sensory additives which are defined as "*any substance, the addition of which to feed improves or changes the organoleptic properties of the feed, or the visual characteristics of the food derived from animals*" (EC No. 1831/2003). Within this category, EOs included under the functional group "flavoring compounds" which are defined as "*substances the inclusion of which in feedingstuffs increases feed smell or palatability*" (EC No. 1831/2003).

As such, depending on the claimed mode of action, many EOs can be sold both in the United States and the European Union as flavoring agents. However, any commercial claims other than feed intake improvement (resulting from imparting/enhancing flavor) require a new review of the safety and efficacy in that intended use, resulting in an AAFCO feed ingredient definition or FDA food additive approval in the United States or an application to European Food Safety Authority for European Union registration as zootechnical feed additive.

TABLE 3 Effect of Essential Oils on Carcass Characteristics with EO Supplementation in Broiler Chickens (% Difference from Control)

Additive Composition	Diet Type	Day	Inclusion (Tonne of Feed)	Dressing Percentage	Breast Meat Yield	Abdominal Fat	References
EO blend (capsaicin, cinnamaldehyde, and carvacrol)	Wheat and barley	48	150 g	–1.26	4.3	–13.3	Jamroz et al. (2003)
		48	300 g	–1.68	3.06	–41.6[a]	
EO blend (capsaicin, cinnamaldehyde, and carvacrol)	Maize	41	100 g	0.56	0.29	–8.64	Jamroz et al. (2005)
	Wheat and barley		100 g	0.27	3.32	–1.22	
EO blend (oregano, laurel leaf, sage leaf, myrtle leaf, fennel seed, citrus peel)	Maize	42	24 g	0.72	–	–	Cabuk et al. (2006)
			48 g	1.21	–		
EO blend (clove, cinnamon ceylanensis, and cinnamon camphora)	Mixed diet (wheat, barley, maize)		100 g	–	2.8[a]	–4.1	Isabel and Santos (2009)
EO blend from copaiba	Maize	40	150 mL	–0.73	–3.56	–	Aguilar et al. (2013)
			300 mL	–0.24	–1.58	–	
			450 mL	0.24	–3.56	–	
			600 mL	0.00	2.77	–	

[a]*The response is significantly different from the control* ($P < 0.05$).

SUMMARY POINTS

- The ban of antibiotics as growth promoters in animal feed in many countries as well as the consumer demand for natural alternatives increased the interest in the use of EOs in poultry feeds.
- Several trials have shown positive effects of EO on nutrient utilization and poultry performance, but with varying degree of response.
- The variable response to EOs in poultry diets has been attributed to the level and type of EO used, basal diet, and the health status of the animals.
- In vitro and in vivo studies showed that specific blends may have potential in controlling subclinical necrotic enteritis and coccidiosis.
- Antioxidant properties of dietary EOs were reported to transfer to poultry meat during storage.
- In vitro studies demonstrated the potential to use EOS in laying hen diets as a natural alternative to synthetic repellents and acaricides for poultry red mite.
- Systematic investigations on the relationship of EO properties (flavor, antimicrobial, antioxidant, etc.) with bird performance and gut function are warranted to optimize utility of EO and to effectively expand the scope of regulatory approvals.

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Chapter 11

Essential Oils as Flavors in Carbonated Cola and Citrus Soft Drinks

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List of Abbreviations

AEDA Aroma extract dilution analysis
GC-MS Gas chromatograph-mass spectroscope
GCO Gas chromatography-olfactometry
GMP Good manufacturing practice
GRAS Generally regarded as safe
HPLC High-performance liquid chromatograph
ISO International Organization for Standardization
LLCE/SAFE Liquid–liquid continuous extraction/solvent-assisted flavor evaporation
SDLC Soft drink licensing company
SPME Solid phase microextraction
TCCC The Coca-Cola Company
TNI Transnational Institute

INTRODUCTION

Essential Oils, Spices, and Aromatic Plants

Essential oils (EOs) are pure or partly purified mixtures of lipophilic substances, usually obtained as a liquid from a named plant part. EOs have taste and odor because they contain volatile aroma-active compounds (i.e., molecules that elicit a distinctive taste and smell). An EO may be called the "oil of" the plant from which it was obtained—usually by distillation, expression, or solvent extraction. An EO is termed "essential" in the sense that it has a distinctive/characteristic aroma, scent, or essence of the plant from which it came. The term *aromatic plant* describes a plant known for one or more EOs.

Most EOs contain hydrocarbons and oxygenated compounds, mainly as terpenes and terpenoids, with molecular weights no larger than 300 Da. EOs are well known for their uses in food and beverages and in medicine and cosmetics (Sellar, 2001; Baser and Buchbauer, 2010). The plant parts (usually dry) bearing EOs are often called spices; these have been widely used since the middle ages, in the days of the alchemists. Spices are generally regarded as safe flavorings in foods and beverages. However, EOs used in nutrition and medicine need to be handled professionally in accordance with strict good manufacturing practices.

The International Organization for Standardization (ISO) has established over 100 standards for EOs. Examples include ISO 3217:1974, which addresses oil of lemongrass; ISO/TR210:1999, on the general rules for packaging, conditioning, and storage of EOs; ISO 7359:1985, which discusses the analysis of EOs by gas chromatography on packed columns; ISO 4720:2009 on nomenclature; and ISO 25157:2013 on the EO of Chinese Kushui-type roses. These ISO standards are listed in the ISO catalog (2013) and are widely used to guide the production, quality control, marketing, and use of EOs worldwide.

Carbonated Soft Drinks

Carbonated soft drinks ("sodas") are cold, nonalcoholic beverages. This chapter focuses on cola and citrus sodas that contain EOs from the following aromatic plants: coca—*Erythroxylum coca* (Erythroxylaceae); cinnamon—*Cinnamomum*

[†] Deceased

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(Lauraceae); nutmeg—*Myristica fragrans* (Myristicaceae); coriander—*Coriandrum sativum* (Apiaceae); neroli—*Citrus aurantium* (Rutaceae); lemon—*Citrus limon* (Rutaceae); lime—*Citrus limetta* (Rutaceae); orange—*Citrus sinensis* (Rutaceae); and vanilla—*Vanilla planifolia* (Orchidaceae). A soda may contain the synthetic equivalent of any of the foregoing flavorings and may contain no more than 0.5% v/v of ethanol (Alcoholfree.co.uk., 2012). Other names by which sodas are called include "fizzy drink" and "mineral," among others (D'Amato et al., 2011; Vaux, 2011).

The first popular soda, Coca-Cola or Coke, produced by The Coca-Cola Company (TCCC), at a stage in its history contained oils from all the plants enumerated above (TCCC, 2013). The self-styled lemon-lime sodas contain lemon and lime oils as the main flavorings, with or without orange oil as a minor component. Orange sodas contain orange oil as the main flavoring, with or without lemon and lime oils as minor components. Lemon, lime, neroli, and orange oils are collectively called citrus oils.

Sodas are prepared from purified water, sugar (or other sweetener), EOs, colors and preservatives, and carbon dioxide (CO_2). Some quantities of natural fruit juice are sometimes incorporated in sodas. In the soda industry, EOs and other strategic ingredients, including colors, are supplied by big soft-drink licensing companies (SDLCs) as concentrates to smaller, licensed bottling companies ("bottlers") (May, 1998; Benson, 2004; TNI Conflict & Debate Paper 13, 2006; TCCC, 2013). This somewhat feudal arrangement has existed since the late nineteenth century and operates worldwide, except in Cuba and North Korea (TCCC, 2013). During the 1970s and 1980s, some emerging economies attempted to change the order of things, but they were unsuccessful.

Some instructive and informative developments in the history of the soda industry include the following:

1. Fanta, a popular orange soda, was invented in Germany in 1940 during World War II, when there was no communication between the US-based SDLC (the Coke Company and eventual owner of Fanta trademark) and the German bottler licensed to it (Mikkelson, 2011).
2. Campa-Cola was invented in India in the 1970s, following the expulsion of the Coke Company from that country (Coca-Cola India, 2013; Campa-Cola Website, 2013). However, following the liberalization policy of Narasimha Rao's premiership, the Coke Company returned to India in 1993 and Campa-Cola ceased to exist.
3. There were several scores of independent bottlers (i.e., bottlers not licensed to any of the US-based SDLCs) that operated in Nigeria in the 1980s. However, by the early 1990s, they had all become defunct or affiliated with a US-based SDLC operating in Nigeria.
4. In Nigeria, the many scores of independent bottlers during the 1980s obtained their concentrates from independent suppliers of concentrates based in Asia, such as Campa Beverages Limited in India (Campa-Cola Website, 2013), and Europe, such as Britvic Soft Drinks in the United Kingdom (Britvic Website, 2013).

Most of the history and dynamics of the soda industry are fairly well documented. Some of the industry's activities, such as SDLC–bottler arrangements, have been around for a long time. The 1980s was quite important in the history of the soda industry worldwide; the author's activities in the beverage industry as a scientific officer during at this time partly informs the writing of this book chapter.

HISTORY OF SPICES AND THE POLITICAL ECONOMY OF THE SODA INDUSTRY WORLDWIDE

A spice is defined as an aromatic plant substance used as flavoring. Spices have been very important in human history, culture, and civilization. Wars have been fought and deals have been made over spices. Europe's desire for "spices of the East" fueled Vasco da Gama's discovery of the sea route to India.

The primary driving force of the industry worldwide is none other than EOs. John Pembroke's famous cola formula (Jackson, 1978) became popular more from the seven spices it contained than from the psychoactive alkaloids in coca leaf. Ever since that formula in 1885—and the later inventions of Pepsi in 1893, 7Up in 1929, Fanta in 1940, Tango in 1950, Mirinda in 1960, and Sprite in 1961—sodas have been a major contributor to the global economy in form of a multibillion dollar industry. In 2012 for example, the Coca Cola Company of USA planned to spend $30 billion to bring about global growth in the company in year 2013. It produces and markets over 500 brands, with a portfolio of $16 billion, consumers in over 200 countries with an average drinking rate of 1.8 billion servings daily. Also in India, Coca-Cola was the leading soft drink till 1977 and after its return in 1993, it has made significant investments to ensure that the beverage is available to more and more people in a country of over a billion persons, reaching even the remote and inaccessible parts of that country. The politico-economic importance and effect of the soda industry cannot be over-emphasized because of the capital and labor intensive nature which has potent multiplier effect both up and down

streams. The Coca-Cola Company for example is one of the world's top 10 private employers having more than 700,000 employees (Melnick and Jackson, 2002).

INDUSTRIAL PRODUCTION OF SODAS

A schematic representation of the processes and the materials required in soda production is shown in Figure 1. Major requirements are water and water treatment chemicals, sugar, CO_2, chemicals used in preservation (e.g., benzoates) and in

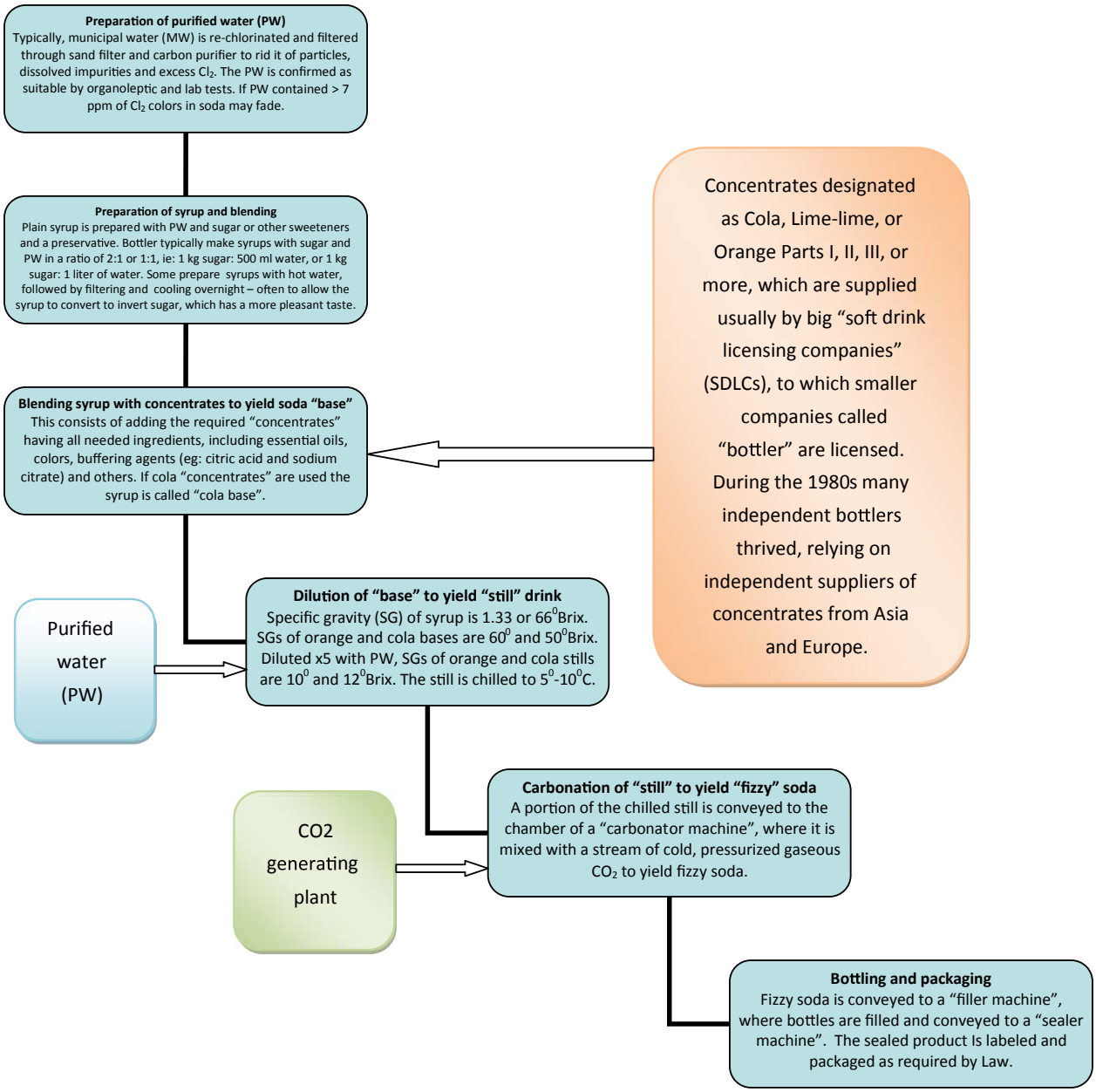


FIGURE 1 Schematic representation of soda production. The sweetener and the syrup produced from it must meet quality control specifications. The specific gravity (SG) of syrup or soda is measured in degrees Brix (°Brix) using a Brix hydrometer. Syrup prepared with refined sugar and water in a ratio of 1 kg–500 ml is given a value of 66 °Brix. Blended orange syrup normally has SG of 60 °Brix, while that of cola or lemon-lime syrup is 50 °Brix. Syrups are diluted 5× with carbonated water. Thus the SG of orange soda is 12 °Brix, while that of soda and lemon-lime is 10 °Brix. Usually orange sodas are given less carbonation than cola and lemon-lime sodas.

sanitation (e.g., sodium bicarbonate), and concentrates. The most strategic inputs of the industry—the EOs—are incorporated in the concentrates.

ESSENTIAL OILS IN COLA AND CITRUS SODA CONCENTRATES

Essential Oils as Flavorings in Sodas

Soda concentrates come as cola, lemon-lime, or orange. They are typically supplied by SDLCs to licensed bottlers. Bottlers not licensed to an SDLC buy their concentrates from independent producers or suppliers. A given soda can have one or more EOs combined into one or more concentrate formulas (containing Parts I, II, III, and more), along with colors and other ingredients, depending upon the brand and supplier. The other ingredients are usually granular substances, such as buffering agents (e.g., citric acid, sodium citrate, phosphoric acid, potassium phosphates) and additional preservatives. During the blending of syrup (Figure 1), the various parts are incorporated in a particular order and method, as described on labels affixed to the said parts. The ingredients contained in cola and citrus parts, including Eos, are given in Tables 1–3.

It is the EOs in concentrates that interact with other flavorings, such as sweeteners, acids, and CO_2, to give a soda its distinctive taste and aroma. The alleged secrets of SDLCs regarding a particular soda lie in the choice and combination of a wide range of EOs (which can have synthetic equivalents).

Essential Oils Used in Cola and Citrus Concentrates

Cola concentrate contains EOs from coca leaf, cinnamon bark, nutmeg seed, coriander seed, neroli flower, lemon peel, lime peel, orange peel, and vanilla seed (Table 1). Lemon-lime and orange soda concentrates contain EOs from all the citrus fruits, including neroli in the case of lemon-lime soda (Table 2). While the dominant oil in orange soda is orange oil (Table 3), those of lemon-lime soda are lemon, lime, and neroli oils. Each of these oils contains scores of different molecular components; some of the leading molecular entities are shown in Table 4 (cola soda concentrate) and Table 5 (citrus soda concentrates). Table 5 further compares and contrasts citrus oils in terms of molecular constituents to explain organoleptic similarities and contrasts between the different citrus oils.

TABLE 1 Ingredients of Cola Concentrate

Ingredient	Remark
Essential oils: • Coca • Cinnamon • Orange • Lemon • Lime • Neroli • Nutmeg • Vanilla Other ingredient: • Kola nut extract (or other sources of caffeine) • Benzoic acid (or other preservatives) • Buffer (e.g., citric acid/Na citrate) • Caramel color • Chelating agent • Emulsifying agent (e.g., gum Arabic) • Glycerin • Natural juice concentrate (in some brands) These ingredients are contained in parts designated as Cola Parts I, II, III, or more depending on brand or region.	**Coke** originally contained caffeine from the kola nut and cocaine from coca leaf and was flavored with vanilla and other ingredients. Today, however, most colas use other flavorings and other sources of caffeine. The original Coca–cola or coke was invented in 1886 in the US by a Pharmacist, Pemberton, whose coke formula was inspired by Mariani's 1863 recipe for Cocawine (Hamblin, 2013). In most countries worldwide colas usually contain most or all the ingredients listed in the left column. Most use sugar or HFCS. Low or zero sugar colas contain little or no syrups or may use sweeteners such as aspartame, sucralose, or others (Poundstone, 1983; D'Amato et al., 2011).

Though called cola, a cola soda may not contain cola extract, or even caffeine. The primary flavorings of colas today are sugar, citrus oils, cinnamon, vanilla, and phosphoric or citric acid. SDLCs or manufacturers of cola concentrates invent distinctively different tastes for each brand by adding trace ingredients like nutmeg. However, the flavorings most associated with colas are vanilla and cinnamon.

TABLE 2 Ingredients of Lemon-Lime Concentrates

Ingredient	Remark
Essential oils from parts of the following fruit: • Lemon • Lime • Neroli • Orange (minor in some brands) Other ingredient: • Benzoic acid (or other preservatives) • Buffer (e.g., citric acid/sodium citrate) • Caffeine (only in some brands) • Calcium disodium EDTA (a chelating agent) • Conc. Natural flavors or juices (in some brands) • Emulsifier (e.g., gum Arabic) • Erythorbic acid (to preserve freshness) • Brominated vegetable oil (in some brands) • Phenylalanine (in some brands as a sweetener) • Yellow 5 (only in some colored brands) • Red 40 (in some colored brands) These ingredients are contained in concentrate parts designated lemon-lime Parts I, II, or III, depending upon brand, country or region.	Lemon-lime sodas are of two kinds—the clear variety and the cloudy. The clear include: 7Up, Sprite, and Sierra Mist. Among the cloudy are: Schweppes bitter lemon and Krest bitter lemon, which contain quinine as additional flavoring. Tripp, a lemon-lime soda of Indian origin was popular in Nigeria during the 1980s, but has since disappeared. Limca, also of Indian origin, is similar to Tripp, and is popular in Nigeria, India and the US. The cloudy appearance of lemon-lime sodas makes them look like real lemon or lime juice. This, in combination with their reputation or status as being free from artificial colors, account for their immense popularity. Although the clear and colorless appearances of Sprite and 7Up do not make them look like lemon or lime juice, their colorlessness bespeaks their non-content of artificial colors. Cloudy lemon-lime sodas are called "lemonade" or "cloudy lemonade" in the UK and most Commonwealth Nations, including Nigeria, where a lemonade brand "Rainbow" was very popular but during the Nigeria Civil War (1967–1970). Because some lemon-lime sodas like Sprite and 7Up are clear and can be mistaken for soda water (i.e., carbonated water), it has become a tradition in the soda industry to present lemon-lime sodas in clear green bottles while soda waters are presented in clear and colorless bottles. Taking a cue from the fact that lemon-lime drinks were popular even before the era of carbonation, some producers currently offer noncarbonated version of their lemon-lime sodas. These include 7Up (Nimbooz) and Minute Maid (Nimbu pani), which are popular in India.

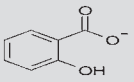
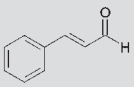
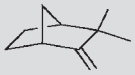
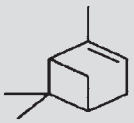
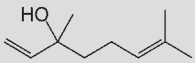
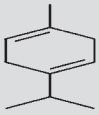
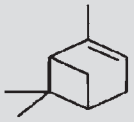
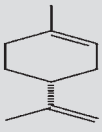
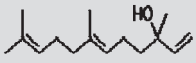
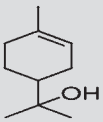
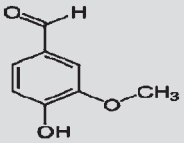
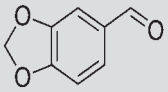
7Up, the oldest of the clear and colorless version of lemon-lime sodas, was invented in 1920 by Charles Grigg, and launched in 1929. It then contained lithium citrate—a mode stabilizing drug, until 1950, when it was discontinued. Attempts to re-formulate 7Up so it can be marketed as "100% natural" climaxed in 2006, with removal EDTA, and replacement of Na-citrate with K-citrate. But the claim was challenged, since the drink had high fructose corn syrup (HFCS). In 2011 however, HFCS was replaced with sugar in the formulation called 7Up Retro (Cadbury-Schweppes, 2008).

TABLE 3 Ingredients of Orange Concentrates

Ingredient	Remark
Essential oils from the following fruit parts: • Orange (as the major citrus oil) (Vora et al., 1983) • Lemon (as the minor citrus oil) • Lime (as the minor citrus oil) • Others (from, e.g., mango, raspberry, apple, pineapple, blackcurrant, and so on) Other ingredient: • Benzoic acid (or other preservatives) • Brominated vegetable oil (in some brands) • Buffer (e.g., citric acid/Na citrate) • Calcium disodium EDTA (to protect flavor) • Carotene (or artificial colors) • Conc. orange juice/other natural flavors • Caffeine (only in some brands) • Emulsifier (e.g., gum Arabic) • Erythorbic acid (for freshness in some brands) • Rosin or gum Arabic • Yellow 5 These ingredients are contained in parts designated as Orange Parts I, II, or more depending upon brand **Flavor variety**: Orange sodas vary with country, brand, and date. About 100 varieties are currently marketed worldwide. In the UK, for example, orange sodas contain orange essence in addition to other essences—lemon, lime, mango, passion fruit, peach, and apricot. **Color**: Artificial colors are allowed in many countries, but in some only natural colors from carrot, pumpkin, or other food plants are used. **Natural fruit juice**: Some brands of orange soda contain small amounts of natural fruit juice.	Fanta, invented in Germany in 1940 is the most popular orange soda worldwide, but the version sold in the US (Fanta Orange) differs from those sold elsewhere (Orange Fanta). The original Fanta had orange juice and a color like orange juice, while Fanta Orange is red-orange and has a candy-like flavor. Fanta Orange was the second drink to be produced by the Coke Company. Some orange sodas contain sodium hexametaphosphate—a sequestrant or chelating agent that complexes with Cu^{2+}, Fe^{2+}, Fe^{3+} and Ni^{2+} that would otherwise be available to catalyst fat peroxidation, an undesirable. Some orange sodas also contain BVO—a densifying/emulsifying agent added to prevent ringing, but causes colors to stick to the tongue. Other popular orange sodas include: Tango, Mirinda, Slice, Sumol, Crush, and Tropicana Twist. **Tango** is an orange soda sold in the UK, Ireland, Sweden, Norway and Hungary, and used to be sold in Nigeria during the 1960s. It was first launched in 1950 by Corona, which was bought over by Beecham in 1958, and later by Britvic in 1987 (Britvic Brand Website, 2013). Like other orange sodas, Tango may contain other flavors—apple, lemon, cherry, and blackcurrant.

Brominated vegetable oils (BVOs) are vegetable oil in which some atoms of hydrogen have been replaced by bromine. The resulting product may have a specific gravity (SG) of up to 1.33. Thus, when a BVO is mixed with a lighter oil like a citrus oil, a mixture with an SG closer to that of water (SG≈1) is produced. Oil-in-water droplets (emulsions) containing BVOs tend to remain suspended in water rather than "ring out"—i.e., separate and float (Bendig et al., 2012). Similar effects can be produced by food additives like sucrose acetate isobutyrate (E444) or glycerol ester of wood rosin (E445). "E" stands for food additive permitted in the European Union.

TABLE 4 Molecular Components of Essential Oils Used in Producing Cola Soda Concentrate

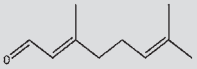
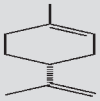
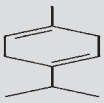
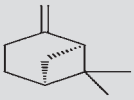
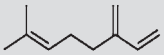
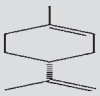
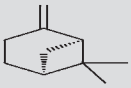
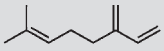
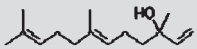
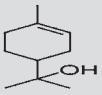
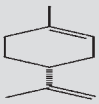
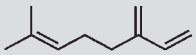
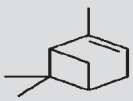
Name of Essential Oil	Key Components	Structures of Key or Other Top Components in Terms of Quantity Present (% w/w)
Coca leaf oil (0.06–0.13% w/w)	The key component is methyl salicylate.	 Methyl salicylate
Cinnamon bark oil	The key component is cinnamaldehyde (65–80% w/w). Others are ethyl eugenol, β-caryophyllene, linalool, and methyl chavicol, limonene, α-terpineol, and so on.	 Cinnamaldehyde
Nutmeg seed oil (5–15% w/w)	The compositions (w/w) are: camphene or sabinene (50%); α-pinene (20%); dipentene, or racemic mixture of limonene (8%); linalool (6%); borneol (6%); λ-terpineol (6%); geraniol (6%); myristcin (4%); eugenol (4%); and safrole (0.6%).	 Camphene  α-Pinene
Coriander seed oil	The main components (w/w) are linalool (77.5%), λ-terpinene (4.6%), α-pinene (4.0%), limonene (1.3%), geraniol (0.6%), and 2-decenal (0.2%).  Linalool	 λ-Terpinene  α-Pinene
Neroli flower oil (0.8–1% w/w) Other citrus oils as in a citrus soda concentrate	The components of Tunisian neroli oil are: limonene (27.5%); nerolidol (17.5%); α-terpineol (14.0%); α-terpinyl acetate (11.7%); farnesol (8.0%); β-ocimene (4.3%); γ-elemene (3.4%); δ-3-carene (2.4%); β-terpinyl acetate (1.7%).  Limonene	 Nerolidol  α -Terpineol
Vanilla seed extract	The volatiles of fermented vanilla seed comprise 85% w/w vanillin, piperonal (heliotropin) and over 100 other aroma compounds.	 Vanillin  Piperonal

Cola concentrates contain lemon, lime, neroli, and orange oils just like some citrus concentrates. Neroli oil has nerolidol as a key component, but other citrus oils contain little or none.

Formulation of Essential Oils into Soda Concentrates

A soda typically consists of over 99% w/w water, sugar, and other ingredients, and less than 0.5% w/w EOs. Because these oils are hydrophobic, a soda is really a very dilute oil-in-water suspension. Therefore, the concentrate must be presented as a concentrated oil-in-water emulsion.

TABLE 5 Molecular Components of Essential Oils Used in Producing Citrus Soda Concentrates

Name of Essential Oil	Key Components (in % w/w)	Structures of Key or Other Top Components in Terms of Quantity Present (% w/w)
Lemon oil	Limonene (54.6); γ-Terpinene (19.1); β-Pinene (14.5); α-Pinene (3.9); Geranial (2.3) and Myrcene (1.5).  Geranial It is noteworthy that both γ-Terpinene (19.1) and geranial (2.3)—also called lemonal or citral, are conspicuous in lemon but below detection or much lower in both lime and orange.	 Limonene  λ-Terpinene  β-Pinene
Lime oil	Limonene (74.8); γ-Terpinene (0.1); β-Pinene (1.4); α-Pinene (0.3) and Myrcene (7.1) (Khan and Abourashed, 2010).  Myrcene It is noteworthy that myrcene is much higher in lime (7.1) than in lemon (1.5) and orange (2.1–4.3).	 Limonene  β-Pinene  Myrcene
Neroli flower oil (0.8–1% w/w)	Tunisian neroli oil has: limonene (27.5%); nerolidol (17.5%); α-terpineol (14.0%); α-terpinyl acetate (11.7%); farnesol (8.0%); β-ocimene (4.3%); γ-elemene (3.4%); δ-3-carene (2.4%); β-terpinyl acetate (1.7%).	 Nerolidol  α-Terpineol
Orange oil	Limonene (94.2); β-Pinene (0.2–1.0); α-Pinene (0.7–1.4); and Myrcene (2.1–4.3) (Coleman et al., 1969).  Limonene It is noteworthy that limonene is much higher in orange (94.2) than in lemon (54.6) and lime (74.8).	Limonene  Myrcene  α-Pinene

All the citrus oils contain limonene and other components in common, (Wang et al., 2012), which partly accounts for their similar aromas. The differences in the concentrations of components partly account for the dissimilarities. Sprite, Sierra Mist and 7UP appear to contain neroli oil judging from their contents of nerol and neral according to the study by Hausch (2010).

The most notable natural emulsifying agent used in the soda industry is gum Arabic. Other natural gums, glycerin, and artificial carboxymethyl cellulose, can also be used. Brominated vegetable oils (BVOs), which have specific gravities (SGs) close to that of syrup (i.e., 1.33) can also be used to stabilize oil-in-water emulsions. As previously stated, concentrates come in parts; at least one of these parts containing one or more EOs will be presented as a syrup-like liquid. Such a liquid cola or orange part may contain, for example, lemon oil, caramel (in the case of cola) or other colors (in the case of orange), glycerin or other polyhydric alcohol, gum Arabic or other emulsifying agent, buffers, and preservatives, including chelating agents. The EO may be presented in a vehicle of vegetable oil or polyhydric alcohol. In countries where BVOs are allowed, orange or other concentrates may contain BVO.

A concentrate normally has an SG greater than 1 so that it can blend more readily with syrup. A typical concentrate can be produced by an SDLC or its proxy as follows:

1. A predetermined quantity of water or other solvent is placed in the tank of an emulsifying blender. This may be followed by the addition of another solvent, such as vegetable oil or glycerin. The resulting mixture is then blended for a specified length of time.
2. A predetermined quantity of an emulsifier (e.g., a 10% w/v solution of gum Arabic in water) is added and blended for a given duration. This may be followed by the addition of and blending with other ingredients, such as BVO, colors, buffers, and chelating agents, and other preservatives previously dissolved or suspended in suitable solvents. An ingredient like caffeine (previously dissolved in water or aqueous solvent) may be added at this point or in a later stage.
3. A predetermined quantity of EO (presented in a suitable vehicle, such as a vegetable oil or polyhydric alcohol) is added to the mixture and blended for a specified duration.
4. Samples of the resulting emulsion are subjected to a number of required tests (by the SDLC or its proxy), which the emulsion must pass before it is packaged and shipped to bottlers. Such tests include determining SG, stability, and other parameters that determine the usability and durability of the emulsion, as well as the suitability and drinkability of the soda produced therefrom by the bottler.

A typical preservative used in the industry to control microbial growth is benzoic acid or a suitable salt thereof. A typical chelating agent used to control peroxidation of oils is ethylenediaminetetraacetic acid or any of its suitable analogues thereof. The reason for using a chelating agent is that it can sequester from solution metallic ions that would otherwise catalyze oil peroxidation, making such oils rancid with offensive organoleptic features. Ingredients used in producing cola and citrus soda concentrates are given in Tables 1–3. The key molecular components of EOs used in colas and in citrus sodas are given in Tables 4 and 5.

ADVANCED EQUIPMENT AND TECHNIQUES USED IN FLAVOR RESEARCH AND SODA TECHNOLOGY

Briefly, advanced equipment and techniques used in soda technology include the following:

1. Liquid–liquid continuous extraction/solvent-assisted flavor evaporation is a key technique used in extracting aroma-active compounds from sodas and similar media (Engel et al., 1999).
2. Gas chromatography-olfactometry is a technique used in detecting aroma-active compounds. It works in conjunction with another technique called aroma extract dilution analysis. This technique has been well described and applied by Hausch (2010).
3. Solid-phase microextraction combined with stable isotope dilution assays is used in quantifying aroma-active compounds (Hausch, 2010; Bertuzzi et al., 2013).
4. A gas chromatograph-mass spectroscope equipped with a comprehensive library is standard requirement for the identification and quantification of aromatic compounds in flavor chemistry (Verzera et al., 2004).
5. It is often essential and convenient to quantify benzoic acid in soda samples using high-performance liquid chromatography.
6. High sensitivity (0.01mg) analytical balance in addition to other such related needs of an advanced analytical laboratory is necessary for the weighing of the small quantities of essential oils and aroma-active compounds found in sodas.

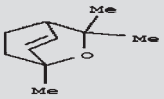
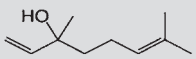
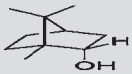
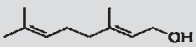
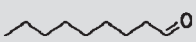
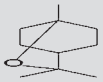
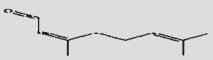
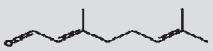
TYPICAL CONCENTRATIONS OF ESSENTIAL OIL MOLECULES IN SODAS

Table 6 shows the typical levels of EO molecules in three lemon-lime sodas: Sprite, Sierra Mist, and 7Up.

CONCLUSION

A search of the literature on sodas reveals that many previous trade secrets are now in the domain of public knowledge. Modern techniques allow scientists to discover the flavor secrets of sodas (i.e., the unique combinations of EOs as flavorings) and to invent new formulations, although questions remain. For example, when a soda contains more than one citrus oil (e.g., lemon, lime, orange, neroli), how much of each is present? This is a challenge because each oil contains scores of aroma-active terpenes and terpenoids (the main molecular entities of EOs that exist as a complex mixture of isomers) and thus are difficult to analyze. In addition, other EOs used in sodas contain similar terpenes and terpenoids as do the citrus oils. Finally, the nature of the techniques used in flavor analysis are such that the analyst

TABLE 6 Typical Concentrations of Essential Oil Molecules in Three Commercial Lemon-Lime Sodas

Molecule	Structure	Concentration of Component in ng/g of De-carbonated Soda as Mean or (Range at 95% Confidence Interval)		
		Sprite	Sierra Mist	7Up
Dehydrocineole		1550 (1360–1740)	2630 (2380–2890)	2750 (2490–3010)
Linalool		220 (202–239)	296 (277–314)	378 (353–403)
Borneol		201 (189–213)	53.8 (52.6–55.1)	58.6 (52.1–65.1)
Octanal		168 (155–181)	449 (411–487)	347 (332–362)
Decanal		61.3 (54.8–67.8)	191 (183–199)	133 (121–145)
Geraniol		47.5 (39.9–55.0)	67.5 (59.3–75.7)	119 (105–132)
Nonanal		33.5 (31.8–35.2)	54.3 (53.7–55.0)	42.1 (35.5–48.6)
Nerol		32.9 (29.5–36.4)	35.0 (29.8–40.2)	49.5 (43.2–55.9)
Isoborneol		32.5 (29.8–35.3)	16.0 (14.4–17.7)	12.2 (10.8–13.6)
Cineole (Eucalyptol)		29.7 (28.3–31.1)	17.4 (16.9–17.9)	15.5 (15.2–15.7)
Geranial (e-Citral)		5.64 (3.36–7.91)	6.58 (4.65–8.52)	10.0 (6.11–13.9)
Neral (z-Citral)		3.58 (2.46–4.70)	3.83 (2.65–5.01)	4.59 (3.56–5.63)

The data are after Hausch (2010), using liquid–liquid continuous extraction/solvent-assisted flavor evaporation (LLCE/SAFE), gas chromatography-olfactometry (GCO) and solid phase microextraction (SPME) combined with stable isotope dilution assays (SIDA). The masses of de-carbonated beverages were: Sprite—97.4 ± 1.55; Sierra Mist—97.8 ± 1.50; and 7Up—99.0 ± 0.18% w/w of their carbonated masses (Hausch, 2010).

or flavorist must continually contend with the possibility of artifacts in the matrix, which distort the true picture of desired components. A major implication of this situation is that the quality control of EOs in sodas is determined not by bottlers but at the level of concentrate formulation. Today's technology permits the production of tastier and safer sodas, using concentrates made with select synthetic flavorings that impart the desired taste and aroma.

SUMMARY POINTS

- Essential oils are obtained from plants containing aroma-active compounds. They are used in food and beverage, cosmetics, and pharmaceutical industries as flavoring agents, fragrance, and medicines.
- Chemically, these aroma-active compounds are terpenes and terpenoids. Each essential oil can characteristically be used to identify the plant source.
- Knowledge of these constituents has been used in the formulation of concentrates for soda flavoring.
- The soda industry has existed since the late nineteenth century and is currently a multibillion dollar industry, impacting global economy.

- Essential oils from various fruit-based spices are key components of the concentrates from which sodas are made, with the addition of other ingredients such as water, sugar, preservatives, colorants, and thickeners.
- Most manufacturers of soda use commercially prepared concentrates or concentrates manufactured by the SDLC or its proxy; thus, they can be considered as bottling companies.
- Cola concentrates are used in cola sodas; lemon-lime sodas use essential oils from lemon, lime, neroli, and orange; whereas orange sodas are made from concentrates containing orange oil as the major component.
- Cutting-edge scientific processes, such as solvent-assisted flavor evaporation and solid-phase microextraction, using high-tech equipment such as gas chromatograph-mass spectroscope and high-performance liquid chromatography, are routinely employed in the formulation, evaluation, and manufacture of the flavor industry.
- The main molecular entities in essential oils exist as complex mixtures of isomers and closely related substances, creating a challenge in their analysis and the ability of the chemist to mix them accurately to create new formulas.

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Chapter 12

Microencapsulation Technology and Essential Oil Pesticides for Food Plant Production

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List of Abbreviation

GRAS Generally recognized as safe

INTRODUCTION

One-third of the world's potential food supplies are estimated to be lost to pre and postharvest pests and diseases. The use of synthetic pesticides has increased crop production; however, they are causing irreversible damage to the environment. Adverse health and environmental effects, insect resistance, or organic food production are arguments against the use of conventional pesticides. The increasing awareness about the impact of agricultural practices is promoting the use of biopesticides over synthetic pesticides (Figure 1(A) and (B)).

For millions of years, plants have evolved sophisticated defensive mechanisms. Diverse groups of chemicals act as barriers to herbivores. Some chemicals are constitutive, while others are induced after attack. Pesticidal compounds exist within almost all classes of secondary metabolites. The most important botanical pesticides present on the market are pyrethrum, neem, and essential oil-based products. Often, essential oils are used in gardens and homes for pest control, as organic alternative to commercial pesticides (Table 1). Essential oil-based products are complex mixtures of low molecular weight, highly volatile secondary metabolites. Due to their versatile nature, they have been applied for various purposes, from contact toxicants to fumigants and as attractants or repellents. Some essential oils and their constituent chemicals are widely used as flavoring agents in foods and beverages and are exempt from pesticide registration. This special regulatory status combined with the availability of essential oils from the flavor and fragrance industries has made commercialization of essential oil-based pesticides possible. While generation of acute toxicities, accumulation in the food chain, long degradation periods, and resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential oil-based pesticides owing to the complex mixtures of constituents. Therefore, natural products are an alternative to synthetic pesticides. Most essential oil chemicals are relatively nontoxic to mammals and vertebrates and meet the criteria for "reduced risk" pesticides. However, to overcome the instability of essential oils, different formulation techniques and methods have been developed in recent years. Microencapsulation, for example, is a method used to protect sensitive materials that can suffer degradation. Microencapsulation techniques are used to prepare microemulsions that slow down the decay of essential-oil-based pesticides and enable controlled and gradual release of such pesticides.

ESSENTIAL OILS

There are approximately 3000 essential oils known (Figure 2), of which 300 are commercially important. Essential oils are complex natural mixtures of secondary metabolites characterized by a strong odor and density lesser than water. Since they are volatile, water vapor can easily extract them. Essential oils contain about 20–60 components at different concentrations.

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FIGURE 1 Different signs in areas where pesticides are used and in pesticide-free areas. (A) Hazardous pesticide in use—warning sign; (B) pesticide-free area—inviting sign.

TABLE 1 Pests and Corresponding Essential Oils with Pesticidal Activity

Pest	Essential Oil
Ants	Peppermint, spearmint
Aphids	Cedarwood, hyssop, peppermint, spearmint
Beetles	Peppermint, thyme
Caterpillars	Peppermint, spearmint
Chiggers	Lavender, lemongrass, sage, thyme
Fleas	Peppermint, lemongrass, spearmint, lavender
Flies	Lavender, peppermint, rosemary, sage
Gnats	Patchouli, spearmint
Lice	Cedarwood, peppermint, spearmint
Mosquitoes	Lavender, lemongrass
Moths	Cedarwood, hyssop, lavender, peppermint, spearmint
Plant lice	Peppermint, spearmint
Slugs	Cedarwood, hyssop, pine
Snails	Cedarwood, pine, patchouli
Spiders	Peppermint, spearmint
Ticks	Lavender, lemongrass, sage, thyme
Weevils	Cedarwood, patchouli, sandalwood

Essential oil used at home as alternative pest management products.

Two or three major components are generally present at concentrations of 20–95%, while others are present in trace amounts. Components present in essential oils can be divided into two groups of distinct biosynthetic origin. The main group is composed of terpenes and terpenoids and the other group of aromatic and aliphatic constituents, all characterized by a low molecular weight (Figure 3). Most essential oils comprise monoterpenes that contain 10 carbon atoms arranged in a ring or in acyclic form, as well as sesquiterpenes which are hydrocarbons comprising 15 carbon atoms. Higher terpenes may also be present as minor constituents. Aromatic compounds occur less frequently than the terpenes and are derived from

FIGURE 2 Image of essential oil. A bottle of essential oil next to the herb from which it is obtained.

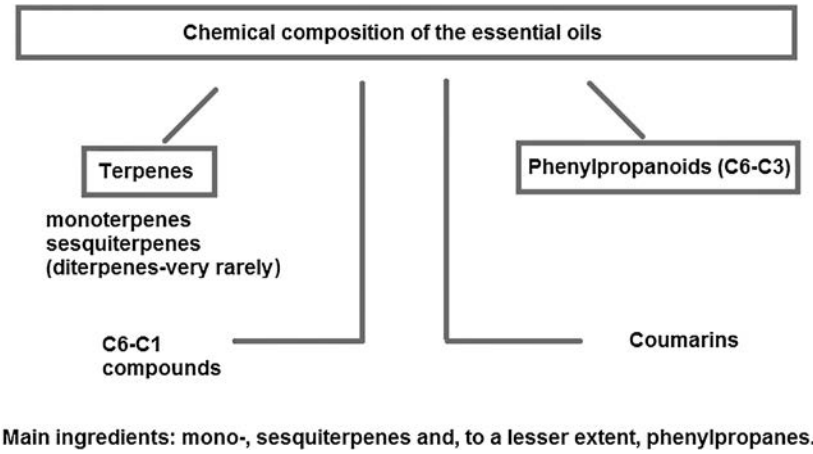

FIGURE 3 Localization of essential oils in plants. Essential oils can be localized endogenously (in cells, cavities, or canals) or exogenously (on the surface).

phenylpropane (Koul et al., 2008). Preliminary studies have shown than oxygenated monoterpenes are more phytotoxic compounds than monoterpene hydrocarbons. The herbicidal and antimicrobial activities of essential oils are correlated with their chemical composition and depend on the dose used as well as the weed or microorganisms against which they are applied, to make it possible to develop specific herbicides and substances for a particular weed or pathogen (Blázquez, 2014).

ESSENTIAL OILS AS PESTICIDES

An important difference between the production of botanical insecticides (complex mixtures of several, often closely related secondary metabolites) and synthetic pesticides (generally based on a single active ingredient) is the standardization of the active ingredient(s), that is, there can be great variability in the quality and composition of botanical pesticides. The source of variability might be natural or might occur as a result of different harvest or extraction methods (Miresmailli and Isman, 2014).

By creating standard criteria for botanical product composition, extraction methods, blending, or fortification, it might be possible to achieve greater uniformity. Compared with synthetic pesticides, botanical insecticides are relatively unstable

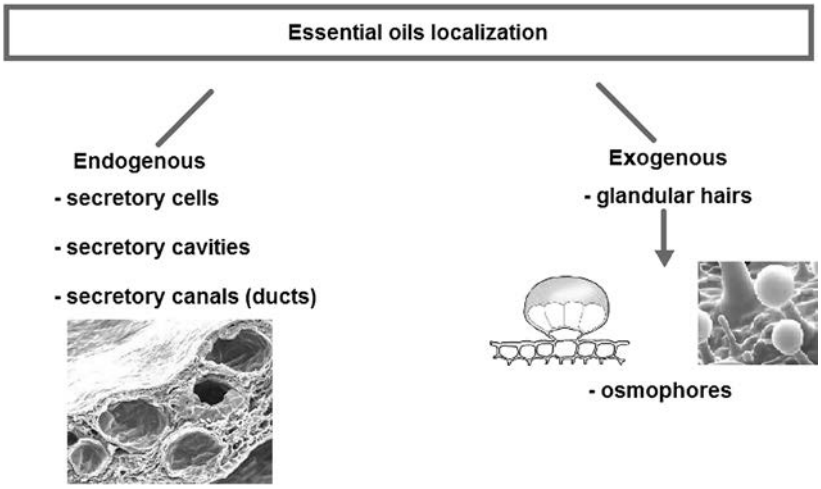

FIGURE 4 Schematic representation of the chemical composition of the essential oils. Terpenes represent the most common compounds in essential oils.

and breakdown faster when exposed to light, temperature, and air. Essential oils are found in special secretory structures either on the surface of the plant or within the plant tissues (Figure 4). Once plant chemicals have been removed from their plant compartments, their constituents become prone to oxidative damage, chemical transformations, or polymerization reactions as a result of destructive extraction methods. As plant extracts age, their quality declines further. The compositional diversity of the botanical extracts and the instability of their constituents can make botanical insecticides unsuitable for applications where residual effects over long periods of time are desirable. Novel technologies that consider the behavior and control level of individual constituents of botanical insecticides smooth the path for a new generation of essential-oil insecticides that are applied in a manner closer to the natural defense methods used by plants. Additionally, botanical insecticides have been successfully used in combination or rotation with synthetic pesticides. Considering the relatively higher cost of botanical insecticides and their limitations, the integrated use of botanical insecticides with other control measures could be a more practicable option (Devi and Maji, 2011).

Essential oils interfere with basic metabolic, biochemical, physiological, and behavioral functions of insects. Genera with such effects are distributed in a limited number of families, such as Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiacecae, Cupressaceae, Poaceae, Zingberaceae, and Piperaceae (Triphati et al., 2009). The point of entry of essential oils is important for their toxic (pesticidal) effect. Essential oils can be inhaled, ingested, or skin absorbed by insects. Plants of families Myrtaceae, Lamiaceae, Asteraceae, Apiaceae, and Rutaceae are considered to show antiinsect activities against specific insect orders like Lepidoptera, Coleoptera, Diptera, Isoptera, and Hemiptera. The physiological actions of essential oils on insects have not been studied enough, but symptoms after treatments suggest a neurotoxic mode of action (Kostyukovsky et al., 2002). Monoterpenoids are targeted to the insect selective octopaminergic receptor. Octopamine has a broad spectrum of biological roles in insects, acting as a neurotransmitter, neurohormone, and neuromodulator and interacts with at least two classes of receptors. A breakdown of nervous system in insects is caused by interruption of octopamine functioning. Accordingly, octopaminergic system of insects represents a target for insect control. As this target point is not shared with mammals (because of the lack of octopamine receptors in vertebrates), most essential oil chemicals are relatively nontoxic to mammals in toxicological tests, and meet the criteria for "reduced risk" pesticides (Bakkali et al., 2008).

Biological effects of essential oils can be the result of a synergism of all molecules or can reflect only those of the main molecules present at the highest levels. Generally, the major components are found to reflect the physical and biological features of the essential oils from which they were isolated, while the extent of their effects is dependent on their concentration. However, it is possible that the activity of the main components is modulated by other minor molecules. Synergistic combinations with pesticidal activity are created to reduce the dose of potentially polluting substances and the risk of developing resistance. Synergistic combination products can have multiple modes of action, respecting the principle that the action of combined product is greater than the sum total of known and unknown chemical components. Yet, it is important to mention that both positive and negative synergism can occur between essential oils. There are commercially available formulations (mixtures of essential oils), directed at octopaminergic sites and therefore classified as generally recognized as safe (GRAS). Formulation of essential oil-based products is an important area. Controlled released formulations allow smaller quantities of pesticides to be used and are highly effective over a longer period of time. The encapsulation process is a suitable method for entrapping essential oils of a very different chemical composition. This method reduces loss of

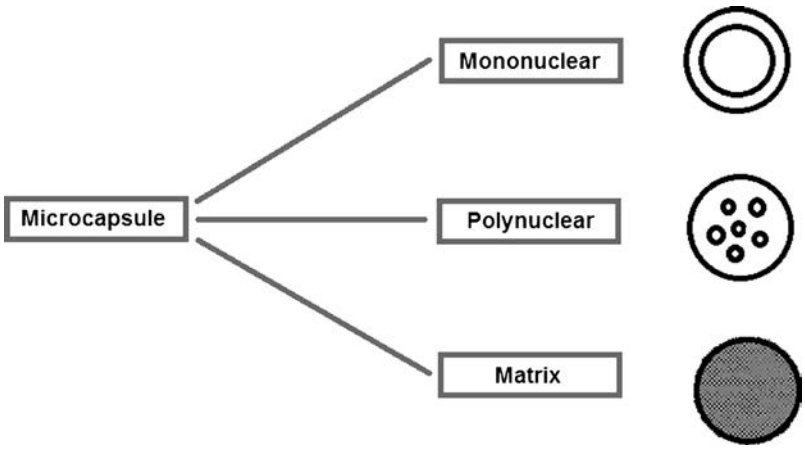

FIGURE 5 Possible morphological structures of microcapsules. Microcapsules can have a variety of structures. Some have a spherical geometry with a continuous shell, while others have an irregular geometry and contain a number of small droplets or particles of core material.

the active principles leading to high loaded microparticles that enable controlled drug release. Microencapsulation means packaging an active ingredient in the form of droplets of solid or liquid material (the core) inside a capsule, that is, continuous film of polymeric material (the shell) ranging in size from one micron to several millimeters. At present, there is no universally accepted size range. However, capsules smaller than 1 μm are often classified as nanoparticles and those greater than 1000 μm as macrocapsules. Commercial microcapsules usually have a diameter between 3 and 800 μm and contain 10–90 wt% core (Devi and Maji, 2011). Substances may be microencapsulated with the intention that the core material be held within capsule for a specific period of time.

Alternatively, encapsulated core material is released either gradually through the capsule walls (controlled release or diffusion) or when external conditions trigger the capsule walls to rupture, melt, or dissolve. The encapsulation efficiency depends upon different factors like concentration of the polymer, solubility of polymer in solvent, rate of solvent removal, solubility of organic solvent in water, etc. Microencapsulation processes are usually categorized into two groups: chemical processes and mechanical or physical processes. These labels can be somewhat misleading, as some processes classified as mechanical might involve or even rely upon a chemical reaction, and some chemical techniques rely solely on physical events (Jyothi et al., 2012). The morphology of microcapsules (Figure 5) can be as follows: mononuclear—contains the shell around the core; polynuclear—have many cores enclosed within the shell; or matrix—the core material is distributed homogenously into the shell material. Additionally, microcapsules can also be mononuclear with multiple shells, or clusters of microcapsules are formed (Devi and Maji, 2011; Jyothi et al., 2012).

Microencapsulation technology has been applied to improve the sustained release effect, stabilization, and easy handling of essential oils. There are two types of wall materials around the core: natural and synthetic polymers. Natural materials, such as chitosan, gelatin, alginate, and poly(L-lactide), have biodegradability and environment-protecting properties. Yet, natural polymers compared with synthetic have low mechanical properties, poor heat stability, and short-term release properties. Synthetic polymers include melamine, urea, etc.

Sustained release is one mode of controlled release system. It can maintain constant concentration of active compounds at target. In the initial stage, the amount of released oil is large and then slowly becomes constant. Release rate of encapsulated volatile essential oils depends on component materials of microcapsules (encapsulated material, emulsifier, and coating material) and various environmental conditions such as temperature, pH, and humidity (Chung et al., 2013).

Taking into account the limitations of some processes and the physicochemical characteristics of essential oils, coacervation is an attractive technique to encapsulate this type of active agents. Coacervation techniques can be divided into two main groups: coacervation in aqueous phase and coacervation in organic phase. Coacervation in aqueous phase can only be used to encapsulate water-insoluble materials (hydrophobic core materials presented in solid or liquid state). Coacervation in organic phase, on the other hand, allows the encapsulation of hydrosoluble material with the use of organic solvents. Coacervation in aqueous phase can further be classified into simple and complex. In simple coacervation, the polymer is salted out by the action of electrolytes or desolvated by the addition of a water-miscible nonsolvent, or by increasing/decreasing temperature. These conditions promote the macromolecule–macromolecule interactions. Complex coacervation is driven by the attractive forces of oppositely charged polymers. The coexistence of a coacervate phase made of concentrated polyelectrolytes and a diluted equilibrium phase depends on pH and ionic strength. Coacervation process can be divided into three steps that occur under continuous stirring. The first step comprises the formation of an oil-in-water emulsion, the second consists of the formation of the polymer coating upon the core material, and the third is the stabilization of

the coating (coating hardening). Coacervation offers numerous possibilities for the encapsulation of various types of active agents (solid or liquid core materials). The retention of the active agent inside the membrane shell is ruled by factors related to the chemical nature of the core, including its molecular weight, chemical functionality, polarity and volatility, shell material properties, and the chosen microencapsulation technique. The most commonly used shell materials in coacervation are polysaccharides and sugars (gums, starches, celluloses, and cyclodextrines); proteins (gelatine, casein, and soy proteins); lipids (waxes, paraffin, and oils); and synthetic polymers (acrylic polymers and polyvinylpyrrolidone). To a lesser extent, inorganic materials such as clays, silicates, and polyphosphates can also be used.

Also, biodegradable polymers have emerged as candidates for the development of carriers. Lately, numerous processes for encapsulation have been developed using aliphatic polyesters, such as polylactic acid and copolymers of lactic and glycolic acids. The biodegradability of these polymers can be modified by incorporating in their main chain a variety of chemical groups such as ethers, anhydrides, etc. The properties of biodegradable polymers are defined by structural features such as molecular weight, nature of the chain end-groups, and copolymer composition. The protection of essential oils in polymer carriers with the purpose of controlled release over a certain period of time has been a topic of substantial research in recent years. Controlled release technologies are used to deliver compounds such as drugs, pesticides, fragrances, or flavors at determined rates. The properties of the polymeric network, such as chain length, flexibility, and mobility, water-uptake and swelling behavior, or potential interactions between polymer and active agent affect the diffusion rate over the polymeric matrix and accordingly, the oil release. Diffusion of active agents occurs when oils pass through the polymer. Several diffusion models have been suggested in the literature to describe the release of an active agent from microcapsules: erosion, diffusion, extraction, and burst (Martins et al., 2014).

Improvement of the efficacy of botanical insecticides can be divided into three directions. The first is better extraction methods with special emphasis on preservation of phytochemical mixtures. The second is associated with the new formulation methods that simulate the chemical compartmentalization in plants. The same techniques that have been successfully used in pharmaceuticals can be used for pesticide formulations upgrade. The third is the development of technologies and delivery methods that provide release control both qualitative and quantitative at the level of individual constituents. Micro- and nanoencapsulation techniques have been examined as means of controlled release of botanical insecticides. These technologies can extend the efficacy of botanical insecticides over longer periods of time (Devi and Maji, 2011).

It is important to stress that natural products are not always safer than synthetic pesticides. For instance, there have been several problems reported with the use of tea tree oil. The most common problematic response is contact dermatitis caused by the formation of degradation products (peroxides, epoxides, and endoperoxides). Another bioactive compound d-limonene, classified as GRAS, is a constituent of number of essential oils like orange, lemon, mandarin, and grapefruit. It also causes dermatitis. Similarly, the essential oil of *Mentha pulegium* (squaw mint) is widely used as insect control agent and contains an active ingredient pulegone, which, upon digestion is oxidized into toxic metabolites including menthofuran. These metabolites bind to proteins causing loss of organ function, seizures, acute poisoning, and death (Triphati et al., 2009).

Based on the above discussion, it can be concluded that rational and carefully designed use of essential oil-based pesticides is necessary for their expanded application in the future.

SUMMARY POINTS

- Synthetic pesticides are related to negative health and environmental effects and insect resistance.
- Monoterpenoids are a major group of chemical compounds in essential oils that interfere with octopaminergic system of insects, which represent a target for insect control.
- Because of the lack of octopamine receptors in vertebrates most essential oil chemicals are relatively safe for use.
- Special regulatory status combined with the availability of essential oils has made commercialization of essential oil-based pesticides possible.
- Microencapsulation mimics the chemical compartmentalization in plants by protection of essential oils from degradation.
- For microencapsulation of essential oils, the coacervation technique is most widely used.

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Chapter 13

Effect of Essential Oils on Organoleptic (Smell, Taste, and Texture) Properties of Food

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List of Abbreviations

BHA Butylate Hydroxy Anisole
CA Caprylic Acid
CPD Convective predrying
EO Essential Oil
GRAS Generally Recognized As Safe
MFC Minimum Fungicidal Concentration
OLP Organoleptic Properties
OEO Oregano Essential Oil
SFE Supercritical Fluid Extraction
VM Vacuum–microwave

INTRODUCTION

Essential oils (EOs) are aromatic and volatile oily liquids formed in special cells or groups of cells, found in leaves and stems, and commonly concentrated in one particular region such as leaves, bark, or fruit. EOs have long served as flavoring agents in food and beverages. Expression, fermentation, or extraction and steam distillation are most suitable methods to obtain them. Temperature, soil conditions, the altitude grown in and the country grown in are main causes for variations between EOs. True EOs are nonoily, despite their name. EOs are all nongreasy, partially water-soluble, inflammable, volatile, soluble in alcohol, and easily absorbed by the human body. EOs are generally recognized as safe and are attracting interest as natural food. Carvacrol and thymol are the main bioactive compounds of oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) oils, respectively, and are characterized by strong antibacterial properties (Gutierrez et al., 2009). EOs have more than 50 components; one to three of them are the main components representing 85–95% of the whole volume, while the others are minor components, representing less than 1%. The chemical character of the compounds influences their antimicrobial efficacy and the mechanism of action on the target organism. The main groups of EOs are terpenes, terpenoids, and aromatic and aliphatic constituents.

PHYSICAL AND CHEMICAL PROPERTIES OF EOS

EOs differ greatly in their odors, refractive indices, optical, and specific rotation properties. They are immiscible with water but soluble in ether, alcohol, and most organic solvents. EOs are not fixed oils, as they can be distilled from their natural sources and they do not consist of glyceryl esters of fatty acids and cannot be saponified with alkalines as in fixed oils. EOs contain hydrocarbons, alcohols, ketones, aldehydes, ethers, oxides, esters, and others, and only a few possess a single component in a high percentage. EOs contain over 200 components which are essential to its odor and flavor. The absence of even one component may change the aroma. EOs of limbo, foliar sheath, and rhizomes are significantly different in their chemical properties, for example, density, refractive index, and rotatory power. These properties contribute to a better characterization of plant EOs (Noudogbessi et al., 2012).

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EFFECT OF EOS ON FOOD ORGANOLEPTIC PROPERTIES (OLP)

EOs extracted from stems, leaves, and flowering tops of some herbal plants are used for flavoring liquors, soft drinks, beverages, and pharmaceutical preparations. Many EOs from herbs and spices are used widely in food as additives. Their principal function is to impart desirable flavors and aromas. Therefore, it is possible that additional safety and toxicological data would be required before regulatory approval for their use as novel food preservatives. EOs and their constituents have been used broadly as flavor ingredients in a wide variety of foods, beverages, and confectionery products (Kalantari et al., 2012). The composition and the presence of EOs can clearly determine the specific aroma of plants and the flavor of condiments. EOs of herbs and spices could also be used as functional ingredients. Sweet basil is used extensively to add a distinctive aroma and flavor to food, such as salads, pizzas, meats, and soups (Viuda-Martos et al., 2011). EOs from leaf oil and berry oil of allspice (*Pimenta dioica*) are used as a flavoring agent in meat products and confectioneries. The maximum permitted level of berry oil in food products is about 0.025% (Krishnamoorthy and Rema, 2004).

Okunola et al. (2007) extracted EOs with *n*-hexane from the seeds of *Piper guineense*, flower buds of *Eugenia aromatica* and kernels of *Monodora Myristica* and added to store grains, these authors assessed the effect of these EOs on the taste, aroma, and appearance of the grains using trained panelists. These authors reported that the cooked grains stored with a lower dosage rate of 5 mL/kg EO were significantly preferred by the panelists. Also, cooked grains treated with nutmeg spiced EO were significantly accepted and preferred (Okunola et al., 2007). The EOs of the leaves, stems, rhizomes, and roots of *Alpinia galanga* is used to flavor various dishes and as food additives in Malaysia, Thailand, Indonesia, and China (Anon, 2002). Lavandin oil, lavender oil, spike lavender oil, and lavender absolute are used as natural food flavors. Their uses in the food industry include baked goods, frozen dairy, soft candy, gelatin, pudding, and nonalcoholic beverages (Lis-Balchin, 2004). The EOs from the ajowan seed are responsible for providing a typical flavor owing to the presence of thymol, stearoptene, cumene, and terpene—"thymene." These constituents give a warm, aromatic and pleasing flavor to food products when used in processed foods, snacks, sauces, and various vegetables (Malhotra, 2004).

Hulankova et al. (2013) proved the effect of oregano essential oil (OEO) and caprylic acid (CA) as a combined additive when used in meat. These authors reported that samples treated with CA showed the lowest attribute scores throughout the storage period and judged as unacceptable. However, samples treated with OEO showed better scores than the control group and stayed above the neutral point (score 5) up to the sixth day of storage. The odor of cooked samples treated with volatile OEO was more pronounced, but still evaluated as acceptable. In overall acceptability the samples treated with mixtures of OEO and CA were not statistically different ($P = 0.91$ and $P = 0.97$), as the better scores in odor were outweighed by worse appearance (color) as the samples treated with CA showed worse color attributes. Finally, the addition of oregano oil improved the flavor, odor, and color of stored minced beef and was almost undetectable after cooking (Hulankova et al., 2013).

Calín-Sánchez et al. (2012) characterized the intense odor notes of fresh sweet basil as fresh (8.1), floral (8.0), and herbaceous (6.4) using convective and vacuum–microwave (VM) drying methods, they noticed a significant increases in the intensities of the dried samples of the following attributes compared with the fresh sample: spicy (mean of 5.3 for convective and VM dried samples compared to 3.6 in fresh sweet basil), hay-like (mean of 3.6 compared with 0.8), sweet (1.5 compared with 0.8), earthy (mean of 2.6 compared with 0.8), woody (mean of 3.5 compared with 0.9), and infusion (mean of 5.0 compared with 2.4) (Figures 1–3). The addition of 1–3% w/w olive leaves improved the organoleptic quality of olive oil, when added to ripe olives prior to extraction to determine their effect on the quality of the resulting olive oil. An increase in *trans*-2-hexenal aldehyde content occurred, giving the pleasant flavor of "fresh cut grass" (Di Giovacchino et al., 1996). The effects of seven EOs (borneol, carvacrol, cinnamaldehyde, eugenol, menthol, thymol, and vanillin (Figure 4) were studied as food preservatives by examining their effects on the growth of activated *Bacillus cereus* inoculated into tyndallized carrot broth. The evaluations of the panelists for carvacrol, cinnamaldehyde, and thymol showed that thymol had a strong odor which left a bad aftertaste and minimized acceptance or liking degree for the product. From this study the authors noticed that low concentration of cinnamaldehyde can improve the taste of carrot broth without any adverse effect on its taste or smell (Valero and Giner, 2006).

Many individual EO components approve food flavorings and impart a certain flavor to foods. Carvacrol is said to produce a "warmly pungent" aroma on fish. Fresh kiwi fruit and honeydew melon when treated with small amounts of carvacrol or cinnamic acid were found to delay spoilage without changing its OLP (Roller and Seedhar, 2002). The European Commission registered EO of carvacrol, carvone, cinnamaldehyde, citral, *p*-cymene, eugenol, limonene, menthol, and thymol to be used as food flavorings without any risk to the health of the consumer. Gutierrez et al. (2008) treated lettuce with oregano at 250 ppm, they found that it was acceptable to a sensory panel and no differences between this lettuce and that washed with chlorinated water. While Wang et al. (2007) treated strawberries with eugenol, thymol, and menthol and found these EOs delayed fruit deterioration and maintained better fruit quality with higher levels of phenolics, sugars, organic acids, flavonoids, anthocyanins, and oxygen radical absorbance capacity than the untreated fruits. The addition of EOs of oregano and

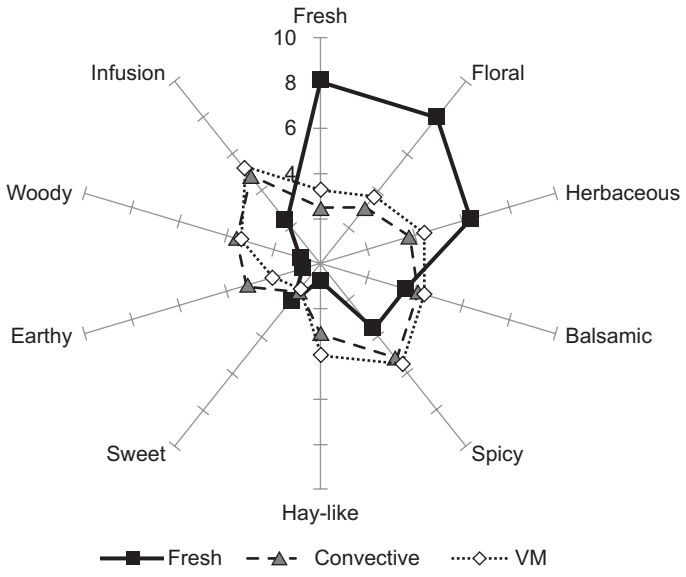

FIGURE 1 Descriptive sensory analysis of aroma attributes in sweet basil samples as affected by the drying method: convective (40 °C) and VM (240 W) drying methods. *Calín-Sánchez et al. (2012)*.

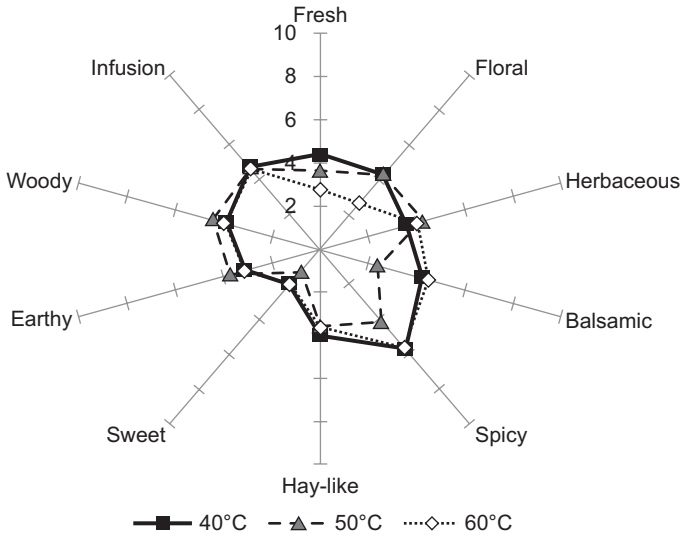

FIGURE 2 Descriptive sensory analysis of aroma attributes in sweet basil samples as affected by the convective predrying (CPD) and vacuum microwave finishing drying method (VMFD) at 50 °C. *Calín-Sánchez et al. (2012)*.

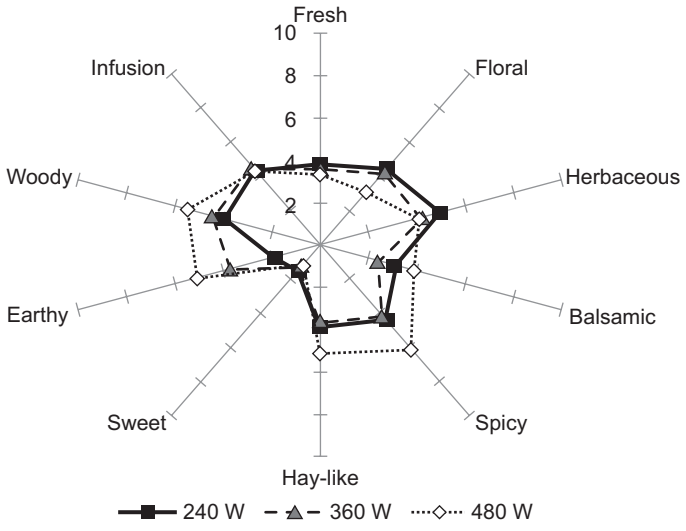

FIGURE 3 Descriptive sensory analysis of aroma attributes in sweet basil samples as affected by the convective predrying and vacuum microwave finishing drying method (CPD–VMFD) at 360 W. *Calín-Sánchez et al. (2012)*.

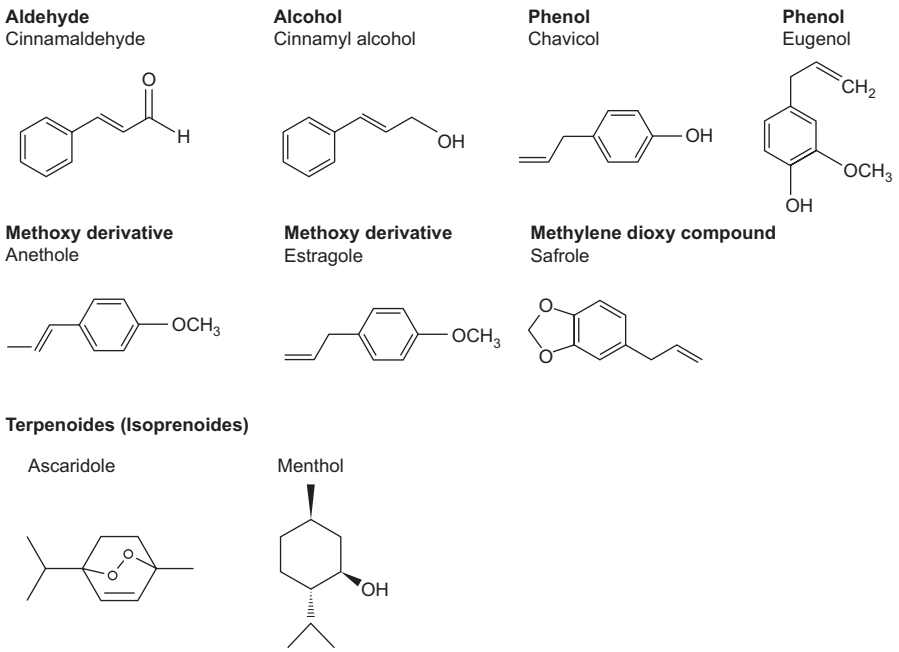

FIGURE 4 Chemical structures of selected components of essential oils. *Bakkali et al. (2008).*

rosemary (Table 1) improves the oxidative and fermentative stability of flavored cheese prepared with a cream cheese base, and prevents lipid oxidation and the development of rancid and fermented flavors. Peroxide and anisidine values, descriptive analysis and fermentation parameters as stability indicators during storage were evaluated for the studied samples. Flavored cheese showed higher stability during storage, and lower acidity and total viable counts and higher pH. Oregano and rosemary EOs demonstrated a protective effect against lipid oxidation and fermentation in flavored cheese prepared with a cream cheese base (Olmedo et al., 2013). de Azeredo et al. (2011) analyzed the sensory scores in vegetables sanitized with *O. vulgare* and *Rosmarinus officinalis* EOs alone and in mixture. They found that the mean of the most evaluated attributes fell between "like slightly" and "neither like nor dislike" on the hedonic scale for samples treated with EOs alone and in mixture. At all assessed storage times no significant difference was found for odor and taste in samples sanitized with the mixture of EOs and control. The time of storage showed no influence on the sensory attributes of vegetables treated with the mixture of EOs. EOs of thyme, summer savory, and clove have good antifungal activity on tomato paste and ketchup. When treated samples were examined by panelists they found that thyme oil was accepted by panelists rather than summer savory and clove. Results showed that all EOs could inhibit the growth of *Aspergillus xavus*. Taste panel evaluations were carried out in a tomato ketchup base which found that the sample with 500 ppm thyme oil was accepted by panelists (Figure 5). To overcome changes in the OLP of the tomato paste, a number of options can be considered. One is to view the EO as a flavor component and not only as a preservative. Second, to incorporate EO into products that already have a strong flavor to mask the presence of the plant EO. A third alternative is to try to use some of the most active components, rather than the whole oil, aiming to reduce changes of the OLP and retain antimicrobial activity (Omidbeygi et al., 2007).

The main constituent of cinnamon leaf oil is cinnamaldehyde which provides the distinctive odor and flavor of cinnamon. Cinnamon EOs give the strong flavor in bakery products and tomato paste (Kalantari et al., 2012). The use of EO vapors may be a potential way of combating the organoleptic effect brought about by direct contact between the food and EO. Cabbage leaf showed obvious physical changes when subjected to EO vapors of lemon, sweet orange, and bergamot for 24-h exposure. The vapors were absorbed into the food matrix, influencing the OLP of the food (Fisher and Phillips, 2006). EOs could be applied as food preservatives to protect the quality and shelf-life of food. Al Otaibi and El Demerdash, (2008) reported that addition of thyme, marjoram, or sage EOs increased the shelf-life of labneh for up to 21 days with acceptable flavor and good appearance without any signs of spoilage organisms. These authors reported that the total scores of labneh containing EOs from thyme, marjoram, or sage decreased with an increase in the concentration of the EOs. Of the different treated labneh, labneh containing 0.2 ppm thyme, marjoram, or sage oils were organoleptically the most acceptable and had a good body and texture similar to that of the untreated control (Al Otaibi and El Demerdash, 2008).

Gao et al. (2014) fumigated button mushrooms (*Agaricus bisporus*) with EOs of clove, cinnamaldehyde, and thyme stored for 16 days. At the end of the storage period, these authors reported that mushrooms fumigated with cinnamaldehyde

TABLE 1 Major Components of Oregano and Rosemary Essential Oils Analyzed by Gas Chromatography–Mass Spectrometry

Oregano Essential Oil		Rosemary Essential Oil	
Components	**g/100g[a] ± SD**	**Components**	**g/100g[a] ± SD**
γ-terpinene	1.1 ± 0.1	Linalool	4.1 ± 0.2
Pinene hydrate (trans)	0.5 ± 0.1	Camphor	35.7 ± 0.4
trans-β-Terpineol	55.5 ± 0.4	Terpinen-4-ol	5.4 ± 0.4
cis-β-Terpineol	3.4 ± 0.3	α-terpineol	7.2 ± 0.2
Borneol	2.1 ± 0.1	Verbenone	26.2 ± 0.4
Terpinen-4-ol	15.9 ± 0.4	β-caryophyllene	15.8 ± 0.3
Linalyl acetate	5.6 ± 0.2	α-humulene	1.4 ± 0.2
Thymol	12.9 ± 0.3	Epi-α-cadinol	0.8 ± 0.2
Carvacrol	0.9 ± 0.2		
Total	**97.7**	**Total**	**96.6**

[a]*Means and standard deviations.*
Olmedo et al. (2013).

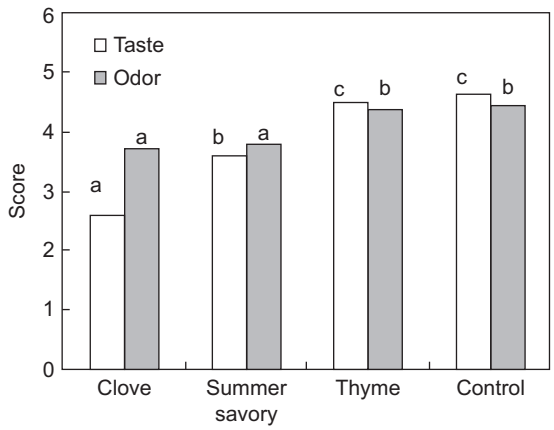

FIGURE 5 Taste panel results of three ketchup sauces prepared by adding 500 ppm of thyme, summer savory, and clove essential oils. Columns with the different letters are significantly different, $P \leq 0.05$. *Omidbeygi et al. (2007).*

browned slightly, but they also had commercial value and edibility. There is no evidence of the role of these natural compounds on this issue, but the well-known antioxidant activity reported for these EOs may reduce dehydration and the occurrence of browned polymers responsible for the mushrooms browning and shriveling activity during the storage period. Thus, fumigation of button mushrooms using EOs improves its quality.

The application of undiluted thyme, oregano, and rosemary EOs to lettuce, carrot discs, cabbage, and dry coleslaw mix led to unacceptable damage to the appearance and unattractive odor (Scollard et al., 2013). Similar results were seen by Uyttendaele et al. (2004) who found that chopped bell peppers tissues can be softened if treated with thyme EO. Undesirable organoleptic effects can be avoided by diluting EOs, by using combinations of EOs, or by using EOs in combination with plant material such as carrot or cabbage shreds (Scollard et al., 2013).

Tserennadmid et al., 2011 reported that using EOs in fruit or milk sensitive to yeast spoilage could be a good alternative to synthetic preservatives, and harmonic taste was achieved by adding lemon EO to clear apple juice, also prolonging storage time. These authors found that the juice taste was positively influenced by lemon EO in all treated products. An unpleasant odor was dominant in clear apple juice, whereas in the cloudy juice it was less strong. Pasteurized milk treated with lemon EO had the most pleasant odor (Tserennadmid et al., 2011). Another study revealed that the addition of lemon EO did not cause a deterioration of the OLP of the apple juice. Moreover, the sensory impact of lemon EO in a freshly squeezed juice could be partially disguised by its richer flavor (Espina et al., 2012).

Chouliara et al. (2007) studied the effect of OEO and packaging method on shelf life and sensory attributes of fresh chicken breast meat stored at 4 °C. The authors found that the color of the treated meat was not clearly affected by oregano oil, but the addition of oregano oil at a concentration of 1.0% imparted very strong taste to the product. The choice of EO and its concentration in a particular food is important because a small amount can cause sensory alterations. The strong aroma of the EOs can affect the OLP of the food but the synergistic combinations of EOs with each other or with other hurdle techniques can reduce this effect (Krisch et al., 2011). Donsì et al. (2011) studied the addition of nanoemulsion T-SL-HPH (a terpenes mixture and soy lecithin (T-SL) encapsulated into nanoemulsions based on food-grade ingredients, prepared by high pressure homogenization (HPH)) at different concentrations to orange and pear juices inoculated with *Lactobacillus delbrueckii* in order to test the microbiological stability as well as the alteration of the OLP of the juices stored at 32 °C. These authors found that the juice concentration and pH were not significantly changed by the addition of the nanoemulsion. This was also observed during the storage period unless significant microbial growth occurred. In contrast, major color deviations were observed when 10 g/L nanoencapsulated terpenes were added to the juices, while the addition of the terpenes mixture at lower concentrations (5.0 g/L and 1.0 g/L) was considered acceptable with it inducing much smaller color deviation. The color remained stable over the storage time for all those systems. Another study on fruit juices was carried by Tyagi et al. (2013) who studied the antiyeast activity of mentha oil and vapors in a mixed fruit juices evaluated by measuring the minimum inhibitory concentration (MIC) and minimum fungicidal concentration against eight food-spoiling yeasts. These authors found that the color of the MIC level treated juices remained acceptable for a longer period than ½ MIC and ¼ MIC level treated samples. The addition of mentha oil did not undesirably alter the odor and color of the mixed juice. In particular, the odor of mixed juice supplemented with mentha oil persisted eight days longer compared with untreated samples. Furthermore, the color remained stable in samples treated with MIC level mentha oil. In ½ MIC level treated samples, juice odor and color persisted up to 4 days, while in ¼ MIC-treated samples juice odor and color persisted up to two days only.

When added to the cakes at different concentrations during processing, *Zataria multiflora* and *Cinnamon zeylanicum* EOs were found to prevent oxidation rate and reduce oxidation products compared with butylate hydroxyanisole. Cake containing these EOs were found to have considerable nutritive and functional advantages. No significant differences were observed between the treated samples in color and texture properties. Finally, results showed that the cakes produced were totally accepted by panelists (Kordsardouei et al., 2013). Supercritical fluid extraction (SFE) is well known as an environmentally friendly technique for separating EOs from the vegetable substrates. Extracts obtained by SFE can retain the organoleptic characteristics of the starting spice material. The addition of aguaribay and cedron essential oils to fried, salted peanuts affected the sensory profile and consumer acceptance as well as it showed protective effect against lipid oxidation (Rubén et al., 2012).

SUMMARY POINTS

- EOs contain over 200 components which contribute to their odor, taste, and color.
- Features of smell, taste, and color comprise the organoleptic properties of EOs.
- EOs and their constituents have been used broadly as flavor ingredients in a wide variety of foods, beverages, and confectionery products.
- The composition and the presence of EOs can clearly determine the specific aroma of plants and the flavor of condiments.
- Organoleptic properties of EOs can be assessed quantitatively by the analysis of specific chemical components.
- Subjective measures can also be used to assess organoleptic properties of essential oil. Measures of aroma have included assessment of fresh, floral, herbaceous, balsamic, spicy, hay-like, sweet, earthy, woody, and infusion properties.
- Addition of some essential oils to foods during processing can prevent their oxidation rate and reduce oxidation products without any changes in color and texture properties.
- An example of the use of EOs in relation to organoleptic properties pertain to those oils extracted from the seeds of *P. guineense*, flowers of *E. aromatica*, and kernels of *M. Myristica*. When added to store grains they can affect positively their taste, aroma, and appearance.

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Chapter 14

Use of Essential Oils in Food Packaging

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List of Abbreviations

AM Antimicrobial
BSA Bovine serum albumin
EOs Essential oils
EVOH Ethylenvinyl alcohol
LDPE Low-density polyethylene
LLDPE Low-density polyethylene
MVP Moderate vacuum packaging
NAI Negative air ions
PET Terephthalate
PVDC Polyvinylidenchloride
SPI Soy protein isolate

INTRODUCTION

A basic function of packaging is the retardation of deterioration, maintenance of quality, and safety of packaged food. A good packaging material should be inert and resistant to hazards, should not allow the molecular transfer to or from packaging materials. A rise in consumer demand for safe, fresh, minimally processed foods, and products with a more reasonable price that offer enhanced shelf-life and/or improved quality has led to the development of new preservation techniques. Active packaging technologies prolong shelf-life and control quality of food product by decreasing microbial, biochemical, and enzymatic reactions through different strategies like the addition of chemical additives/preservatives, removal of oxygen, moisture control, temperature control, or a combination of these. One option able to reduce, inhibit, or retard the growth of microorganisms that may be present in the packed food or packaging material itself is to use packaging with antimicrobial properties.

ANTIMICROBIAL PACKAGING SYSTEMS

Antimicrobial (AM) systems are active packaging in which the package, the product, and the environment interact to kill or inhibit the spoilage and the pathogenic microorganisms that may contaminate packaged food products such as *Listeria monocytogenes*, *Escherichia coli O157*, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens*, *Aspergillus Niger*, and *Saccharomyces cerevisia*. New AM packaging systems are produced by direct physical mixing of active agents in the matrix of the packaging films during manufacturing, or covalently linked AM agents in the main chain of a macromolecule as side-chains coating the packaging films. Food packaging materials used are synthetic films or edible films. Many synthetic AM compounds have been evaluated in synthetic polymeric materials by various researchers such as polyethylene, polypropylene, polyvinylchloride, polyester, and polyamide. Among these polyethylene and polypropylene are excellent moisture barriers, but must be coated or laminated with synthetic polymers including ethylenvinyl alcohol (EVOH) and polyvinylidenchloride copolymers to provide an oxygen barrier. The resulting polymeric structures, while effective in minimizing the permeation of oxygen, water vapor, and smell, are characterized by their poor reuse due to difficulties in separating each layer for its individual recycling. Biopolymers can be made from materials with film-forming ability such as: hydrocolloids (proteins, polysaccharides, and alginate), lipids (fatty acids, acylglycerol, waxes), and composites. Polymer–clay nanocomposites such as montmorillonite have received significant attention as an alternative to conventional filled polymers because of their ability for nanoscale dispersion, which gets significant progress in mechanical strength, thermal

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stability, water solubility, functional and barrier properties compared with microscale polymer composites. Typically, AM packaging systems can be regarded as migrating or nonmigrating with the distinction depending on the specific AM agent used and on its interactions with the packaging and food matrix. There are three systems of AM agent activity: release, immobilization, and absorption systems. In a release system, the AM agent (solute or gas) is liberated from the packaging film into the package headspace and onto the food. The immobilization system suppresses the growth of microorganisms on the contact surface. It involves packaging materials in which the AM agent is immobilized within the material. Immobilization systems are considered less effective in the case of solid foods compared with liquid foods because there is generally less contact between an AM package and a solid food product. Antimicrobial agents may be incorporated directly into packaging materials or may be used in vapor form. The vast majority of these AM agents, however, are synthetic materials, such as quaternary ammonium salts, potassium sorbates, sodium benzoates, sodium propionates, sulfites, chlorides, triclosa, nitrites, silver zeolites, copper ions, acetic acid from vinegar, and benzoic acid from cranberries. Unfortunately the synthetic antimicrobial agent may give an off-flavor and consumers are worried about the potential side-effects. To assist in resolving these problems, AM packaging systems have started to use natural AM additives. These agents are classified in naturally occurring polymers (chitosan) and antimicrobial agents from plant (essential oils (EOs)), animal (lysozyme, lactoferrin), and microbial sources (niacin, natamycin) (Davidson and Zivanovic, 2003). The EOs extracted from plant sources, such as flowers, rhizome, roots, bark, leaves, seeds, peel, fruits, wood, resin, berries, and whole plants, contain 85–99% volatile and 1–15% nonvolatile components. The volatile constituents consist of various mixtures including aldehydes (cinnamaldehyde), terpenoids, esters, ketones, acids, alcohols (cinnamic alcohol), methoxy derivatives (anethole, estragole), phenols (eugenol), and methylene dioxy compounds (myristicine). The diversity of EO components is enormous and represents a broad range of compounds. Some are potent antimicrobials, other present interesting antifungal and antioxidant properties. EOs have several targets in the cell. The absorption of these compounds into cellular membranes can eventually lead to disruption of membrane permeability and ion channel function in nerve cells, leading to cardiac and respiratory depression. There are regulatory limitations on the accepted daily intake of EOs or EO components, due to their toxicity related to their lipophilicity and therefore their capacity to pass very efficiently across membranes. Moreover, the food application of EOs can be difficult because of its volatility, reactivity and strong odor (Bakkali et al., 2008). Furthermore, they are insoluble in water, and for certain applications a controlled release is required. Therefore common goals in the development of EO formulations are to protect the EOs from degradation or from losses by evaporation, to achieve a controlled release, and to facilitate handling. Possible formulations include liquid forms (emulsions, micelles, liquid solutions etc.), semiliquid forms (gels, liposomes etc.), and solid forms (microcapsules or microcomposites). Generally volatile oils must be added in small amounts to foods and combined with other preservation technologies such as low temperature, low dose irradiation, high hydrostatic pressure, and modified atmosphere packaging (MAP) in order to prevent deterioration of foodstuffs' sensory properties and obtain the antimicrobial effect. The mode of action of AM agents incorporated in a packaging material is influenced by the controlled and slow release of the agent onto the food surfaces. The absorption mode removes the essential factors of microbial growth from the food systems and inhibits the growth of microorganisms. The EOs antimicrobial potency is reduced in foods with lower water activity and is influenced by the presence of fat, carbohydrate, protein, salt, pH, storage temperature, and the amount of oxygen within the packaging. For example, bovine serum albumin (BSA) neutralizes the antimicrobial activity of thymol, yet oil of clove and tea tree oil were not substantially affected by organic matter or BSA (Davidson and Naidu, 2000). Cilantro oil showed considerable antilisterial action in broth, yet became ineffective against *L. monocytogenes* when used with glycerol monolaurate or lecithin in a gelatin gel coating on ham (Gill et al., 2002). Concerning pH, it is generally accepted that a low pH improves the action of EOs by increasing their hydrophobicity. Nevertheless, this general statement was not confirmed for oregano and thyme EOs which have very high antibacterial activity at pH 4.33–5.32 as well as clary sage, juniper, lemon, and marjoram EOs that have maximal antiyeast activity at pH 7, but it are good also in the acidic range, thus indicating that the ionic form of the EO components do not play a major role for the acidophilus yeasts. Storage temperature is another key factor for the antimicrobial activity of EOs, likely due to a higher partition coefficient of oils and to an enhanced fluidity of the bacterial membrane.

SYSTEMS FOR DELIVERING ANTIMICROBIALS

Figure 1 outlines the main systems for delivering EOs as antimicrobials.

Encapsulation of EOs

Encapsulation is a process in which small particles are enclosed in solid carriers to increase their protection, reduce evaporation, promote easier handling, and control their release during storage and application. EOs exist in liquid form at room

FIGURE 1 Systems for delivering antimicrobials.

temperature. Therefore, the first step of encapsulation techniques consists of emulsifying or dispersing EOs in an aqueous solution. Encapsulation techniques can be divided into three classes: chemical processes like molecular inclusion or interfacial polymerization; physicochemical techniques like conservation and liposome encapsulation; and physical processes like spray drying, spray chilling/cooling, cocrystallization, extrusion, or fluidized bed coating. EOs are entrapped in microcapsules and nanocapsules. Microcapsules are spheres with diameters ranging between a few micrometers and a few millimeters. The size and structure of forming microparticles depend on the wall materials and on the physicochemical properties of the core used. Natural and synthetic polymers are utilized as wall materials. The microcapsules may guarantee excellent protection of EOs, but in general they do not affect antimicrobial activity. Nanoencapsulation is the coating of various substances within another material at sizes in the nanoscale (253.8–415.2 nM). This system is able to increase the passive cellular absorption mechanisms, to reduce mass transfer resistances and to increase antimicrobial activity due to its subcellular size.

Essential Oils Combined with Paper

Paper is the oldest "flexible packaging" material used in food and beverage packaging. Papers and boards are sheet materials made from cellulose fibers derived from wood by using sulfate and sulfite. The fibers are then treated with slimicides and strengthening agents to produce the paper product. Paper and paperboards are biodegradable and have good printability. Coatings such as waxes or polymeric materials can be used to improve their poor barrier properties. Common paper packages include: polyethylene terephthalate, Kraft paper, sulfite paper, greaseproof paper, chipboard, and fibreboard. Barrier properties and the capacity to protect foods depends largely on the permeability of the packaging material to gases and vapors which can be realized with a single layer of polymer or the use of multilayered films including different polymers, coating, and metal foils. Preservative agents must be applied to paper packaging. Many approaches have been proposed to control microbial growth including the use of:

- bacteriocin into polyamide resin in i-propanol/n-propanol against *Micrococcus flavus*;
- nisin into low-density polyethylene (LDPE) film for controlling food spoilage;
- extracts of *Rheum palmatum* and *Coptis chinensis* into LDPE films or silver-substituted inorganic zirconium against aerobic bacteria, lactic acid bacteria, and yeast;
- linalool or methyl chavicol into linear LDPE films against the growth of *E. coli*;
- clove (*Sygzium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), and oregano (*Origanum vulgare*) EOs in the vapor phase into paraffin to inhibit *Candida albicans*, *Aspergillus flavus*, *Eurotium repens*, *Penicillium nalgiovense*, and *Penicillium roqueforti* (Rodriguez et al., 2007).

Essential Oils Combined with Edible Film

Studies looking at the antimicrobial properties of films based on milk proteins, chitosan, or alginates incorporated with various EOs (oregano, rosemary, garlic, pimento, cinnamon) have shown good results. In that regard, chitosan is a valuable

component for producing biodegradable packaging films because it is able to form a film and it has antioxidant and antimicrobial properties. One great advantage of materials based on biopolymers is that they can be used as vehicles for additives, like antioxidant and/or antimicrobial agents, vitamins, flavors, and pigments, thus acting as the compound-releasing packaging that help to improve the quality and increase the shelf-life of the foods. Some studies have considered the incorporation of aqueous plant extracts into the preparation of gelatin edible films, and the formulation of plastic films incorporated with clove EO. The effect of catfish gelatin coating containing Origanum oil on *Salmonella typhimurium* and *E. coli* was also reported.

Essential Oil Vapor and Negative Air Ions

Studies suggest that EOs in vapor phase could be highly effective against surface pathogens and food spoilage bacteria at relatively lower concentrations. The vapor phase approach appears to be promising as a control protocol and could be applied in active packaging, creating a protective atmosphere with minimum organoleptic alteration of the packaged foodstuffs. Synergistic effects between negative air ions (NAIs) and EOs were also observed. Investigations conducted since the mid-1990s have reported that NAI biocidal effects depends on several possible mechanisms of electrical origin (i.e., the action of the ions, the production of ozone, or the action of the electric field) (Fletcher et al., 2007). It was demonstrated that the combination of an ionizing source, such as a candle flame, with a bactericidal volatile has a greater effect on surface-borne bacteria than either treatment alone. The free electrons react with the oxygen to generate the oxygen radicals which promote the transport of ionized EO molecules through the aqueous film and increase the contact with the cell membrane. The potential application of NAI in preventing the decay of fruits and vegetables has been demonstrated (Kumar et al., 2012) in Table 1.

LEGAL ASPECTS OF THE USE OF EOs IN FOODS

In 1958, the United States Food and Drug Administration (FDA) published the food additives amendment in the code of federal regulations. Over the years this panel has periodically reviewed. The crude EOs classified as generally recognized as safe by the FDA include among others clove, marjoram, thyme, nutmeg, basil, mustard, and cinnamon. However, there are regulatory limitations on the accepted daily intake of EOs or EO components due to their toxicity. In Europe, linalool, thymol, eugenol, carvone, cinnamaldehyde, vanillin, carvacrol, citral, and limonene have been accepted by the European Commission (2002/113/EC, 2002; 2004/1935/EC, 2004; 89/107/EEC, 1989) for use as flavorings in food products. Moreover, in November 2011, an official definition of antimicrobial active packaging was introduced in "EU Guidance to the Commission Regulation (EC) No 450/2009 of 29 May 2009."

FOOD PACKAGING PROJECTED TO EXTENDING SHELF-LIFE OF MAIN FOOD CATEGORIES

The potential use of EOs as natural antimicrobials and antioxidants has been reported in meat, fish, dairy product, fruits, vegetables, and cereal products. The literature also provides the possibility of encountering a number of potential synergisms to increase EO effectiveness (Table 2).

ANTIMICROBIAL ACTIVITY OF EOs IN FOOD SYSTEMS PROPOSED TO ENHANCE MEAT QUALITY

It is generally supposed that the high levels of fat and/or protein in meat protect the bacteria from the action of the EOs. Thyme and oregano EOs stand out as having better antimicrobial potential than others for meat applications. Their preservative properties could be attributed to the presence of phenolic compounds, particularly thymol and carvacrol able to sustain a high sensory quality of meat by inhibiting lipid oxidation and bacterial growth such as aerobic bacteria, lactic acid bacteria (facultative anaerobes able to grow under high concentrations of CO_2), Enterobacteriaceae (considered as a hygiene indicator), and *Pseudomonas*. Scientists have suggested several schemes to increase the shelf-life of meat. Among these it is the combined use of nisin with EOs (oregano or thyme oil) in order to decrease the risk related to the presence of *Listeria* sp., Gram-negative, and Gram-positive bacteria in chicken meat. Another advance was the utilization of EO (*Inula graveolens*, *Laurus nobilis*, *Pistacia lentiscus*, and *Stagmomantis montana*) with MAP to reduce risk associated with *Campylobacter* in chicken meat (Djenane et al., 2012) or to enhance lamb meat shelf-life by using thyme EO with MAP (Karabagias et al., 2011). Another method proposed is to incorporate EOs into edible films. Emiroğlu et al. (2010) added thyme and oregano EOs in soy edible films to improve antimicrobial activities against *S. aureus*, *E. coli*, *Pseudomonas* spp.,

TABLE 1 Systems for Delivering Essential Oils and Their Potential Application

Technology	Essential Oil	Potential Application	Activity against	Source
Essential oils combined with paper	Cinnamon EO in paraffin coating	Preservation of fruit and vegetables	*Candida albicans* *Aspergillus flavus*	Rodríguez, A., et al. (2007). Progress in organic coatings. 60 (1), 33–38.
	Cinnamon EO to solid wax paraffin	Preservation of fungi	*Rhizopusstolonifer*	Rodríguez, A., et al. (2008). J. Agric. Food Chem. 56 (15), 6364–6369.
Essential oils combined with edible film	Milk protein-based edible films containing oregano–pimento (1:1) essential oils mix	Preservation of whole beef muscle	*Escherichia coli O157:H7* *Pseudomonas* spp.	Oussalah, M., et al. (2004). J. Agric. Food Chem. 52 (18), 5598–5605.
	Chitosan films enriched with 1 and 2% oregano EO	Preservation of meat product	*Listeria monocytogenes* *Escherichia coli O157:H7*	Svetlana, Z., et al. (2005). J. Food Sci. 70 (1), M45–M51.
	Edible film made from the apple puree solution with oregano, cinnamon, and lemongrass oils	Preservation of fresh-cut salad mixes	*Escherichia coli O157:H7*	Rojas-Graü, M.A., et al. (2006). J. Agric. Food Chem. 29;54 (24), 9262–9267.
	Gelatin–chitosan film incorporating clove essential oil	Preservation of fish during chilled storage	*Pseudomonas fluorescen*, *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Listeria innocua*, *Escherichia coli*, *Lactobacillus acidophilus*	Gómez-Estaca, J., et al. (2010). Food Microbiol. 27 (7), 889–896.
	Whey protein film with cinnamon essential oil	Food preservation	*Lactobacillus lactis*, *Pseudomonas putida*, *Streptococcus agalactiae*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus subtilis*, *Candida albicans*	Bahram, S., et al. (2013). J. Food Proc. Preserv., in press.
	Whey protein isolate-edible films with oregano or clove EO	Preservation of meat and poultry products	*Listeria innocua*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Pseudomona fragi*	Fernández-Pan, I., et al. (2012). J. Food Sci. 7 (7), M383–M390.
EO combined with NAI	*C. Citratus*, *M. arvensis*, *M. piperita* and *E. globulus* oils		*Pseudomonas fluorescens*	Tyagi A., et al. (2010). Micron 41, 797–805.

and coliform bacteria in ground beef patties during refrigerated storage. While Zivanovic et al. (2005) used chitosan–oregano EO films to inhibit *L. monocytogenes* and *E. coli* in bologna slices. Finally, the effectiveness of capsulated rosemary oil activity against *Clostridium botulinum* spores in a vacuum packed and in a pasteurized minced pork product has been studied (Ismaiel and Pierson, 1990).

ANTIMICROBIAL ACTIVITY OF EOs IN FOOD SYSTEMS PROPOSED TO ENHANCE FISH QUALITY

In fish, just as in meat products, a high fat content reduces the effectiveness of antibacterial EOs. The EOs of clove (*Syzygium aromaticum* L.), fennel (*Foeniculum vulgare* Miller), cypress (*Cupressus sempervirens* L.), lavender (*Lavandula angustifolia*), thyme (*Thymus vulgaris* L.), herb-of-the-cross (*Verbena officinalis* L.), pine (*Pinus sylvestris*), and rosemary (*Rosmarinus officinalis*) were tested for their antimicrobial activity on *L. monocytogenes*, *Salmonella enteritidis*, *E. coli*, and *S. aureus* in various agar mediums. The use of EOs added to biofilms, MAP, or vacuum packaging was proposed to improve fish shelf-life.

TABLE 2 Antimicrobial Activity of Essential Oils

Food Categories	Essential Oil	Combined Use with	Activity against
Meat	Thyme oil		Acid bacteria Enterobacteriaceae *Pseudomonas*
	Oregano oil		Acid bacteria Enterobacteriaceae *Pseudomonas*
	Oregano or thyme oil	Nisin	*Listeria* sp. Gram negative Gram positive
	Oregano and thyme oils	Soy edible films	*S. aureus*, *E. coli* *Pseudomonas* spp. Coliform bacteria
	Inula graveolens, *Laurus nobilis*, *Pistacia lentiscus*, and *S. montana*	MAP	*Campylobacte*
	Chitosan oil		*Listeria monocytogenes* *Escherichia coli*
	Rosemary oil		*Clostridium botulinum*
Fish	Clove oil		*Listeria monocytogenes Salmonella enteritidis* *Escherichia coli* *Staphylococcus aureus*
	Fennel oil		
	Lavender oil		
	Thyme oil		
	Herb-of-the-cross oil		
	Pine oil		
	Rosemary oil		
	Citosan oil	Sunflower protein films	*Pseudomonas fluorescens* *Shewanella putrefaciens* *Photobacterium phosphoreum* *Listeria innocua, Escherichia coli,* *Lactobacillus acidophilus*
Dairy product	Clove oil		*Salmonella enteritidis*
	Clove and cinnamon oils	Vanillin	*Listeria monocytogenes, Escherichia coli*
Juice	Mixture of terpenes and D-limonene nanoencapsulated		*Saccharomyces cerevisiae, Escherichia coli* *Lactobacillus delbrueckii*

The gelatin–chitosan and the sunflower protein films incorporated with the clove EO were tested. The first system inhibited the growth of *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Listeria innocua*, *E. coli*, and *Lactobacillus acidophilus*. The second system, applied to the preservation of refrigerated sardine, showed a retard in lipidic autooxidation and an impediment to the total mesophiles growth. As regards the application of MAP combined with EO, two studies have reported their positive effects on processed fish. Kostaki et al. (2009) described the use of thyme oil in combination with two different gas mixtures (40% CO_2/50% N_2/10% O_2; and 60% CO_2/30% N_2/10% O_2) in order to preserve the quality and shelf-life of fresh filleted sea bass during refrigerated storage ($4 \pm 0.5\,^{\circ}C$) for a period of 21 days. Del Nobile et al. (2009) referred to the possibility to improve the quality of blue fish burgers by using thymol and extracts of grapefruit seed and lemon in combination with MAP. Finally, thyme and garlic oil were combined to vacuum packaging to enhance the shelf-life under refrigeration of hot smoked rainbow trout (Shentian, 2012) and oregano oil was added vacuum packaging to preserve Mediterranean octopus (Atrea et al., 2009).

ANTIMICROBIAL ACTIVITY OF EOs IN FOOD SYSTEMS PROPOSED TO ENHANCE DAIRY PRODUCT QUALITY

Dairy products are milk and any of the foods made from milk, including butter, cheese, ice cream, yogurt, and condensed and dried milk. The use of EOs can be considered a tool for increasing the safety of pasteurized milk and its derivatives. A high water and/or salt level facilitates the action of EOs. Experimental studies have demonstrated that pasteurized milk will support growth of *L. monocytogenes* and *E. coli O157:H7*, microorganisms that could grow in milk under refrigeration temperatures. Vanillin (a low molecular weight that is the primary component of *Vanilla* genus) and mixtures of clove and cinnamon leaves with vanillin showed effective antimicrobial action against these pathogens. The aldehyde group of vanillin is believed to be responsible in part for its antimicrobial activity. Instead, a reaction between carvacrol and proteins is a limiting factor in the antibacterial activity against *B. cereus* in milk (Pol et al., 2001), as well as in diluted low-fat cheese, when clove oil is used as an antimicrobial against *S. enteritidis* (Smith-Palmer et al., 2001).

ANTIMICROBIAL ACTIVITY OF EOs IN FOOD SYSTEMS PROPOSED TO ENHANCE MINIMALLY PROCESSED FRUIT AND VEGETABLE QUALITY

Whole fruits and vegetables are perishable because they are affected by postharvest fungal diseases, insect infestation, physiological activities that occur in them after harvest, and other pathological breakdown processes. MAP active packaging was used to prolong organoleptic and functional properties together with safety of fruit products. Unfortunately, CO_2 concentration inside packages could not be high enough to act as a fungicide or bactericide, therefore the role of EOs serves to enhance these characteristics. The antimicrobial activity of EOs in vegetable dishes benefits from a decrease in storage temperature and/or a decrease in the pH of the food. The effect of EOs on increasing or maintaining total phenolics and total antioxidant activity has also been found using thymol in table grapes or thymol, eugenol, and menthol in strawberry and sweet cherry. Noteworthy in this respect is the work presented by Arrebola et al. (2010) in which the authors, in order to reduce the taste resulting from the use of EOs, combined an antagonist (*Bacillus amyloliquefaciens*) with lemon EO and MAP to further improve the performance of packaging. Galeta et al. (2013) proposed improving the stability of a mixed vegetable salad with an antimicrobial active packaging consisting of a polypropylene EVOH copolymer film that released oregano EO or control combined with MAP. Another strategy was recommended by Yage et al. (2012). They investigated the effect of cinnamon oil fumigation and moderate vacuum packaging on the shelf-life of fresh-cut lotus root at 4 °C. The result showed that the combined treatment samples prevented product retarded the microbiological deterioration of fresh-cut lotus root. Two schemes were proposed for incorporating EO into fruits in order to increase the antioxidant status and reduce bacterial growth: the addition of EOs to films both edible pectin, and paraffin-based paper packaging or their incorporation into salt. The zeolite-EO composition can be sprayed over growing crops or freshly cut fruits. The use of paraffin-based paper packaging combined to cinnamon EO was most recommended by Rodriguez-Lafuente et al. (2010) to extend the shelf-life of the cherry tomato. The use of garlic oil incorporated into soy protein isolate-coated oriented polypropylene/polyethylene packaging was proposed to extend the shelf-life of fresh sprouts (Gayani et al., 2009).

ANTIMICROBIAL ACTIVITY OF EOs IN FOOD SYSTEMS PROPOSED TO ENHANCE JUICE QUALITY

The use of some EOs in fruit juices is not recommended because of their adverse effect on the sensory properties. Thus, combinations with other preservation methods are needed to diminish their impact on food flavor. A novel approach for EOs use in foods was proposed by Donsì et al. (2011) who used a nanoencapsulation system (sunflower and palm oils as organic phases; glycerol monooleate, soy lecithin, Tween 20 and Cleargum(R) as emulsifying agents) for the entrapment of a mixture of terpenes and D-limonene. They examined the antimicrobial activity of this system toward *Saccharomyces cerevisiae*, *E. coli*, and *Lactobacillus delbrueckii* and found a higher effect of nanoencapsulated compounds in pear and orange juices in preserving the sensorial properties of juices.

ANTIMICROBIAL ACTIVITY OF EOs IN FOOD SYSTEMS PROPOSED TO ENHANCE CEREAL-BASED FOOD QUALITY

In bakery products, fungi are the most common spoilers. In unpreserved bread a shelf-life of 3–4 days may be expected especially if the hygiene in the factory is not sufficiently high. The spoilage of bakery products is mainly caused by molds

and yeasts, only occasionally by bacteria. Fungi are responsible for off-flavor formation and production of mycotoxins and allergenic compounds, which may be formed even before growth is visible. An active packaging with cinnamon EO combined with MAP was tested to increase the shelf-life of gluten-free sliced bread. The results showed that active packaging considerably increases the shelf-life of packaged food maintaining the sensory properties of the gluten-free bread. Another strategy was proposed by Suhr and Nielsen (2005). They combined MAP with vaporous mustard oil to inhibit spoilage organisms in wheat and rye breads. The results were the desiderata inhibition, but a high mustard oil dosing, was required for wheat bread with possible sensory quality implications. Finally, active paper package based on the incorporation of cinnamon EO to solid wax paraffin as an active coating of bread is proposed by Rodríguez et al. (2008). The use of a cellulose film with oregano EO was suggested for pizza packaging remains of great interest, since oregano spice is a pizza ingredient and the packaging would not compromise the product flavor and aroma.

The addition of citrus EOs to a low-density polyethylene films was suggested by Dias et al. (2013) as flavoring films to use as biscuit packaging due to its ability to prevent changes in water vapor permeability and mechanical properties through time. Lime EO was proposed as natural antimicrobial preservative in cream-filled cakes and pastries and it can be used to decrease the risk of food poisoning associated with consumption of this product (Jafari et al., 2011).

SUMMARY POINTS

- AM packaging systems use both synthetic and natural AM additives.
- There are three systems of AM agent activity: release, immobilization, and absorption systems.
- The essential oils antimicrobial potency is reduced in foods with lower water activity and is influenced by the presence of fat, carbohydrate, protein, salt, pH, storage temperature, and the amount of oxygen within the packaging.
- Systems for delivering antimicrobials are microencapsulation, paper and edible film, and vapor.
- The strategies proposed to enhance meat shelf-life are the combined use of EOs with nisin, biofilm, or vacuum packaging.
- The strategies proposed to enhance fish shelf life are the combined use of EOs with biofilm, MAP, vacuum packaging.
- Strategies proposed to enhance fruits and vegetable shelf-life include the combined use of EOs with biofilm or MAP.
- The strategies proposed to enhance cereal-based food product shelf-life are the combined use of EOs with biofilm, paper film, or MAP.

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Chapter 15

Essential Oils Added to Edible Films

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List of Abbreviations

EF Edible film
EO Essential oil

INTRODUCTION

Consumer interest in acquiring foods that are safer, more nutritious, and healthier, combined with concerns regarding the environment have led to research efforts into edible films as an alternative to fulfill these requirements. There are abundant renewable sources to be used as edible film-forming agents, which may potentially reduce the need of films made from synthetic materials. Edible films have the potential to expand the possibility of improving the quality of heterogeneous foods by controlling the migration of moisture, fat, flavors, and colors among food components or to the outside, and therefore maintain the quality of food after opening its package. Edible film applications have advantages such as biocompatibility, aesthetic appearance, barrier properties, and functionality, such as the addition of antioxidants and antimicrobial compounds, as well as other functional additives (Cha and Chinnan, 2004; Marsh and Bugusu, 2007).

Considering that in some foods, microbial growth develops on their surfaces and this growth is the main cause of their deterioration, the development of antimicrobial edible films is a promising research area. Nevertheless, direct application of antimicrobial agents in foods by spraying, in the form of powder, or other methods have limited benefits due to the active substances being neutralized by contact or quickly diffusing from the surface into the product (Cagri et al., 2004). The addition of antimicrobials like essential oils (EOs) into edible films has many advantages such as the need for low concentrations of antimicrobial and low diffusion rates. Hence, the aim of this chapter is to describe and discuss the scientific basis of edible film formation, and several applications that have been developed due to the addition of different EOs to edible films.

GENERAL ASPECTS OF EDIBLE FILMS

Edible films and coatings are defined as continuous matrices that may be made of proteins, polysaccharides, and/or lipids. Edible films and coatings are not intended to replace synthetic packaging foods designed for long storage. Their utility lies in their ability to act as an accessory that improves food quality, extending shelf-life, and the possibility of increasing the efficiency of food packaging by incorporating various substances that enhance the functional properties of edible films and coatings. The difference between an edible film and a coating lies in the preparation method. Coatings are formed directly on the surface of food while films are made separately (Srinivasa and Tharanathan, 2007).

Edible films and coatings have three main components: polymer(s), solvent, and plasticizers. Edible films are prepared from polymers as their major component, such as hydrocolloids or lipids, and composite films. When made from hydrocolloids, a range of proteins and polysaccharides such as starch, alginate, cellulose derivatives, chitosan, or agar derivatives can be used (Table 1). Waxes, acylglycerols, and fatty acids are the commonly used lipids. Composite films are a mixture of hydrocolloids and lipids, together with other ingredients that may vary in their concentration, that determines their mechanical and barrier properties (Cha and Chinnan, 2004). It is necessary to use a solvent that is safe and suitable for food; generally water with different pH values to solubilize the polymer is used and, on rare occasions, ethanol solutions. Finally, the main objective of the plasticizer is to emulsify phases that are not miscible, in addition to imparting particular characteristics to the film such as flexibility and resistance. Plasticizers soften the rigid film structure by increasing the

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TABLE 1 Polymers Frequently Used for Edible Film and Coating Formation

Alginates	Cellulose	Gelatin	Sunflower proteins
Amaranth	Chitosan	Maltodextrins	Triticale
Arabic gum	Collagen	Pectin	Wheat gluten
Carragenin	Corn zein	Soy protein	Whey protein
Casein	Fatty acids	Starch	Waxes

mobility of the polymer chains, reducing the intermolecular forces, and improving mechanical properties such as elongation. It is important that the plasticizer has a low molecular weight, a high melting point, and is miscible in the polymer (Cooksey, 2005). Food-grade plasticizers like sorbitol, glycerol, mannitol, sucrose, and polyethylene glycol are commonly applied. Water can be used as a plasticizer but affects the film moisture content. In films made from lipid and hydrocolloid mixtures, plasticizers reduce film gloss and interfer with inter and intramolecular bonds due to hydrogen bonds between the lipid phase and the hydrocolloid causing a decrease in strength force and glass transition temperature (Cagri et al., 2004; Srinivasa and Tharanathan, 2007).

EDIBLE FILM FORMATION AND FUNCTIONAL PROPERTIES

Different types of bonds (ionic, covalent, hydrogen bonding, electrostatic interactions, and van der Waals forces) are generally involved in film formation among polymer chains to form a semirigid network that traps the solvent. There are two kinds of forces at the molecular level: a cohesive force that acts between polymer molecules and an adhesion force that exerts between the film and the support. High molecular weight polymers have enough cohesive strength and coalescing capacity to produce film structures with different solvents. Cohesion affects film properties such as density, compactness, porosity, permeability, flexibility, and opacity. Cohesion forces depend on polymer structure, solvent, temperature, and type and concentration of plasticizer. Cohesion and adhesion are related to polymer chain structure, chemical composition, molecular weight, structural regularity of the chains, branches, and polar group distribution. Polymer chains enhance film cohesion and rigidity. Polar groups reduce molecular diffusion but promote arranged side chain formation. Molecular order is promoted by crosslinks due to denaturation and by the incorporation of some additives. Polymer functionality is also related to solvent characteristics, polymer solvation, and chain extension that improve film formation (Srinivasa and Tharanathan, 2007).

There are different techniques to form edible films: solvent removal, thermal gelation, and solidification. The first is used to produce hydrocolloids films, and it forms a homogeneous structure stabilized by intermolecular chemical and physical interactions. In the second technique, heat treatment is applied which forms with the plasticizer a rigid and stable gel structure. In solidification, macromolecules are dissolved in a solvent along with the plasticizer and homogenized; the mixture is placed in dishes to form a thin layer and dried to release it from the dish surface (Cagri et al., 2004).

Polysaccharide films (Table 2) are selectively permeable to CO_2 and O_2, therefore, slow respiration of many fruits and vegetables could be enhanced by limiting oxygen; these hydrophilic films attract water vapor, preventing condensation of water, which is a potential source for microbial growth (Cha and Chinnan, 2004).

TABLE 2 Functional Properties of Edible Films and Coatings

Delay moisture migration	Impart additional integrity to food structure
Retard gas transport (O_2 and CO_2)	Retain aroma volatiles
Retard fat and oil migration	May contain additives such as antioxidants and antimicrobials
Improve food properties	

ESSENTIAL OILS USED IN EDIBLE FILMS

In order to provide microbiological stability to foods, edible films can incorporate antimicrobial agents, which include benzoates, propionates, parabens, sorbates, curing agents, enzymes, bacteriocins, and EOs among many others. These

compounds extend product shelf-life and reduce the risk of growth of pathogens in food surfaces (Cagri et al., 2004; Min and Krochta, 2005; Lin and Zhao, 2007; Ozdemir and Floros, 2008).

It is clear that consumers demand more natural or mildly processed products with long shelf-life but without chemical preservatives. In this search, a wide range of natural preservative systems from animals, plants, and microorganisms are being studied. Naturally occurring antimicrobial compounds like EOs are abundant in the environment; while some of these antimicrobial systems are currently employed for food preservation, many are just being studied for their possible use in foods. When EOs are incorporated to edible films they are expected to migrate onto food surface or stay retained in the film, both cases determine the antimicrobial effectiveness of the edible film. EO migration depends on electrostatic interactions between the antimicrobial and the polymer chains, ionic osmosis phenomena, possible structural changes induced by the EO, and the environmental conditions to which edible films are exposed (Min et al., 2008). EO diffusion on edible films is influenced by the polymer, plasticizer, elaboration process, surface food characteristics such as pH and water activity, hydrophilic film properties, temperature, and storage time (Lin and Zhao, 2007; Ozdemir and Floros, 2008). EOs with different chemical properties interact with film structure, polymer, and plasticizer, and prevent the EO migration (Cha and Chinnan, 2004; Min et al., 2008). In the case of protein-based polymers, some EO major components could be trapped within the film (due to amino acid–EO interactions); as a result, the antimicrobial activity could be affected. To counteract this effect, high concentrations of EO must be added to the film, promoting saturation of the system and thus allowing the presence of free molecules of EO. Films with similar composition, like those from whey proteins, present better retention of cyclic molecules (thymol and carvacrol) compared with linear molecules (sorbic acid). For this reason, films with linear molecules present higher rates of diffusion, therefore, greater antimicrobial action (Cagri et al., 2004). When EO content increases, the greater molecular contact between methyl groups and oil compounds may weaken the polymer chain aggregation forces, making the matrix more open. Barrier properties of films added with EO increase when its concentration increases. This could be due to the relatively hydrophilic nature of the main components of these EOs and their negative effect on the cohesion forces of the methyl group matrix. Bonilla et al. (2012) discovered that at low EO concentration, the effect of the overall increase in the hydrophobic nature of the matrix predominated over the effect of the loss of matrix cohesion, limiting water vapor transport.

Numerous studies have examined the antimicrobial effectiveness of different EOs incorporated in edible films against multiple food-borne pathogenic and spoilage microorganisms. These studies are divided into two major groups: in vitro and in vivo (food applications).

In vitro Studies

Valuable research studies regarding EO added to edible films are listed in Table 3. Zivanovic et al. (2005) incorporated different concentrations of EOs of oregano, basil, or anise into chitosan films and observed an inhibitory effect on *Listeria monocytogenes* and *Escherichia coli* O157:H7. Rojas-Graü et al. (2007) reported similar results for alginate–apple films added with lemongrass, cinnamon, or oregano EOs. Maizura et al. (2007) applied lemongrass EO to starch films in which water vapor and gas barrier properties were unaffected. Avila-Sosa et al. (2010) added Mexican oregano (*Lippia berlandieri* Shauer) EO to amaranth, chitosan, or starch edible films. Chitosan and starch films inhibited fungal growth of *Aspergillus niger* and *Penicillium* spp. at EO concentrations below 1%. *Zataria multiflora* Boiss EO was added to sodium caseinate edible films (Broumand et al., 2011), being more effective on Gram-positive (*Staphylococcus aureus*) than Gram-negative bacteria (*E. coli* O157:H7 and *Salmonella thyphimurium*). Sánchez-González et al. (2011a) incorporated different concentrations of bergamot, lemon, or tree tea EO into chitosan and hydroxypropylmethylcellulose films. Only tea tree EO had a bacteriostatic effect for *E. coli, S. aureus*, and *L. monocytogenes*. The nature and amount of EO, structure of the film, and possible interactions between polymers and active constituents of EOs affected the antimicrobial activity of the films. Ahmad et al. (2012) added bergamot or lemongrass EO to gelatin films. Films with lemongrass EO showed inhibitory effect in a concentration dependent way against *E. coli, S. aureus, L. monocytogenes*, and *S.* Thyphimurium, while bergamot EO only inhibited *L. monocytogenes* and *S. aureus*. Both EOs did not inhibit *Pseudomonas aeruginosa*. Aguirre et al. (2013) used oregano (*Origanum vulgare* L.) EO on triticale protein films; higher antimicrobial activity was reported against Gram-positive bacteria and lower antimicrobial activity on Gram-negatives such as *P. aeruginosa*. Pires et al. (2013) investigated the antimicrobial properties of citronella, coriander, tarragon, and thyme EOs added to hake protein films. Studied films added with thyme EO presented bactericidal activity on *Shewanella putrefaciens*, films with citronella EO inhibited *Pseudomonas putida* and films added with tarragon EO were effective against *Brochothrix thermosphacta*. Shojaee-Aliabadi et al. (2013) made κ-carragenin films incorporated with *Satureja hortensis* EO; these films were capable to inhibit *S. aureus, B. cereus, E. coli, S.* Thyphimurium, and

TABLE 3 In vivo Studies of Edible Films with Added Essential Oils

Essential Oils	Edible Films	Microorganisms Inhibited	References
Anise, basil, and oregano	Chitosan	*Escherichia coli* O157:H7 and *Listeria monocytogenes*	Zivanovic et al. (2005)
Cinnamon, lemongrass, and oregano	Alginate-apple puree	*Escherichia coli* O157:H7 and *Listeria monocytogenes*	Rojas-Graü et al. (2007)
Lemongrass	Starch	*Escherichia coli* O157:H7 and *Listeria monocytogenes*	Maizura et al. (2007)
Mexican oregano	Amaranth, chitosan, and starch	*Aspergillus niger* and *Penicillium* spp.	Avila-Sosa et al. (2010)
Zataria multiflora Boiss	Caseinate	*Staphylococcus aureus, Escherichia coli* O157:H7 and *Salmonella thyphimurium*	Broumand et al. (2011)
Bergamot, lemon, and tea tree	Chitosan and hydroxypropylmethylcellulose	*Staphylococcus aureus, Escherichia coli*, and *Listeria monocytogenes*	Sánchez-González et al. (2011b)
Bergamot and lemongrass	Gelatin	*Staphylococcus aureus, Escherichia coli, Listeria monocytogenes*, and *Salmonella thyphimurium*	Ahmad et al. (2012)
Oregano	Triticale protein	*Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*	Aguirre et al. (2013)
Citronella, coriander, tarragon, and thyme	Hake protein	*Shewanella putrefaciens, Pseudomonas putida*, and *Brochotrix thermosphacta*	Pires et al. (2013)
Cinnamon	Casava starch	*Penicillium commune* and *Eurotium amstelodami*	Souza et al. (2013)
Satureja hortensis	κ-carragenin	*Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella thyphimurium*, and *Pseudomonas aeruginonsa*	Shojaee-Aliabadi et al. (2013)

P. aeruginosa by direct contact and in vapor phase. Souza et al. (2013) added cinnamon EO to cassava starch edible films that exhibited antifungal activity against *Penicillium commune* and *Eurotium amstelodami*, molds commonly found in bread products.

In vivo Studies

Research has also been developed regarding films added with EOs and applied as coatings to different foods (Table 4). Oussalah et al. (2004) coated whole beef muscle with a milk protein-based film added with oregano and pimento EOs, inhibiting *E. coli* O157:H7. These researchers also developed, for the same food, alginate coatings added with oregano and cinnamon EOs. Results showed that whole beef muscle shelf-life was prolonged by 10% and there was a 0.95 to 1.34-log cycle reduction of *Pseudomonas* spp. (Oussalah et al., 2006). Oregano (*O. vulgare* L.) EO was applied to chitosan coatings in bologna (Chi et al., 2006), increasing the storage time and microbial inhibition (*Pseudomonas* spp.). No evidence of phenolic substance migration to food was found in these products and, in both cases, food products achieved a good level of sensory acceptance.

Sánchez-González et al. (2011b) applied chitosan or hydroxypropylmethylcellulose films added with bergamot EO to table grapes. The most recommended coating for muscatel grapes is chitosan. Despite only slightly contributing to sample color, these films showed antimicrobial activity and greatest control of respiration rates with a reasonably good control of water loss during storage. Total mesophilic, yeast, and mold populations decreased on fruit surfaces. Maqbool et al. (2011) controlled anthracnose on banana and papaya during cold storage using arabic gum edible coatings added with lemongrass and cinnamon EOs. Their study revealed that 0.4% cinnamon oil was the optimal concentration in controlling decay (80%) and recommended its use as a biofungicide for controlling postharvest anthracnose on major tropical fruits. Perdones et al. (2012) prepared chitosan coatings with added lemon EO and applied it to strawberries, enhancing antifungal activity during

TABLE 4 Applications (In vitro Studies) of Edible Coatings with Added Essential Oils

Essential Oils	Edible Coatings	Food Matrices	References
Oregano and pimento	Milk protein	Whole beef muscle	Oussalah et al. (2004)
Oregano and cinnamon	Alginate	Whole beef muscle	Oussalah et al. (2006)
Oregano	Chitosan	Bologna	Chi et al. (2006)
Bergamot	Chitosan and Hydroxypropylmethylcellulose	Table grapes	Sánchez-González et al. (2011b)
Cinnamon and lemongrass	Arabic gum	Banana and papaya	Maqbool et al. (2011)
Lemon	Chitosan	Strawberries	Perdones et al. (2012)
Clove	Sunflower protein	Fish patties	Salgado et al. (2013)

cold storage against *Botrytis cinerea*. Salgado et al. (2013) applied sunflower protein coatings with clove EO on fish patties, inhibiting deteriorative microorganisms and prolonging the shelf-life of patties.

CONCLUDING REMARKS

Application of EOs to edible films and coatings provides opportunities to develop new food preservation systems. Compared with direct application of EO to food surfaces, EO films and edible coatings are more effective. In addition to these antimicrobial properties, the application of EO edible films could improve the sensory, mechanical, and nutritional properties of food, as well as protecting it (generally small pieces), and highlights the importance of additional studies regarding applications of EO edible films and coatings.

SUMMARY POINTS

- Edible films and coatings may act as an accessory to improve food quality and extend shelf-life.
- Edible films have the possibility to incorporate EOs and provide microbiological stability to the food.
- Application of EOs to edible films and coatings provides opportunities to develop new food preservation systems.
- EO films and edible coatings are more effective than direct application of EO to food surfaces.
- Antimicrobial effectiveness of different EOs incorporated in edible films against multiple food-borne pathogenic and spoilage microorganisms has been demonstrated.

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Chapter 16

Essential Oils in Food Applications: Australian Aspects

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INTRODUCTION

Aromatic plants and their essential oils (EOs) have been used since ancient times in food, agriculture, medicine, cosmetic applications, as condiments and spices, in therapeutic uses, as antimicrobials, as flavoring agents, and in storage as insecticidal agents (Bakkali et al., 2008). The history of using EOs as traditional medicines in Australia also dates back thousands of years. Reported studies on the use of emu bush (*Eremophila longifolia*) by indigenous Australians as a sterilizing agent by burning of aerial parts of the plant over hot fire embers suggests antimicrobial properties of the volatiles present in this plant material (Sadgrove and Jones, 2013). The landing of the first fleet by Captain Cook in 1788 also led to the discovery of the Australian tree, *Eucalyptus piperita*, or the Sydney peppermint and the close resemblance of the EO from this tree to that of *Mentha piperita* from England known for its peppermint aroma (G.T.M., 1937). *Eucalyptus* (family *Myrtaceae*), a native of Australia represented by over 700 species, is one of the commercially important sources of EO globally and is used extensively in the food, flavor, fragrance, and pharmaceutical industries (Batish et al., 2008). Some of the other genera within the Australian *Myrtaceae* family with EOs of economic importance are *Melaleuca*, *Callistemon*, *Leptospermum*, *Kunzea*, *Backhousia*, *Syzygium*, *Baeckea*, and *Angophora*. This chapter reviews the application of Australian native EOs in food and agriculture and their chemical properties.

AUSTRALIAN NATIVE ESSENTIAL OILS

EOs are formed by aromatic plants as secondary metabolites; they are volatile and made up of a range of complex natural compounds, characterized by a strong odor. There are several methods for extracting EO, including steam and hydrodistillation, and liquid carbon dioxide. The extraction product can vary in quality, yield, and composition depending on climate, soil composition, plant organ, age, and vegetative cycle stage (Angioni et al., 2006; Bakkali et al., 2008). A study on the EOs from *Leptospermum* species revealed significant differences in the oil yield for *Leptospermum polygalifolium wallum* based on locational variation. *Leptospermum liversidgei* showed higher percentage oil yield in spring, in contrast to all other species that showed higher percentage yields in the summer (Windsor and Brooks, 2012).

The complex mix of natural compounds within EOs can be divided into two groups. The main group is made up of terpenes and terpenoids, and the second group of aromatic and aliphatic compounds, all of low molecular weight (Bakkali et al., 2008). The complex mix can contain about 20–60 components at different concentrations. They are generally characterized by two or three major components at fairly high concentrations compared with the other components which are found in trace amounts. For example, α-terpinene (10%), γ-terpinene (21%), and terpinen-4-ol (43%) are the major components of *Melaleuca alternifolia* EO; piperitone (43%) and α-phellandrene (30%) in *Eucalyptus dives*; anethole (90%) in *Syzygium anisatum*; and citral (97%) in *Backhousia citriodora*. The chemical composition of some EOs belonging to the family *Myrtaceae* is given in Table 1.

ANTIMICROBIAL AGENTS IN FOOD APPLICATIONS

Consumers are looking for fewer synthetic additives in food and are aware of clean labels and preservation using natural additives, and this underpins the need of finding alternative sources of safe and effective preservatives (Burt, 2004).

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TABLE 1 Major Chemical Components of Selected Australian *Myrtaceae* Native EOs

Common Name of EO	Latin Name of Plant Source	Major Chemical Components	% Composition	References
Tea tree	*Melaleuca alternifolia*	α-Terpinene	10.0	Callander and James (2012)
		γ-Terpinene	20.9	
		Terpinen-4-ol	43.0	
Lemon scented tea tree	*Leptospermum petersonii*	Citral	55.1	Kurekci et al. (2013)
		Citronellal	18.1	
Lemon myrtle	*Backhousia citriodora*	Neral	42.6	Kurekci et al. (2013)
		Geranial	54.6	
Broad leaf eucalyptus	*Eucalyptus dives*	Piperitone	42.9	Oussalah et al. (2006)
		α-phellandrene	30.0	
Narrow leaf melaleuca	*Melaleuca linariifolia*	Terpinen-4-ol	30.2	Oussalah et al. (2006)
		γ-Terpinene	18.6	
		1,8-cineole	14.2	
Nerolidol niaouli	*Melaleuca quinquenervia nerolidolifera*	Nerolidol	90.2	Oussalah et al. (2006)
Lemon Ironbark	*Eucalyptus staigeriana*	1,8-cineole	34.8	Gilles et al. (2010)
		Neral	10.8	
		Geranial	10.8	
Strawberry gum	*Eucalyptus olida*	(E)-Methyl cinnamate	99.4	Gilles et al. (2010)
Lemon scented gum	*Eucalyptus citriodora*	Citronellal	68.9	Lee et al. (2008)
Bottle brush plant	*Callistemon lanceolatus*	1,8-cineole	65.0	Shukla et al. (2012)
		α-pinene	20.9	
Blue gum	*Eucalyptus globulus*	1,8-cineole	45.4	Tyagi and Malik (2011)
		Limonene	17.8	
Anise myrtle	*Syzygium anisatum*	anethole	89.9	Blewitt and Southwell (2000)

EOs fall into this group of natural additives as they are obtained from plants and antimicrobial activity against a broad spectrum of bacteria and fungi has been well demonstrated. There has been an increase in the reported studies using EOs as natural preservatives to extend the shelf-life of food. EOs are also used as fumigants in the postharvest treatment of fungal diseases in fruit to fulfill the needs of consumers and regulatory agencies addressing the issues of food safety and the environment. Furthermore, the advantage of using EOs is their bioactivity in the vapor phase, a feature that is important for use as possible fumigants for stored product protection (Tripathi and Dubey, 2004). The effectiveness of EO of Australian lemon myrtle (*B. citriodora*), cinnamon bark (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*), thyme oil (*Thymus vulgaris*), clove bud (*Eugenia caryophyllata*), valerian (*Valeriana officinalis*), and Australian tea tree oil (*M. alternifolia*) in controlling brown rot caused by the fungus *Monilinia fructicola* in nectarines was evaluated. This study revealed lemon myrtle EO as the most potent fumigant to control the mycelium growth and spore germination (Lazar-Baker et al., 2011).

Callistemon lanceolatus was assessed for antifungal properties, aflatoxin inhibition, antioxidant activity, and phytoxicity against fungi isolated from chickpea seeds. *Aspergillus flavus* was the most predominant fungi, followed by *Aspergillus niger* and *Penicillium citrinum*, and to a lesser extent *Fusarium* sp., *Mucor* sp., *Chaetomium* sp., *Drechslera* sp., and *Trichoderma* sp. All the fungal species were completely inhibited at 0.918 mg/mL of the EO but required a higher concentration of 1.285 mg/mL of 1,8-cineole alone. Similarly, aflatoxin production was inhibited at 0.546 mg/mL of the EO and 0.918 mg/mL of 1,8-cineole. This suggests that the enhanced antifungal and aflatoxin inhibitory effects of *C. lanceolatus*

EO could be due to the minor components having a synergistic effect with the major components rather than 1,8-cineole as the major component. This study also confirmed the antioxidant activity of *C. lanceolatus* EO, with no adverse effects on seed germination suggesting its nonphytotoxic nature (Shukla et al., 2012). These results indicate the potential of using *C. lanceolatus* EO as a plant-based food preservative for use in extending the storage life of pulses and legumes.

Botrytis cinerea (gray mold) and *Rhizopus stolonifer* (soft rot) are the two major fungal pathogens affecting strawberries during storage. Tea tree EO significantly reduced spore germination and mycelia growth of these fungi. Treatment of tea tree EO, vapor concentration at 0.9 g/L in air for 3 h, is suitable for strawberry preservation. Tea tree EO is classified as generally regarded as safe by the United States Food and Drug Administration (Shao et al., 2013).

The antimicrobial activity of *Eucalyptus globulus* EO was evaluated against food spoilage microorganisms in liquid and vapor phases. The minimum inhibitory concentration (MIC) varied from 2.25 to 9 mg/mL for bacterial and fungal strains, and from 1.13 to 2.25 mg/mL for yeast strains. A significantly higher antimicrobial activity was observed in the vapor phase (Tyagi and Malik, 2011).

The inhibitory effect of 60 EOs on *Pseudomonas putida* a food spoilage bacteria in meat was evaluated. *Melaleuca linariifolia* showed a high antibacterial activity with a minimum inhibitory concentration of 0.2%, indicating its potential use in meat preservation (Oussalah et al., 2006). The EOs of three *Eucalyptus* species, namely *Eucalyptus olida*, *Eucalyptus staigeriana*, and *E. Dives* were evaluated for antimicrobial properties against Gram-positive *Enterococcus faecalis* and *Staphylococcus aureus*, Gram-negative *Escherichia coli* and *Psuedomonas aeruginosa*, and the yeast *Candida albicans*. Gram-positive bacteria were more sensitive to the EOs than Gram-negative. *Eucalyptus staigeriana* was the most effective in inhibiting the growth of the microorganisms, especially against the Gram-positive bacteria. This suggests the potential applications of *E. staigeriana* as a natural antimicrobial in food (Gilles et al., 2010).

Eliminating or reducing *Campylobacter jejuni*, a common cause of human gastrointestinal illness, from the chicken gut before slaughter is one possible approach that may lead to the production of carcasses free from microorganisms. Plant antimicrobials such as EOs are being evaluated for anti*Campylobacter* activity. A study to assess the antimicrobial activity of three EOs, *M. alternifolia*, *B. citriodora*, and *Leptospermum petersonii*, were tested against *C. jejuni*. All three EOs revealed strong anti*Campylobacter* activity and was comparable to streptomycin and ampicillin two standard antibiotics. This study suggests the potential of using EOs to control *C. jejuni* in poultry (Kurekci et al., 2013). A study by Wilkinson and Cavanagh (2005) assessed the antimicrobial activity of 18 Australian EOs from six genera (*Syzygium*, *Callitris*, *Eremophilia*, *Eucalyptus*, *Melaleuca*, and *Thyptomine*) against *E. coli*, *Salmonella typhimurium*, *S. aureus*, *P. aeruginosa*, *Alcaligenes faecalis*, and the yeast *C. albicans*. All bacteria, with the exception of *P. aeruginosa* were susceptible to one or more of the EOs at 100% with only *Eremophila mitchelli* inhibiting the growth of any bacteria at 1%. In a similar study by Sultanbawa et al. (2010), eight Australian EOs, blue mallee (*Eucalyptus polybractea*), blue gum (*E. globulus*), narrow leaf peppermint (*Eucalyptus australiana*), lemon ironbark (*E. staigeriana*), lemon scented gum (*Eucalyptus citriodora*), broad leaf eucalyptus (*Eucalyptus dives*), lemon myrtle (*B. citriodora*), and anise myrtle (*S. anisatum*) were tested at concentration levels from 0.0006 to 1.25% (v/v). The EOs were evaluated against the following food spoilage microorganisms: *Proteus vulgaris*, *P. aeruginosa*, *Enterobacter aerogenes*, *Acinetobacter baumannii*, *Shewanella putrefaciens*, *Saccharomyces cerevisiae*, *Geotrichum candidum*, and food-borne pathogens *Bacillus cereus*, *Listeria monocytogenes*, *E. coli*, and *S. aureus*. MIC values are given in Table 2.

Lemon myrtle EO indicated the greatest inhibitory effect against the tested microorganisms. It was most effective against the yeasts which were completely inactivated at concentrations of 0.08% (v/v). Gram-negative bacteria required a higher concentration for inhibition than Gram-positive. *Pseudomonas aeruginosa* required the highest concentration, >1.25% (v/v), for inhibition.

INSECT REPELLENTS IN AGRICULTURE

Increasing problems with today's fumigants and the detrimental effects they have on the environment and insect resistance, especially in stored grains, make it necessary to seek environmentally friendly alternatives. Aromatic plants in different preparation formats such as powders, solvent extracts, EOs, and whole plants are being evaluated for their insecticidal activity, as repellants, antifeedants, and insect growth regulators. However, it is suggested that the fumigant activity of these plants can have a greater potential than grain protectants for large-scale storage applications (Isman, 2000). A study of 42 EOs extracted from seven Australian *Myrtaceae* genera (*Eucalyptus*, *Melaleuca*, *Callistemon*, *Leptospermum*, *Kunzea*, *Baeckea*, and *Angophora*) tested against three stored grain insects: *Sitophilus oryzae*, *Tribolium castaneum*, and *Rhyzopertha dominica*. Six EOs, *Eucalyptus nicholii*, *E. codonocarpa*, *Eucalyptus blakelyi*, *Callistemon sieberi*, *Melaleuca fulgens*, and *Melaleuca armillaris*, and their major constituents (1,8-cineole) showed the most potent fumigant toxicity against the selected insects (Lee et al., 2004).

TABLE 2 MIC (% v/v) of Essential Oils for Complete Inhibition of a Range of Food Spoilage and Pathogenic Organisms

Plant Extract	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus cereus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Enterobacter aerogenes*	*Acinetobacter Baumannii*	*Shewanella putrefaciens*	*Listeria monocytogenes*	*Geotrichum candidum*	*Saccharomyces cerevisiae*
Blue mallee	0.78	0.78	1.25	1.25	>1.25	1.25	0.63	0.63	1.25	0.63	0.31
Blue gum	0.39	0.78	1.25	1.25	>1.25	1.25	0.63	0.63	0.63	0.31	0.16
Narrow leaf peppermint	0.39	0.39	1.25	0.63	>1.25	1.25	0.31	0.31	0.63	0.16	0.08
Lemon ironbark	0.78	1.56	0.31	0.63	>1.25	0.63	0.63	0.63	0.31	0.31	0.16
Lemon scented gum	0.10	1.56	0.31	>1.25	>1.25	>1.25	>1.25	1.25	0.63	0.63	0.31
Broad leaf eucalyptus	0.39	0.78	0.63	0.31	>1.25	0.63	0.31	0.31	1.25	0.16	0.16
Lemon myrtle	0.16	0.31	0.16	0.31	>1.25	1.25	0.31	0.16	0.16	0.08	0.04
Anise mrytle	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	0.63	1.25	>1.25	0.16	0.08

Antimicrobial activity measured using a microtiter plate assay (Sultanbawa et al., 2009) and the minimum inhibitory concentration determined. Concentration levels from 0.0006–1.25% (v/v) were tested. Data are presented as the mean (n = 6) and the coefficient of variation (CV) was less than 10%.

Flystrike is a debilitating and painful disease in sheep and can have adverse effects on trade. *Lucilia cuprina* is the main cause of flystrike in Australia, South Africa, and New Zealand. Tea tree oil (*M. alternifolia*), known for its insecticidal properties, repellent effects, and antimicrobial and antiinflammatory effects, was tested as a possible treatment for protection against flystrike. The results of this study were promising, showing the possible use of the EO at a concentration of 1–2.5% to control flystrike by killing and repelling larvae, protecting by preventing oviposition and ovicidal action (Callander and James, 2012). Additionally, Australia has well-established quality control procedures for this EO.

FLAVORING AGENTS IN FOOD AND BEVERAGES

Some of the most popular EOs used as flavoring agents in food and beverages are anise myrtle, lemon myrtle, Eucalyptus, and Tasmanian pepper leaf (*Tasmannia lanceolata*). The native mint from the genus *Prostanthera* has been used to a limited extent as an ingredient in the production of cordials, syrups, sauces, and chutneys. The advantage of the native mint is the contribution of a clean, strongly minty eucalyptol fragrance. The main component contributing to these flavor notes are1,8-cineole, α-pinene, β-pinene, and globulol for the three species *Prostanthera rotundifolia*, *Prostanthera lasianthus*, and *Prostanthera incise* (Fulton, 2000).

In eucalyptus EOs, depending on the species, the main component in the oil can be 1,8-cineaole, also known as eucalyptol. An interesting scenario of incorporating *Eucalyptus* EO in wines has been reported by the Australian Wine Research Institute investigating the source of 1,8-cineole and how it gets into the wine. Over three vintages, the relationship between grape composition and eucalyptus trees grown close to the vines and the impact it has on the character of wines was investigated in Western Australia, Victoria, and South Australia. It was concluded that having the eucalyptus trees close to the vines increased the concentration of 1,8-cineole in wines and enhanced the "camphor," "fresh," and "minty" notes in wine, giving the opportunity to create wines that express a "terroir" with market appeal (Capone et al., 2012). This example also opens doors for other food applications where exotic and novel flavors of Australian EOs can be used to create a unique Australian cuisine.

ISSUES AND CHALLENGES OF USING EOS IN FOOD PRODUCTION

The challenge of using plant extracts such as EOs in food production is the lack of reproducibility of their activity due to the diversity of compounds that are present in plants (Negi, 2012). These plant-derived compounds have variations due to different cultivars, growing conditions, harvesting times, and environmental factors. After harvesting the stability of the phytochemicals is determined by the extraction methods and also storage conditions (Harbourne et al., 2013).

In Australia, standards have been established for some EOs to ensure consistent quality. Tea tree EO, mainly composed of α-terpinene, γ-terpinene, and 4-terpineol, has an Australian standard for "terpinen-4-ol type" (AS 2782-1985), which sets a minimum content of terpinene-4-ol of 30% and a maximum 1,8-cineole content of 15% (Altman, 1998). The composition of the EO is also specified under International Organization for Standardization standard 4730 (Oil of melaleuca-terpinen-4-ol type). Lemon myrtle EO standard for Australia AS 4941, first published in 2001, is under revision to reflect the current commercial quality of the oil (Lassak, 2012). Such standards ensure a consistent product and prevent adulteration an advantage in the use of natural products, known for variability in chemical composition.

SUMMARY POINTS

- Australian native EOs have broad spectrum antimicrobial activity against food-related bacteria.
- There is scientific evidence to support the use of these EO as natural preservatives in food and beverage applications.
- These EOs can be used as plant-based food preservatives for extending the storage life of fruits, pulses, and legumes.
- They can be used as environmentally friendly alternatives to chemical insect repellents.
- Australian native EOs are used globally as flavoring agents.
- New and exciting EOs with biologically active molecules within Australia and worldwide are still to be discovered.
- Innovative applications of these EOs are yet to be explored.

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Part II

Named Essential Oils

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Chapter 17

African Cardamom (*Aframomum danielli*) Oils

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INTRODUCTION

Aframomum danielli (Hook.f.) K. Schum belongs to the genus Zingiberaceae and grows particularly in West and Central African countries. Morphologically, *A. danielli* resembles the Indian large cardamom (*Amomum subculatum* Roxb.); hence, it is been referred to as African cardamom (Adegoke et al., 1998).

Martins et al. (2001) examined the composition and antimicrobial activity of the essential oil of *A. danielli* obtained from São Tomé and Principe. Menut et al. (1991) also examined the characteristics of the oil of *A. danielli* obtained from elsewhere. The essential oil of *A. danielli* obtained from Nigeria has also been examined by Adegoke et al. (1998).

Although there are some data available on the characteristics of the oil from the seeds of *A. danielli*, there is a paucity of data on the uses of the oil in food science. Furthermore, most studies have focused on the oils from seeds of *A. danielli*. However, Afolabi (2010) reported on the characteristics of the oils from the flower and leaf of *A. danielli* and also noted the possible applications of the oils in food science.

This chapter highlights the possible ways by which oils from the seeds, leaves, and flowers of *A. danielli* can be used in food science in addition to the general characteristics, components, and bioactivities of *A. danielli* in food systems (Adegoke, 2013).

BOTANICAL ASPECTS

Scientific name: *Aframomum danielli* (Hook.f.) Schum

Species and Geographical Distribution

The genus *Aframomum* has more than 10 species that are restricted to some regions in Africa. *Aframomum* species are ecologically different from other members of the family Zingiberaceae; for example, *Aframomum longilgulatum* is found in forests in Cameroon, *Aframomum ellioti* and *Aframomum longiscarpum* (Hook) K. Schum can be found in Cote D'Ivoire (Diomande et al., 2012), and *A. danielli* occurs mostly in West and Central African countries (Menut et al. 1991).

Description

Aframomum danielli (Hook.f.) K. Schum is a large, robust, perennial plant that is about 3–4 mm tall (Figure 1) and is usually found under shades in plantations (such as cocoa plantations) near riverine areas. Morphologically, *A. danielli* (African cardamom), which belongs to the genus *Aframomum* of the family Zingiberaceae (Dalziel, 1948), resembles Indian cardamom (*Amomum subculatum* Roxb.) (Adegoke et al., 1998).

Aframomum danielli forms an extensive rhizome system with colored flowers and peduncles, which are covered with sterile bracts on the outside of the inflorescence (Tane et al., 2005). The plant grows naturally under the cover of rainforest trees as weeds (Purseglove et al., 1981). The flowers of *A. danielli* are green; the fruit is flask-shaped and has several seeds that are aromatic, olive-brown, and shiny with a turpentine taste, measuring about 3 mm in diameter (Figure 2).

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Cultivation and Harvesting

Whether "true" or "false" cardamom (Heath, 1981; Purseglove et al., 1981), cultivation and conditions necessary for the growth of cardamom are basically very similar. *Aframomum danielli* (false cardamom) needs sufficient natural shade for growth and will thrive in a well-drained, loamy, and moist soil. However, the soils to be used for the cultivation of *A. danielli* should have received a minimum amount of disturbance. Both the seeds and rhizomes of *A. danielli* can be planted; however, for productive purposes, the rhizomes are preferred for productive planting.

FIGURE 1 *Aframomum danielli* plant. *Adegoke, G.O., 2013. Aframomum danielli: Components and Bioactivities in Food Systems, Lambert Academic Publishing, Saabrucken. ISBN: 978-3-659-45718-0.*

FIGURE 2 Seeds of *Aframomum danielli*. *Adegoke, G.O., 2013. Aframomum danielli: Components and Bioactivities in Food Systems, Lambert Academic Publishing, Saabrucken. ISBN: 978-3-659-45718-0.*

If the seeds of cardamom are to be used for planting, they should be collected from ripe capsules of vigorous, high-yielding, disease-free plants, then washed in water and sown immediately; alternatively, they may be mixed with ash and dried for 2–9 days before sowing (Pattanshetti and Prasad, 1973). Fruits are harvested individually at the correct stage of ripeness; immature fruits produce uneven, shriveled, and badly colored samples (Pruthi, 1980; Purseglove et al., 1981).

George (1974) noted that cardamom seeds will germinate 5–7 weeks after sowing and seedlings can be transplanted when they are 3–4 months old and about 15 cm high at a spacing of 15–45 cm apart into suitable nursery beds. Maturity of the seeds may take up to 3–5 years. When matured, ripened seeds are collected before they dry to avoid splitting during drying; thereafter, they are sun-dried.

USAGE AND APPLICATIONS

Aframomum danielli (African or "false" cardamom) has some historical use for relieving "postpartum" pain and for flavoring traditional Nigerian dishes, such as "pepper soup."

Aframomum danielli has been reported to inhibit the growth of *Salmonella enteriditis, Staphylococcus aureus, Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus ochraceus* (Adegoke and Skura, 1994). Ochratoxin A (OTA) in raw materials used for the production of kunu zaki (a nonalcoholic beverage) was reduced from 50 to <1.5 mg/kg with 1.5%, 2.0%, and 2.5% of Daniellin. Daniellin is a patented product made from *A. danielli* (Adegoke, 2006).

In a dose-dependent manner, *A. danielli* inhibited the growth of *Listeria monocytogenes* and has a greater listericidal effect than rosemary (Adegoke et al., 2000). The powder of *A. danielli* has been reported to have some protective effects on liver cells (Adegoke et al., 2002). In addition, the antioxidant extracts prepared from *A. danielli* are more effective than butylated hydroxytoluene and α-tocopherol in stabilizing refined peanut oil against lipid oxidation (Adegoke and Gopalakrishna, 1998).

When mayonnaise samples were treated with 200 ppm of antioxidant extracts of *A. danielli* and butylated hydroxylanisole (BHA), antioxidant effectiveness found with *A. danielli* and BHA were 83.3% and 76.56%, respectively Etti et al., 2012).

Using the powder of *A. danielli* to treat maize and soybean with storage under ambient conditions, the commodities remained stable for 15 months without insect infestation and mouldiness (Adegoke et al., 2002). Preservative activities of *A. danielli* have also been reported with roselle juices (Ashaye et al., 2013).

Chemical Composition

The oil of *A. danielli* obtained by hydrodistillation has some physico-chemical properties that are similar to Asian cardamom (Table 1). Using gas chromatography and gas chromatography–mass spectrometry, the monoterpenes and sequiterpenes in the oil of *A. danielli* from Nigeria were found to be similar to those obtained from Cameroon (Menut et al. 1991) and the Indian cardamom (Adegoke et al., 1998).

TABLE 1 Physico-chemical Properties of Oil of *Aframomum danielli*

Characteristics	*A. danielli*	*Amomum subculatum*
Moisture (%)	11.64	12.00
Essential oil (%, v/w)	3.20	3.60
Color	Colorless	Colorless
Odor	Eucalyptus	Eucalyptus
Specific gravity	0.9230	0.9176
Refractive index	1.462	1.461
Acid value	1.12	1.27
Aldehydes (%)	1.02	Nil
Solubility in 90% ethanol	1 in 3 vols	1 in 3 vols
Evaporation residue (%)	0.82	0.78

Adegoke, G.O., et al., 1998. Flavour Fragr. J. 13, 349–352.

TABLE 2 Some Major Constituents of the Oil of *Aframomum danielli*

Compound	%
α-Pinene	4.3
Camphene	0.1
β-Pinene	13.2
1,8-Cineole (including limonene)	59.8
γ-Terpinene	0.3
Terpinolene	0.1
Linalol	0.1
α-Campholenic aldehyde	0.8

Adegoke, G.O., et al., 1998. Flavour Fragr. J. 13, 349–352.

According to the findings of Afolabi (2010), the major constituents of the oil from the flower of *A. danielli* are α-terpineol (21.2%), 1,8-cineole (18.6%), caryophyllene oxide (14.4%), β-caryophyllene (11.2%), L-linalool (6.1%), and α-humulene (5.6%). Some major constituents of the oil of *A. danielli* seeds are shown in Table 2.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Control of Foodborne Pathogens

Illness and death from diseases caused by unsafe food (Hird et al., 2009) exist all over the world. For example, it is estimated that foodborne diseases result in 76 million illnesses and 5000 deaths each year in the United States (Mead et al., 1999), and pathogenic micro-organisms are associated with these foodborne illnesses.

The monoterpenes of *A. danielli* have antimicrobial effects on some food spoilage microorganisms. For example, α–terpinene (312 μg/mL) reduced the population of *Candida tropicalis* from 10^5 to 10^3 (Adegoke et al., 2000). Furthermore, (+)-limonene had a minimum inhibitory concentration of 78 μg/mL for *A. parasiticus*. Antimicrobial effects of the terpenes from the oil of *A. danielli* are shown in Table 3. Kimbu et al. (1979) found that some diterpenes isolated from *A. danielli* showed some antifungal activities.

OTA, a mycotoxin produced by *A. ochraceus* and *Penicillium viridicatum* and *Penicillium cyclopium,* is nephrotoxic, immunosuppressive, and genotoxic (Adegoke, 2004). *Aspergillus flavus* and *A. parasiticus* are molds that produce mycotoxins (Adegoke, 2004). Adegoke and Letuma (2013) have described some strategies for the prevention and reduction of mycotoxins in foods in developing countries.

Using the essential oils from the seeds of *A. danielli*, Aroyeun and Adegoke (2007) found that the percentage reduction efficiency in OTA increased with concentration of the oil used. Using the oil at 2000 and 3000 ppm, OTA was not detected in cocoa powder (Table 4).

Synergistic Activities

Synergistic activities between the essential oil components of *A. danielli* and hydrostatic pressure have been reported. Adegoke et al. (1996) found that when *Saccharomyces cerevisiae* was exposed to terpinene (75 μg/mL) and hydrostatic pressure (1800 kg/cm^2), there was a growth reduction of six log cycles when compared to two log cycles found with *S. cerevisiae* exposed to only hydrostatic pressure. Furthermore, in another experiment, Adegoke et al. (1997) found a dose-dependent reduction (Table 5) with *S. cerevisiae* exposed to limonene and hydrostatic pressure (1800 kg/cm^2).

Antibrowning Effects

Food browning, which can either be enzymatic or nonenzymatic, is important in the food industry because it results in food quality loss and food safety problems (Friedman, 1996).Using L* values (increase) and a* and b* values (decrease) of treated apples. Antibrowning effects were found when using the seeds (Adegoke et al., 2000) and the essential oil from

TABLE 3 Minimum Inhibitory Concentrations (MICs) and Minimal Fungal Concentrations (MFCs) of Terpenes of *Aframomum danielli* for Some Food Spoilage Yeasts and Aflatoxigenic Molds

	Terpenes							
	α-Terpinene		(+)-Limonene		α-Pinene		1,8-Cineole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Yeasts								
Torulopsis candida	4.9[a](1)	9(2)	39(2)	156(3)	78(4)	312(5)	78(4)	312(5)
Kluveromyces fragilis	39(2)	312(5)	312(5)	312(5)	78(4)	312(5)	78(4)	312(5)
Kluveromyces thermotolerans	78(4)	312(5)	39(2)	156(3)	78(4)	312(5)	156(3)	312(5)
Candida tropicalis	156(3)	156(3)	312(5)	312(5)	156(3)	312(5)	156(3)	312(5)
Pichia pastoris	312(5)	625(6)	39(2)	156(3)	78(4)	312(5)	78(4)	312(5)
Hansenula anomala	312(5)	1250(7)	312(5)	312(5)	156(3)	625(6)	156(3)	625(6)
Molds								
Aspergillus flavus	625(6)	1250(7)	312(5)	625(6)	ND[b]	ND	ND	ND
Aspergillus parasiticus	312(5)	312(5)	78(4)	312(5)	ND	ND	ND	ND

[a]*Data are μg/mL, with values in parentheses being the mean of three replications; thus (1) = 4.1 ± 1.4; (2) = 32.5 ± 11.3; (3) = 130 ± 45.0; (4) = 65 ± 22.5; (5) = 260 ± 90.1; (6) = 520 ± 180.4; (7) = 1041 ± 360.4.*
[b]*ND = not determined, as previous studies showed weak reactions with molds.*
Adegoke, G.O., et al., 2000. Flavour Fragr. J. 15, 147–150.

TABLE 4 Effects of *Aframomum danielli* Spice Oils on OTA Reduction in Cocoa Powder

	Essential Oil (ppm)					
Spike Levels (ppb)	500	%(RE)	1000	%(RE)	2000	3000
100	30	70	10	90	ND	ND
120	40	66.7	10	91.7	ND	ND
140	40	71.1	10	92.9	ND	ND
160	50	68.8	10	93.8	ND	ND
180	40	77.8	10	94.4	ND	ND
200	50	75	10	95	ND	ND

RE, reduction efficiency; ND, not detected.
Aroyeun, Adegoke, 2007. Afr. J. Biotechnol. 6, 612–616.

TABLE 5 Combination Effects of Hydrostatic Pressure (1800 kg/cm^2) and Monoterpenes on Survival of *Saccharomyces cerevisiae*

	S. cerevisiae (log CFU/mL)[a]	
Terpene (μg/mL)	Pressure	No Pressure
−(+)-Limonene		
0	6.8	8.3
220	5.9	8.7
440	5.6	8.0
2200	3.9	6.3
α-Terpinene		
0	7.0	9.0
37	6.4	9.0
75	4.1	9.0
150	2.5	8.6

[a]*Mean of three viable counts.*
Adegoke et al. (1997).

the flowers and leaves of *A. danielli*, respectively (Afolabi, 2010). Figure 3 shows the antibrowning effects of the oil from the flower and leaf of *A. danielli* on mashed apples.

Food Preservation

Food preservation is important in increasing/enhancing the shelf life of food and ensuring food safety. Extending the shelf life of foods is based on controlling enzymes or chemically active molecules in food, controlling microbial deteriorative processes, and avoiding faulty postharvest handling practices (Adegoke and Olapade, 2012). Thus, Fasoyiro (2007) used petroleum ether and ethanol fractions obtained from the seeds of *A. danielli* to preserve soybean, cowpea, and maize and found that infestation of the food commodities decreased with an increase in the concentration of the fraction used.

Evwierhurhoma (1998) washed, drained, and treated mature, green sweet oranges (*Citrus sinensis*) with the essential oil of *A. danielli* followed by storage under ambient conditions (temperature: 26 ± 1 °C; relative humidity: 75% ± 5%) for 35 days. The author noted that when compared with untreated controls, treated orange samples had very high retention rate of vitamin C with storage time (Table 6), ripened without deterioration (Table 7), and showed no spoilage or no losses (Table 8).

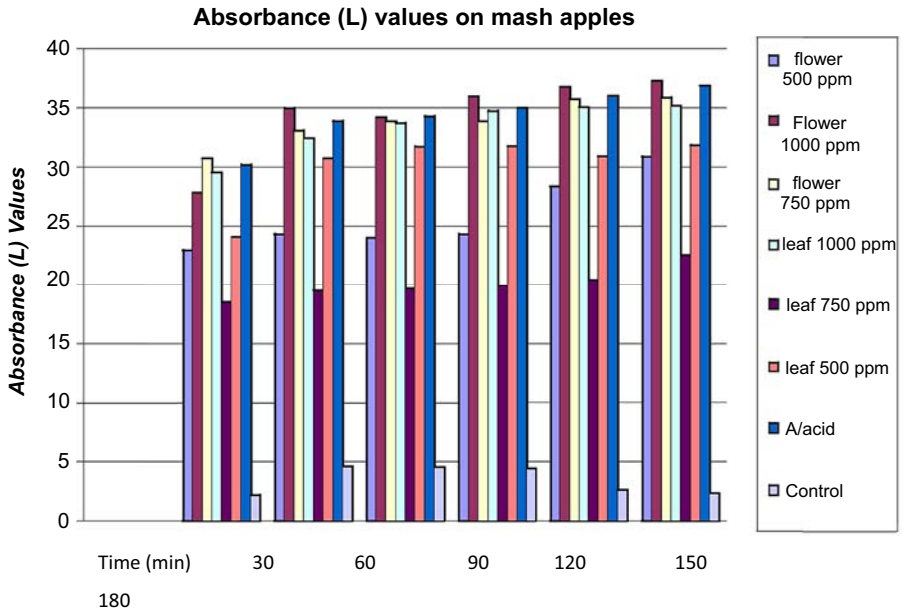

FIGURE 3 Antibrowning activities of *Aframomum danielli* flower and leaf essential oils on mashed apples. *Afolabi, M.O., 2010. Antimicrobial, Antioxidative, Antibrowning, and Food Preservative Properties of Bioactive Components of Aframomum danielli Leaf and Flower (Ph.D. thesis). University of Ibadan, Ibadan, Nigeria.*

TABLE 6 Vitamin C Values of Oranges Stored at Room Temperature for 35 Days

	Duration of Storage (Days)					
Treatment	**0**	**7**	**14**	**21**	**28**	**35**
Control	72.35 ± 0.10	55.65 ± 0.04	14.87 ± 0.20	44.52 ± 0.10	38.96 ± 0.10	33.39 ± 0.03
0.5%		60.42 ± 0.48	59.80 ± 0.04	51.52 ± 0.05	45.44 ± 0.05	41.92 ± 0.04
1.0%		64.74 ± 0.00	61.40 ± 0.04	55.65 ± 0.00	50.59 ± 0.01	44.96 ± 0.05
(DIA) 1.5%		64.95 ± 0.02	62.00 ± 0.00	58.02 ± 0.12	52.33 ± 0.08	46.40 ± 0.10
2.5%		65.88 ± 0.05	62.75 ± 0.06	59.41 ± 0.02	55.42 ± 0.08	48.21 ± 0.04
0.5%		60.62 ± 0.10	57.49 ± 0.10	52.41 ± 0.05	47.05 ± 0.01	41.90 ± 0.15
(VTA) 1.0%		64.78 ± 0.21	61.41 ± 0.10	55.95 ± 0.00	48.93 ± 0.11	45.71 ± 0.10
1.5%		65.04 ± 0.01	62.11 ± 0.01	60.20 ± 0.01	53.11 ± 1.00	48.46 ± 0.06
2.5%		65.91 ± 0.00	63.05 ± 0.10	60.44 ± 0.01	55.72 ± 0.10	50.28 ± 0.02
ET		66.44 ± 0.02	63.48 ± 0.02	60.73 ± 0.03	58.62 ± 0.10	52.47 ± 0.10

Values are the means of three replicates, ± standard deviation.
DIA, dipping in aqueous solution of *Aframomum danielli*; VTA, vacuum treatment with aqueous solution of *A. danielli*; ET, essential oil treatment.
Evwierhurhoma (1998).

SUMMARY POINTS

- The volatile oil constituents of *A. danielli* seeds consist mainly of monoterpenes.
- The oil of *A. danielli* is a rich source of natural 1,8-cineole.
- Some constituents of *A. danielli* oil possess broad-spectrum antimicrobial activities.
- α-Terpinene and –(+)-limonene exert more antimicrobial effects when used in combination with hydrostatic pressure.
- The oils from the flower and leaf of *A. danielli* have antibrowning properties.
- Postharvest losses in oranges (*C. sinensis*) can be controlled using *A. danielli* oil.

TABLE 7 Color Changes in Oranges Stored at Room Temperature for 35 Days

	Duration of Storage (Days)					
Treatment	**0**	**7**	**14**	**21**	**28**	**35**
Control	G	G	G/Y	Y/G	R	RD
0.5%	G	G	G/Y	G/Y	R	RD
(DIA) 1.0%	G	G	G/Y	G/Y	R	R
1.5%	G	G	G	G/Y	Y/G	R
2.5%	G	G	G	G/Y	Y/G	R
0.5%	G	G	G/Y	Y/G	R	RD
1.0%	G	G	G/Y	G/Y	R	RD
1.5%	G	G	G	G/Y	Y/G	R
(VTA) 2.5%	G	G	G	G/Y	Y/G	R
ET	G	G	G	G/Y	Y/G	R

G = green, G/Y = more green than yellow, Y/G = more yellow than green, R = ripe, RD = ripe but deteriorating.
DIA, dipping in aqueous solution of *Aframomum danielli*; VTA, vacuum treatment with aqueous solution of *A. danielli*; ET, essential oil treatment.
Evwierhurhoma (1998).

TABLE 8 Postharvest Losses of Oranges Stored at Room Temperature for 35 Days

	Duration of Storage (Days)				
Treatment	**7**	**14**	**21**	**28**	**35**
Control	+	+	+	10%	40%
0.5%	+	+	+	+	20%
(DIA) 1.0%	+	+	+	+	10%
1.5%	+	+	+	+	10%
2.5%	+	+	+	+	+
0.5%	+	+	+	+	20%
(VTA)1.0%	+	+	+	+	+
1.5%	+	+	+	+	+
2.5%	+	+	+	+	10%
ET	+	+	+	+	+

+, no spoilage, and hence no losses.
DIA, dipping in aqueous solution of *Aframomum danielli*; VTA, vacuum treatment with aqueous solution of *A. danielli*; ET, essential oil treatment.
Evwierhurhoma (1998).

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Chapter 18

Agarwood (*Aquilaria malaccensis*) Oils

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List of Abbreviations

cm centimeter
FRIM Forest Research Institute Malaysia
kg kilogram
m meter
TCM Traditional Chinese Medicine
TIM Traditional Indian Medicine

INTRODUCTION

At a glance, the agarwood tree is just like any other ordinary plant growing in the evergreen tropical forest. However, this plant has a very treasured property—its resin or agarwood. Agarwood (gaharu) is the resinous heartwood produced by a number of *Aquilaria* species. There have been 19 acknowledged species known to produce agarwood; however, not all *Aquilaria* trees are able to produce these prized resin. The genus *Aquilaria* are now listed by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Appendix II), including all agarwood-related products.

The distribution of *Aquilaria* species in Malaysia is shown in Table 1 (Chang et al., 2011). *Aquilaria malaccensis* or karas tree is one of the well-known species. The resinous heartwood is formed as a result of pathology or a wounding procedure. The resin formation may also be a form of natural biological respond to fungal infection.

The use of agarwood has been reported in many ancient cultures and some of the earliest civilizations. The Egyptians have been using it for more than 3000 years. In addition, the Chinese, Japanese, Koreans, and Indians all mentioned the importance of agarwood in their ancient texts pertaining to use in food and traditional medicine.

The use of agarwood flourished in other Southeast Asian countries, such as Laos, Cambodia, Vietnam, Thailand, Indonesia, and Malaysia, because of their ancient trade routes and the ever-popular demand for agarwood essential oils in particular. Different quality essential oils can be produced from the leaves, branches, or chips and flakes from the trunk of agarwood, although the oils are mainly produced from its resinous wood. High-quality oil extracted through the delicate distillation process can fetch a global market price of USD 30,000 per kilogram of pure and superior material (Barden et al., 2000).

BOTANICAL ASPECTS

Agarwood (scientifically known as *Aquilaria malaccensis* Lamk.) is from the family Thymelaeaceae (Figure 1). It is commonly found growing in the plains of primary and secondary forest habitats. It also grows well along hillsides and ridges, up to an altitude of 750 m. The distribution of the trees extends from the Indian continent to Myanmar, the Malay Peninsula and Borneo Island, and the Philippines. Other agarwood-producing species include *Aquilaria crassna*, *Aquilaria agallocha*, *Aquilaria sinensis*, *Aquilaria filaria*, and *Aquilaria subintegra* (Chung and Purwaningsih, 1999).

Agarwood is a medium-to-large evergreen tree with a towering height of 20–40 m. The tree trunk can grow to a diameter of 180 cm and is usually straight without branches. The trunk is supported by thick buttress up to 2 m high, and the bark is whitish in color and smooth. It has simple alternate leaves with elliptical blades. The small fragrant flower is pale greenish or yellowish in color and can be found in small clusters at the leaf axil. Its fruit is green and ovoid in shape. When the fruits ripen, their leathery capsules crack open into two halves, producing seeds hanging out on long appendages. The seeds are blackish-brown in color, ovoid in shape, and densely covered with red-brown hairs (Hashim and Ahmad Zuhaidi, 2011).

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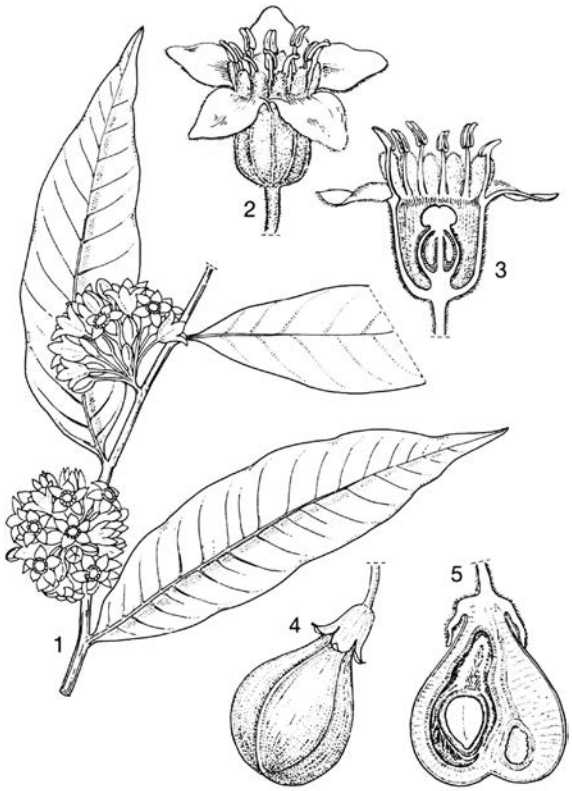
TABLE 1 Distribution of *Aquilaria* Species in Malaysia

Species	Local Name	Distribution
Aquilaria malaccensis	Karas, kekaras, chandan, engkaras	Peninsular Malaysia, Sabah, and Sarawak
Aquilaria microcarpa	Engkaras	Sabah and Sarawak
Aquilaria hirta	Chandan buluh	Peninsular Malaysia
Aquilaria rostrata	–	Peninsular Malaysia
Aquilaria beccariana	Gaharu tanduk	Peninsular Malaysia, Sabah, and Sarawak

Chang et al. (2011).



Aquilaria malaccensis Lamk – 1, flowering branch; 2, flower; 3, longitudinal section through flower; 4, fruit; 5, longitudinal section through fruit.

FIGURE 1 Botanical description of *Aquilaria malaccensis* Lamk. *(Chung and Purwaningsih, 1999).*

The tree begins to produce flowers and fruits at the age of 6–7 years. This occurs during the dry season. In the Malesian region, trees were observed to flower between the months of July and August, followed by mature fruits in the period of November and December. A medium-sized tree will produce about 1.5 kg of seed in a good year and is estimated to produce up to 19,000 seeds per season. Germination process starts between 7 and 15 days after sowing. Most seedlings disperse within the range of 5 m from the adult plant. The seeds are recalcitrant, and their viability decreases when the moisture content drops (Hashim and Ahmad Zuhaidi, 2011).

USAGE AND APPLICATIONS

Agarwood has been widely used in the multicultural society of Malaysia, mainly by the Malays, Chinese, and Indians in their traditional cures. There were many known uses of agarwood in the local Malay traditional medicine. It is also

commonly known as Gaharu, which originates from the Sanskrit word *Aguru*, meaning "heavy." The wood was reported to be used to treat sickness after childbirth. It was used in formulation with other herbs for the treatment of rheumatism, body pains, and smallpox. Other reports also showed that the wood was regarded as stimulant and carminative medicine, and it was also used as a tonic during pregnancy for the female reproductive organs. It was added into special formulations with other herbs and spices to treat typhoid fever, thrush, hemorrhoid, and bladder stones and to prevent body odor. It was also reported to increase one's appetite. The waste or the discarded soft wood has been used to treat jaundice and body pains. The roots and leaves were rarely used, but there are reports of them being used as a prescription for dropsy. A mixture of parts of the leaves, roots, and wood was reportedly used in the traditional treatment for drug addiction (Burkill, 1966; Perry, 1980; Harun, 2006).

In traditional Chinese medicine (TCM), properties such as pungent, sweet, and bitter are considered as important elements in the treatment of ailments. Interestingly, the Chinese character "Chén Xiāng" (沉香) literally means sinking incense or fragrance, which describes the sinking heavy aromatic heartwood when placed in water. The heartwood has been prescribed as a traditional cure for colds, gastric pains, colic problems, vomiting, and hiccough. Other uses were to treat chest and abdomen pains, frequent urination, asthma, wheezing, fatigue, hypertension, and diarrhea. It was also commonly applied for nervous system disorders, such as numbness and paralysis. Some Chinese medical formulations containing agarwood have been used to treat nausea, morning sickness, and constipation. The heartwood was also believed to possess aphrodisiac properties and has been used as a tonic and stimulant (John, 1976; Wee and Hsuan, 1990; Dan and Andrew, 1993; Daniel, 2001).

Agarwood is also known as Agar-i-hindi in Hindi, Agara in Bengali, Agalura-che in Persian, Aguru in Sanskrit, and Udaaghulukhi in Arab. In traditional Indian medicine (TIM), preparations from agarwood for medicinal uses come in the form of paste, decoction, and incense. The heartwood was believed to have many beneficial medicinal properties, such as sedative, cardiotonic, anti-inflammatory, antileprotic, depurative, deodorant, carminative, detoxifying, and rejuvenating. Based on its various properties, agarwood was known to be mixed with other ingredients in formulations for rheumatism, gout, bronchitis, and headache. The heartwood is useful for treating bad breath, indigestion, skin diseases, leprosy, inflammations, arthritis, weak heart, abnormally low body temperature, and sexual debility (Rustomjee, 1986).

A report on TCM and TIM stated the uses of the lignum and resin of agarwood as an astringent, febrifuge, dyspepsia, and diuretic, as well as a treatment for skin disorders and ectoparasites (Van Wyk and Wink, 2004). Agarwood is also known as jinkoh in Japanese. The high-quality grades are used in meditation and religious ceremonies due to their sedative and calming properties (Takemoto et al., 2008).

USAGE AND APPLICATIONS IN FOOD SCIENCE

The herbal and food industries are always searching for new innovative food ingredients and healthcare ingredients with proven biological effects. Due to the emergence of new technology and market demand, innovative food products have been developed. The function of traditional food products has evolved to include wellness and healthcare. Studies in nutritional sciences have discovered many bioactive food ingredients with proven anti-inflammatory and antioxidant properties, thus making the food industry more attractive to the health-conscious consumer (Zhou et al., 2008).

Presently, *Aquilaria* species are collected from the wild; however, there are efforts to plant on a large scale for later harvesting for resin and essential oil production. To date, several inoculation methods have been developed to induce resin production within a short period; however, the quality of the agarwood is still of either a low- or medium-quality grade.

Agarwood resins contain essential oils, which are mainly used as perfumery ingredients. The unique aroma of the agarwood oils are the result of complex mixtures of sesquiterpenes, oxygenated compounds, and chromone derivatives, such as α-agarofuran, β-agarofuran, agarospirol, jinkohol, jinkohol II, and valerianol (Naef, 2011). Research studies have also shown that *Aquilaria* species possessed antioxidant, antimicrobial, and laxative properties (Huda et al., 2009; Dash et al., 2008; Hara et al., 2008). Agarwood has also been studied for its relaxing and sedative effects.

The leaf oils of agarwood have also been studied, although they contain less essential oil than agarwood resins (Nor Hasnida et al., 2011). A study on *A. sinensis* showed various chemical compositions and the relative amount of essential oils in healthy trees, wild agarwood, and chemically induced agarwood (Chen et al., 2011). Some of the essential oil components and antimicrobial activities are shown in Tables 2 and 3, respectively.

Although there are no scientific studies reporting on the use of agarwood oils in food science, the resinous wood is often used in flavoring drinks and herbal tea, perhaps due to its aromatic and sometime bitter taste. *Aquilaria* leaves are often made into tea, and drops of agarwood oil are added to give a subtle aroma to the tea. Some of the chemical compounds identified from the *Aquilaria* leaves are polyphenols, which may be responsible for its high antioxidant properties.

Agarwood and its oils are highly prized in the perfumery and scent industry. In the global trade, the forms of agarwood are the wood itself in chips, flakes, or powdered form, as well as oil and finished products such as perfume,

TABLE 2 Chemical Compositions and Relative Amounts of the Essential Oils from Agarwood Originated from *Aquilaria sinensis* Stimulated by the Chemical Method (S1), Wild Agarwood (S2), and Healthy Tree (S3)

No.	Compounds	RI	Relative Amount (%)			Identification
			S1	S2	S3	
Sesquiterpenes and Aromatics						
1	Benzylacetone	1257	7.91	2.34	–	RI, MS
2	Vanillin	1418	–	–	0.46	RI, MS
3	α-Humulene	1464	0.30	–	–	RI, MS
4	α-Selinene	1493	0.45	3.71	–	RI, MS
5	α-Agarofuran	1535	–	0.24	–	RI, MS
6	Elemol	1556	0.32	0.71	–	RI, MS
7	5β,7βH,10α-Eudesm-11-en-1α-ol	1583	0.54	0.66	–	MS
8	Caryophyllene oxide	1588	2.22	2.12	–	RI, MS
9	Isoaromadendrene epoxide	1606	0.94	2.77	–	RI, MS
10	γ-Eudesmol	1632	1.06	2.84	0.50	RI, MS
11	Hinesol	1638	6.34	0.34	–	RI, MS
12	Agarospirol	1643	0.80	4.03	0.85	RI, MS
13	Cubenol	1647	2.21	1.97	–	RI, MS
14	*cis*-Z-α-Bisabolene epoxide	1651	0.83	0.78	–	RI, MS
15	(–)-Aristolene	1654	0.61	4.70	1.31	MS
16	Guaiol	1661	9.34	10.67	2.18	MS
17	Eudesm-7(11)-en-4α-ol	1666	4.35	2.09	–	RI, MS
18	Aromadendrene oxide (1)	1674	1.27	1.41	–	RI,MS
19	α-Copaen-11-ol	1686	4.06	10.22	–	RI, MS
20	4,4,11,11-Tetramethyl-7-tetracyclo-[6.2.1.0(3.8)0(3.9)] undecanol	1690	0.53	1.50	–	MS
21	Bicyclo[4,4,0]dec-2-ene-4-ol,2-methyl-9-[prop-1-en-3-ol-2-yl]	1697	3.11	0.52	–	MS
22	Diepi-α-cedrene epoxide	1701	6.00	0.38	–	MS
23	Aromadendrene oxide (2)	1705	–	1.88	–	RI, MS
24	Baimuxinal	1707	2.44	14.78	1.52	MS
25	Selina-3,11-dien-14-al	1733	5.50	0.38	–	RI, MS
26	Guaia-1(10),11-dien-9-one	1753	10.89	–	–	RI, MS

27	1,2,5,5,8a-Pentamethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol	1755	–	5.82	–	RI, MS
28	6-Isopropenyl-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone	1769	1.65	0.54	–	RI,MS
29	Eremophila-7(11),9-dien-8-one	1811	4.54	5.42	2.11	RI, MS
Fatty Acid and Alkanes						
30	Tetradecanoic acid	1772	–	–	2.36	RI, MS
31	Nonanoic acid	1278	–	–	1.50	RI, MS
32	*n*-Decanoic acid	1371	–	–	0.52	RI, MS
33	Pentadecanoic acid	1878	–	–	4.87	RI, MS
34	*cis*-9-Hexadecenoic acid	1955	–	–	2.87	RI, MS
35	*n*-Hexadecanoic acid	1982	0.30	0.06	49.47	RI, MS
36	Hexadecanoic acid, ethyl ester	1996	–	–	1.13	RI, MS
37	Eicosane	1999	0.22	0.58	–	RI, MS
38	Heptadecanoic acid	2073	–	–	0.37	RI, MS
39	Heneicosane	2100	0.51	–	1.09	RI, MS
40	Oleic Acid	2153	–	–	10.18	RI, MS
41	Docosane	2200	0.80	–	0.53	RI, MS
42	Tricosane	2300	0.97	–	0.80	RI, MS
43	Tetracosane	2400	0.79	–	0.86	RI, MS
44	Pentacosane	2500	0.70	–	0.87	RI, MS
45	Hexacosane	2600	0.62	–	0.80	RI, MS
46	Heptacosane	2700	0.45	–	0.57	RI, MS
47	Octacosane	2800	0.20	–	0.54	RI, MS
Others						
48	2-Hydroxycyclopentadecanone	1851	0.24	0.30	2.32	RI, MS
49	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1869	0.69	–	0.85	RI, MS
50	Dibutyl phthalate	1962	2.13	1.23	2.47	RI, MS
51	1,2,3,4-Tetrahydro-1-nonylnaphthalene	2021	0.64	–	–	MS
52	8,9-Dehydro-9-formyl-cycloisolongifolene	2082	0.56	0.98	–	MS
53	γ-Palmitolactone	2111	–	–	0.99	RI, MS
54	4,8,12,16-Tetramethylheptadecan-4-olide	2357	–	–	0.42	RI, MS
55	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	2541	–	–	0.39	RI, MS

Compounds are listed in the order of elution; RI is the retention index which was calculated against C_8–C_{40} *n*-alkanes on the nonpolar VF-5MS column; MS = mass spectrum.

TABLE 3 Screening Results for Antimicrobial Activity of Agarwood Oils Originated from *Aquilaria sinensis* Stimulated by the Chemical Method (S1), Wild Agarwood (S2) and Healthy Tree (S3)

Agarwood Oils		*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*
S1	AWD	8.14 ± 0.05	10.29 ± 0.20	11.68 ± 0.26
	MIC	6.25	3.125	0.39
	MBC	25	6.25	12.5
S2	AWD	7.00 ± 0.02	11.25 ± 0.02	11.57 ± 0.02
	MIC	6.25	0.195	0.195
	MBC	12.5	6.25	12.5
S3	AWD	7.52 ± 0.26	8.10 ± 0.24	9.39 ± 0.30
	MIC	12.5	1.56	0.78
	MBC	>25	12.5	>25
Gentamicin	AWD	23.08 ± 0.88	21.45 ± 1.77	23.73 ± 0.32
	MIC	0.487	0.487	0.487
	MBC	0.487	0.487	0.487

AWD = agar well diffusion method, where the diameters of the inhibition zone, including the well diameters, are 6 mm; MIC = minimum inhibitory concentration, where the values of the three oil samples are given in mg/mL, and the values of gentamicin are given in μg/mLp; MBC = minimum bactericidal concentration, where the values of the three oil samples are given in mg/mL, and the values of gentamicin are given in μg/mL.

incense, and medicine. As stipulated in the Chinese Pharmacopoeia, agarwood with higher alcohol soluble extractive content (surpassing the 10% requirement for TCM) is highly sought by industries. Forest Research Institute Malaysia (FRIM) has developed an agarwood classification based on resin content for quality grading of the agarwood (Nor Azah et al., 2013).

In the food and beverages sector, there is little usage of agarwood or its oil as food ingredients for daily consumption. However, apart from agarwood, the leaves were boiled in water, concentrated, and freeze dried. The extracts were then employed in various kinds of products such as herbal tea, beverages, noodles, biscuits, and confectionaries.

Herbal Tea

The natural *Aquilaria* leaves are made into tea. Some leaves come in the form of loose-dried leaves, and some are cut and packaged into small teabags or sachets for the convenience of the consumer. It is believed to be a good alternative not only to hot caffeinated coffee or tea and also as a thirst quencher when served cold or chilled. *Aquilaria* tea is consumed in Vietnam and Malaysia. Sometimes, all parts of the plant are used in making the herbal tea.

Source: agarwood-embun.blogspot.com.

Wine

In the ancient Chinese historical records and literature, medicated wine was produced by mixing wine and selected medicinal herbs. These formulations were prescribed when necessary to treat illness, including gynaecological conditions, post-surgical treatment, and tonic function. In Taiwan, agarwood is added as the fine ingredient in medicinal wines, such as the famous Chu-yeh Ching and Wu Jia Pi.

Noodles and Biscuits

In Malaysia, herbal manufacturers have incorporated agarwood into the ingredients of their noodle products. A local agarwood tree farming company has produced noodles to be consumed with agarwood mixture-based soup. It is promoted as a health food because of the known traditional medicinal properties of agarwood, as well as providing aroma and flavor to the food. Other products produced include biscuits, ice creams, and herbal rice.

Source: kosmo.com.my; May 23, 2012.

Source: kosmo.com.my; May 23, 2012.

Herbal Infusions and Beverages

Agarwood can be mixed with honey and other herbal plant ingredients to produce premixed herbal mixtures and beverages. It is mainly consumed for its medicinal properties and for giving energy, although these claims have not been substantially proven by scientific studies.

Research on Agarwood by Forest Research Institute Malaysia

To ensure the production of quality and safe herbal raw materials for food and herbal product applications, FRIM is extensively conducting studies on the standardization of the plant extracts and quality grading of agarwood. Apart from aroma profiling of the oils via headspace–microextraction–gas chromatography mass spectrometry technique and chromatographic fingerprint profiling, electronic noses were used for the quality control of *Aquilaria* agarwood products and oils. It is hoped that these current methods can be used in addressing the quality of the oils in the products.

SUMMARY POINTS

- *Aquilaria* trees produce agarwood, which can be distilled for its essential oils. Both ingredients are employed in perfumes, food, and beverages.
- Although it is not a food ingredient that is consumed daily, its medicinal and aromatic properties have attracted new uses in food products.
- Agarwood is now a commodity in Vietnam and Thailand, so further research is essential in developing new and innovative products from *Aquilaria* species.

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Chapter 19

Ajwain (*Trachyspermum ammi* L.) Oils

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List of Abbreviations

ATP Adenosin triphosphate
ESBLs Extended-spectrum-β-lactamases
H_2O_2 Hydrogen peroxide
LAB Lactic acid bacteria
MBC Minimum bactericidal concentration
MIC Minimum inhibitory concentration
US-FDA United States-Food and Drug Administration

INTRODUCTION

Researchers are attempting to replace synthetic preservatives with natural botanicals and essential oils due to the established drawbacks of synthetic preservatives. Experimentation has revealed that natural methods of preservation are more beneficial than their unnatural counterparts. Essential oils have proven to be better than synthetics in preventing the growth of pathogens and delay of spoilage in food. Moreover, these essential oils do not have the harmful health risks that come with the use of synthetics. Moreover, essential oils are environmentally friendly, degradable, and cheaper than chemical preservatives. Thus, essential oils should be at the forefront of the food industry (Das et al., 2012).

Botanicals are environmentally sustainable because their manufacturing process is clean, inexpensive, and produces very little waste, even on an industrial scale. Steam extraction and hydrodistillation are two common high-output methods of essential oil extraction, both of which only produce two byproducts: biodegradable, compostable biomass and water vapor. Both of these methods require only simple machinery and a source of plant material, such as rosemary (whose essential oil is very effectively extracted by steam); they produce a potent product containing all the terpenes and phenols that make essential oils effective and economic (Boutekedjiret et al., 2003).

The seeds of *Trachyspermum ammi* (L.)—commonly known as ajwain or caraway—have been reported for wide range of medicinal applications. They show antifungal, antioxidant, antibacterial, cytotoxic, antilithiasis, nematicidal, anthelmintic, and antifilarial activities, which has generated interest in the use of ajwain as a food preservative. Among its other constituents, volatile oil (thymol, γ-terpinene, *para*-cymene, α- and β-pinene, α-thujen, myrcene, 1,8-cineole, and carvacrol) has effective antimicrobial and antifungal activity, especially thymol and carvacrol. Ajwain oil contains about 50% thymol, which is a strong germicide, antibacterial, antispasmodic, and fungicidal. Although antimicrobial studies have been carried out for ajwain oil against many bacterial and fungal species, food spoilage has been credited to broader range of microorganisms. Hence, it is confined to be used synergistically with other essential oils. Furthermore, ajwain oil possesses antioxidant properties; the high percentage of oxygenated monoterpenes, particularly thymol, and the high level of g-terpinene and *p*-cymene of ajwain oil might be related to its high antiradical activity. In fact, thymol and carvacrol have been considered to be safe by the European Commission and US Food and Drug Administration (FDA) and are classified as flavoring agents; hence, they undergo regulatory requirements as additives for food preservation.

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BOTANICAL ASPECTS

Trachyspermum ammi (L.) Sprague ex Turrill, commonly known as ajwain or caraway, is an erect, glabrous, or minutely pubescent, branched annual herb, 60–90 cm tall, belonging to the family Apiaceae. Other synonyms are *Ammi copticum* (L.), *Carum copticum* (L.), and *Trachyspermum copticum*. It is native to Egypt and widely grown all over Iraq, Iran, Afghanistan, Pakistan, and India, in arid to semi-arid regions where soils contain high levels of salts. *T. ammi* L. contains striated stems, with an inflorescence that is a compound umbel. Each umbel contains 16 umbellets, each with up to 16 actinomorphic, white, and small flowers with five petals, five stamens, and ovary inferior. Its leaves are rather distant and pinnately divided, with a terminal and seven pairs of lateral leaflets. Fruits are ovoid, muricate, aromatic cremocarps that are grayish-brown; the mericarps are compressed, with distinct ridges and tubercular surface and one-seeded (Joy et al., 2001).

Microscopically, transverse section of fruit exhibits two hexagonal structures linked by carpophores. Epicarp is made of a single layer of tangentially elongated tabular cells, while mesocarp is slightly thick-walled, with polygonal tangentially elongated cells having some vittae. Fruiting bodies and vascular bundles appear as groups of thick-walled elongated cells; the endosperm consists of thin-walled cells filled with embryos, small circular oil globules, and is composed of thin-walled polygonal cells. The powder exhibits oil-globules and groups of endosperm cells (Ayurvedic Pharmacopoeia of India, 1999–2011).

Seeds of *T. ammi* L. are widely used in India and eastern Asia, both in diet and in traditional medicine. They contain fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone, and mineral matter (7.1%) (Bairwa et al., 2012). Moazeni et al. identified 18 compounds in the essential oil, representing 99.54% of the total oil. The major constituents were thymol (50.07%), γ-terpinene (23.92%), and *p*-cymene (22.9%), along with α- and β-pinenes, α-thujen, myrcene, 1,8-cineole, and carvacrol, present in very low concentrations (Moazeni et al., 2012; Zachariah, 2008).

Trachyspermum ammi L. is known as a popular aromatic herb and an important commercial product for the food/flavoring industries. The fruits are edible and usually used as spice in India, Middle East Asia, and some parts of America. The seeds possess remarkable digestive and antiseptive properties and are used in traditional medicine, primarily to control bowel disorders such as indigestion, flatulence, colic, and diarrhea (Khan et al., 2010). They are also used as a stimulant, stomachic, carminative, aromatic, antispasmodic, antihypertensive, antiseptic, antiparasitic, antiscorbutic, antihistamine, vermicide, emmenagogue, sialagogue, and anti-inflammatory (Aftab et al., 1995; Kamal et al., 2012).

Ajwain oil contains around 50% thymol, which is a strong germicide, antibacterial, antispasmodic, and fungicide (Bairwa et al., 2012). The diuretic properties of *T. ammi* L. seeds have been widely reported and are used in some drug formulations for kidney stone treatments (Kaur et al., 2009). The seeds are also used in traditional ethno-veterinary for treating diarrhea, indigestion, and constipation in animals. It is given orally to animals in case of weakness and to increase milk-yielding capacity (Sharma et al., 2012).

USAGE AND APPLICATION IN FOOD SCIENCE

Ajwain Oil as an Antibacterial Agent

The bacteria that are involved in specific food spoilage and food products are summarized in Table 1. Mayaud et al. evaluated the antimicrobial activity of 13 essential oils, including ajwain oil, against antibiotic-resistant bacterial strains using two methods: the agar dilution method and the time-killing curve method. The ajwain oil was effective against *Escherichia coli*, *Enterobacter cloacae*, *Salmonella*, *Citrobacter*, *Hafnia alvei*, *Vibrio cholerae*, *Klebsiella*, *Pasteurella multocida*, *Bordetella bronchisepta*, *Acinetobacter baumanii*, *Stenotrophomonas maltophilia*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococci*, *Streptococci*, *Enterococci*, *Listeria monocytogenes*, and *Corynebacterium* with varying degrees of sensitivity (Table 2). They classified the bacteriostatic efficacy of tested essential oils, including ajwain oil, based on chemical composition: phenols, aldehydes, and monoterpenols were more potent than the essential oil containing oxides and hydrocarbons. This classification is in general agreement with previously reported results (Mayaud et al., 2008).

Kaur et al. studied the antibacterial activity of aqueous and organic seed extracts of *T. ammi* L. using agar diffusion assay, minimum inhibitory concentration, and viable cell count studies; their antibacterial effect was compared with some standard antibiotics. The phytochemical constituents of the extracts were alkaloids (4.23%), flavonoids (8.58%), tannins (22.77%), and saponins (0.71%). Although they did not study the presence of monoterpenoids, this has been identified and reported elsewhere. The aqueous extracts have shown variable zones of inhibition (11–25 mm) for all the tested bacteria except *Klebsiella pneumoniae* 1 and 2 and *Pseudomonas aeruginosa* 1, which were completely resistant (Table 2). Organic

TABLE 1 Food Products and Bacteria Responsible for Their Spoilage

Food and Food Products	Different Types of Bacterial Species Involved in Spoilage
Fish	Shewanella, Pseudomonas, Photobacterium, Shewanella
Smoked fish	Lactic acid bacteria (LAB), Enterobacteriaceae, Photobacterium
Marinated fish	LAB
Meat	Pseudomonas, *Lactobacillus curvatus* (LAC), Enterobacteriaceae, Brocothrix, Clostridia
Meat products	*L. curvatus* (LAC), Enterobacteriaceae, Brochotrix
Milk	Pseudomonas, Bacillus
Raw vegetables	Erwinia, Pseudomonas
Eggs	Pseudomonas, Enterobacteriaceae
Fruits	LAB
Mayonnaise salads	LAB
Beer	LAB
Wine	LAB
Cereals	LAB
Nuts	LAB

Species of bacteria involved in food spoilage.

extracts showed similar results as observed in the case of aqueous extracts with some variations. The extracts prepared in hexane and acetone gave relatively better inhibitory zones, ranging from 9 to 30 mm. Indeed, the strains that exhibited considerably good sensitivity to *T. ammi* L. extracts were selected further to determine minimum inhibitory concentration (MIC) of aqueous and acetone extract of seeds. The higher value of MIC was observed for the aqueous extract (MIC: 60–80 mg/mL), whereas lower value of MIC was shown for the solvent extract (MIC: 5–15 mg/mL) against Gram-positive bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus* and Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, and *Shigella flexneri* (Table 2).The viable cell count for hot water extracts (200 mg/mL) of the *T. ammi* L seeds against strains depicted complete killing of bacteria, mainly within a 10-h time span. Their bactericidal nature was further confirmed when no regrowth occurred, even after 24 h of incubation (Kaur et al., 2009). While comparing the antibacterial potential between aqueous and acetone extracts of *T. ammi* (L.) seeds with standard antibiotics, acetone extract implicated statistically significant activity in comparison to its aqueous extract ($p < 0.05$), while an insignificant difference was observed for *Anethum graveolens* and *Foeniculum vulgare* extracts.

Khan et al. (2010) investigated different solvent extracts of *T. ammi* (L.) seeds to determine their efficacy against multidrug-resistant (MDR) strains of five extended-spectrum-β-lactamase (ESBL)-producing strains of *E. coli* (nosocomial infection) confirmed by polymerase chain reaction and reference strains of *Streptococcus mutans* ATCC-700610 and *Streptococcus bovis* ATCC-9809. Alkaloids, amino acids, proteins, sterol terpenes, and glycosides were identified in the solvent extract. The MIC and minimum bactericidal concentration (MBC) were tested by using a broth microdilution method. Various solvent extracts of *T. ammi* seeds were found to be bactericidal against five ESBL-producing strains of *E. coli*, *S. mutants*, and *S. bovis* with MIC (1250–5000 μg/mL) and MBC (312.5–˃5000 μg/mL) shown in Table 2 (Khan R et al., 2010). In this study, the authors qualitatively identified terpinene as one of the components; these findings are in agreement with other studies reporting that thymol and carvacrol, a monoterpenoid phenol, are the major components of ajwain oil responsible for antimicrobial action.

Terpenoids are terpenes that undergo biochemical modifications via enzymes that add oxygen molecules and move or remove methyl groups. Naturally occurring compounds such as thymol and carvacrol can reduce bacterial resistance to common drugs, such as penicillin (Palaniappan and Richard, 2010). Thymol and carvacrol were found to be more effective in killing bacteria (Bairwa et al., 2012). Another study showed that thymol and carvacrol were the most effective

TABLE 2 Antibacterial Activity of Ajwain (*Trachyspermum ammi* L.) Oil and Seeds Extract

Parts/Extracts of *T. ammi* Seeds	Susceptible Bacterial Species			References
Hot water extract		**MBC (mm)**	**MIC (mg/mL)**	Kaur et al. (2009)
	Enterococcus faecalis	22	60	
	Staphylococcus aureus	23	70	
	Escherichia coli	17	80	
	Klebsiella pneumoniae 1	–	–	
	K. pneumoniae 2	–	–	
	Pseudomonas aeruginosa 1	–	–	
	P. aeruginosa 2	24	80	
	Salmonella typhi	–	80	
	Salmonella typhimurium 1	–	–	
	S. typhimurium 2	12	80	
	Shigella flexneri	15	80	
Solvent extract (acetone)	*E. faecalis*	28	05	Kaur et al. (2009)
	S. aureus	30	05	
	E. coli	23	10	
	Klebsiella pneumoniae 1	13	–	
	K. pneumoniae 2	12	–	
	P. aeruginosa 1	15	–	
	P. aeruginosa 2	25	05	
	S. typhi	18	10	
	S. typhimurium 1	14	–	
	S. typhimurium 2	24	05	
	S. flexneri	24	05	
Solvent extract (acetone)		**MBC (µg/mL)**	**MIC (µg/mL)**	Khan R et al. (2010)
	E. coli 1 ESBL	5000	2500	
	E. coli 2 ESBL	>5000	2500	
	E. coli 3 ESBL	5000	2500	
	E. coli 4 ESBL	2500	5000	
	E. coli 5 ESBL	2500	2500	
	Streptococcus mutans	1250	1250	
	Streptococcus bovis	2500	2500	
Ajwain (*T. ammi* L.) oil		**MIC % v/v**		Mayaud et al. (2008)
	E. coli	0.37 ± 0.15		
	Enterobacter cloacae	0.2 ± 0.07		
	Salmonella	0.31 ± 0		
	Citrobacter	0.26 ± 0.07		
	Hafnia alvei	0.24 ± 0.08		
	Vibrio cholerae	0.16 ± 0		

TABLE 2 Antibacterial Activity of Ajwain (*Trachyspermum ammi* L.) Oil and Seeds Extract—cont'd

Parts/Extracts of *T. ammi* Seeds	Susceptible Bacterial Species			References
	Klebsiella	0.31 ± 0		
	Pasteurella multocida	0.27 ± 0.07		
	Bordetella bronchisepta	0.47 ± 0.16		
	Acinetobacter baumanii	0.27 ± 0.15		
	Stenotrophomonas maltophilia	0.16 ± 0		
	Aeromonas hydrophila	0.14 ± 0.04		
	Branhamella catarrhalis	ND		
	P. aeruginosa	7.4 ± 2.5		
	S. aureus	0.24 ± 0.08		
	Coagulase–staphylococci	0.25 ± 0.07		
	Streptococci	0.31 ± 0.14		
	Enterococci	0.47 ± 0.15		
	Listeria monocytogenes	0.47 ± 0.15		
	Corynebacterium	0.27 ± 0.06		
Ajwain (*T. ammi* L.) oil		**MBC (mm)**	**MIC (ppm)**	Gandomi et al. (2013)
	Bacillus cereus	35	500	
	S. aureus	33.5	500	
	L. monocytogenes	36	500	
	S. typhimurium	34	500	
	E. coli	39	500	
Ajwain (*T. ammi* L.) oil			**MIC (µg/mL)**	Paul S et al. (2011)
	S. aureus ATCC 6538		162.5	
	S. aureus KCTC 1916		175	
	E. coli O157:H7ATCC 43888		450	
	Bacillus subtilis ATCC 6633		12.5	
	P. aeruginosa KCTC 2004		12.5	
	S. typhimurium KCTC 2515		225	
	E. coli ATCC 8739		462.5	
	Enterobacter aerogenes KCTC 2190		12.5	
	Salmonella enteritidis KCYC 12021		250	

The above values shows minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum bactericidal zone (mm) of Ajwain (*Trachyspermum ammi* L.) oil against bacterial strains.

components against *Aeromonas hydrophila*, followed by *Brochothrix thermosphacta* and *E. coli*. (Klein et al., 2013). The antibacterial activity of ajwain oil and seed extracts are shown in Table 2.

Mode of Action

Thymol is a phenolic monoterpenoid and one of the major constituents of ajwain oil. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different position on the phenolic ring. The antimicrobial action of phenolic compounds, such as thymol and carvacrol, are anticipated to cause structural and functional damage to the cytoplasmic

membrane. Studies have shown that thymol interacts with cell membranes. This interaction affects membrane permeability, leading to a loss of membrane potential and leakage of potassium ions and adenosine triphosphate. Thymol integrates at the polar head-group region of a lipid bilayer, causing alterations to the cell membrane. Also, it interacts with membrane proteins and intracellular targets. It forms a complex with membrane-bound or periplasmic proteins by means of hydrogen bonds and hydrophobic interactions. It also impairs the citrate metabolic pathway and affects many enzymes directly or indirectly involved in the synthesis of ATP (Hyldgaard et al., 2012).

The antimicrobial action of carvacrol, a phenolic monoterpenoid, is similar to that of thymol. It causes structural and functional damage to the cell membrane. It has been demonstrated to affect the outer membrane of Gram-negative bacteria. Disintegration of the outer membrane caused release of lipopolysaccharides from Gram-negative bacteria. Although carvacrol affects the outer membrane, its site of action is thought to be the cytoplasmic membrane, resulting in passive transport of ions across the membrane. Besides interaction with membranes, carvacrol has been proposed to interact with membrane proteins and periplasmic enzymes, but the evidence for this is limited (Sikkema et al., 1995; Hyldgaard et al., 2012).

Ajwain Oil as an Antifungal

Molds not only contaminate our air but also contaminate our food. As the molds grow on food, they produce enzymes that break down the food, resulting to spoilage. Details of food spoilage and the mold and fungal species involved are listed in Table 3 (Hocking, 2006; Filtenborg et al., 1996). Gilani et al. (2013) formulated 5% ajwain oil into dermal cream and tested against molds (*Aspergillus oryzae, Aspergillus niger, Penicillium digitatum*, Mucor) and yeast (*Candida utilus*) in rabbits, comparing the antifungal activity with an iodine tincture. The gas chromatographic analysis of ajwain oil revealed the presence of monoterpenes, hydrocarbons, and oxygenated fraction. The identified components of the essential oil were α-thujene (0.2983%). α-pinene (0.1509%), *p*-cymene (0.185%), myrcene (1.7638), limonene (20.5294%), α-terpinene (18.9218%), Δ careen (0.1561%), carveol acetate (0.1462%), carvone (0.441%), thymol (55.308%), α-terpineol (0.2404%), carveol (0.2813%), and bergapten (0.3705%). They found that the antifungal and wound-healing activity of ajwain oil was similar to iodine tincture (Table 4). These findings generate speculation that ajwain oil can protect cereals, nuts, fruits, vegetables, meat, eggs, fish, and vegetables from spoilage exhibited by *Aspergillus* and *Penicillium species* (Table 3).

Uniyal and co-workers assessed the antifungal action of ajwain oil against *A. niger*, *Aspergillus fumigatus,* and their spores using an agar well diffusion assay and gaseous contact method. They found antifungal activity against *A. niger* and *A. fumigatus* with inhibition zones of 20 ± 1.5 and 27 ± 1.0 mm, respectively. The gaseous contact assay revealed complete spore inhibition of both species (Uniyal et al., 2012). Similarly, Bansod and Rai studied ajwain oil as a preservative for antimycotic activity against *A. fumigatus* and *A. niger*; MICs of oils (%v/v) were determined by an agar dilution method, with MIC and MCC data (%v/v) obtained by the broth microdilution method (Bansod and Rai, 2008). The MIC and MCC values are given in Table 4. These authors classified ajwain as having low antimyotic activity when compared with other potent essential oils from *Cymbopogon martini, Eucalyptus globulus,* and *Cinnamomum zylenicum*. The different observations obtained by Uniyal et al. and Bansod are due to the assay techniques used. Agar diffusion technique assesses qualitative data only, as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium. Agar and broth dilution methods are also commonly used. The results obtained by each of these methods may differ as many factors vary between assays—differences in microbial growth, exposure of microorganisms to plant oil, the solubility of oil or oil components, and the use and quantity of an emulsifier.

Antifungal activities of various essential oils have been reviewed (Tabassum and Vidyasagar, 2013; Kamal et al., 2012). Tabassum et al. classified and described antifungal essential oils on the basis of the family of plant of origin. *Trachyspermum ammi* (L.) belongs to Apiaceae (or Umbelliferae), commonly known as the carrot or parsley family, which is a family of mostly aromatic plants. Ajwain oil is composed of 26 identified components, which account for 96.3% of the total amount. Thymol was found to be a major component along with *p*-cymene, γ-terpinene, β-pinene, and terpinen-4-ol. Ajwain oil exhibited a broad spectrum of fungitoxic activity against fungi, such as *A. niger, Fusarium moniliforme,* and *Curvularia lunata*; absolute mycelial zone inhibition was found to at a 6-μl dose of the oil (Tabassum and Vidyasagar, 2013). Kamal et al. listed the medicinal uses of *T. ammi* (L.). The volatile constituents of *T. ammi* seeds inhibited 72–90% growth of 10 fungi (*Acrophialophora fusispora*, *Curvularia lunata*, *Fusarium chlamydosporum*, *Fusarium poae*, *Myrothecium roridum*, *Papulaspora* sp., *Alternaria grisea*, *Alternaria tenuissima*, *Drechslera tetramera,* and *Rhizoctonia solani*; Table 4). These findings reinforce that phenolic components, such as thymol and carvacol in ajwain oil, exhibit antifungal activity depending on the concentration used (Kamal et al., 2012).

TABLE 3 Types of Food and Fungi Involved in Spoilage

Type of Food	Molds and Fungi
Molds commonly found on cereals, nuts, and their products	*Aspergillus candidus*
	Aspergillus flavus
	Aspergillus glaucus
	Aspergillus niger
	Aspergillus ochraceus
	Aspergillus parasiticus
	Chrysonilia sitophila
	Fusarium spp., e.g., *Fusarium graminearum*
	Penicillium citreoviride
	Penicillium citrinum
	Penicillium expansum
	Penicillium islandicum
	Penicillium stoloniferum
	Penicillium verrucosum
	Rhizopus stolonifer
Molds commonly found on high-sugar foods	*Aspergillus glaucus*
	Penicillium corylophilum
	Wallemia sebi
Molds commonly found on fruits and vegetables	*Alternaria* spp.
	A. niger
	Botrytis cinerea
	Cladosporium spp.
	Fusarium spp.
	Gloeosporium spp.
	Penicillium digitatum
	Penicillium expansum
	Penicillium italicum
	Rhizopus stolonifer
	Sclerotinia spp.
Molds commonly found on animal products such as meat, eggs, fish, and milk	*Aspergillus* spp., e.g., *Aspergillus versicolor*
	Eurotium spp., e.g., *Eurotium herbariorum*
	Penicillium spp. e.g., *Penicillium commune*
	Scopulariopsis spp.
Wilt disease on tomato and brinjal plants	*Fusarium oxysporum* f. sp. *lycopersici and*
	Fusarium solani f. sp. *Melonga*.

Species of fungi involved in food spoilage.

TABLE 4 Antifungal Activity of Ajwain (*Trachyspermum ammi* L.) Oil and Seeds Extract

Source	Antifungal Activity			References
Ajwain (*T. ammi* L.) oil		**MFC (mm)**	**Spore inhibition**	Uniyal et al. (2012)
	Aspergillus niger	20+1.5	Yes	
	Aspergillus fumigatus	27+1.0	Yes	
Ajwain (*T. ammi* L.) oil		**MFC (mm)**	**MIC (ppm)**	Gandomi H et al. (2013)
	Penicillium citrinum	>80	2000	
	Penicillium chrysogenum	>80	2000	
	Aspergillus flavus	>80	2000	
	A. niger	>80	1000	
	Aspergillus parasiticus	>80	3000	
Ajwain (*T. ammi* L.) oil		**MFC (µg/ml)**	**MIC (µg/ml)**	Khan R et al. (2010)
	Candida albicans 1	2500	2500	
	C. albicans 2	1250	1250	
	C. albicans 3	2500	2500	
	Candida glabrata	5000	2500	
	Candida krusei	>5000	2500	
	Candida tropicalis 1	2500	1250	
	C. tropicalis 2	5000	1250	
Ajwain seeds ethanolic, benzene and petroleum ether extract	*A. niger*	Benzene and petroleum ether extract better fungitoxic activity		Rizki et al. (1997)
	A. flavus			
	F. solani			
	Aspergillus alternata			
	Helminthosporium sp.			
Ajwain (*T. ammi* L.) oil		**Fungitoxicity**		Singh et al. (1979); Kamal et al. (2012)
	Acrophialophora fusispora	72–90% inhibition		
	Curvularia lunata			
	Fusarium chlamydosporum			
	Fusarium poae			
	Myrothecium roridum			
	Papulaspora sp.			
	Alternaria grisea			
	A. tenuissima			
	Drechslera tetramera			
	Rhizoctonia solani			
Ajwain (*T. ammi* L.) oil		**Fungitoxicity**		Singh et al. (1986)
	Epidermophyton floccosum	900 ppm (Ajwain)		
	Microsporum canis	(*T. ammi* L.) oil		
	Trichophyton mentagrophytes	1000 ppm (Thymol)		

The above values show minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) of Ajwain (*Trachyspermum ammi* L.) oil against fungal strains.

Dwivedi and Enespa et al. (2012) studied aqueous extraction of *T. ammi* seeds for protection against wilt disease on tomato and brinjal plants, at three concentrations—25, 50, and 75% (v/v)—using an in vitro poisoned food technique against *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium solani* f.sp. *Melonga.* The antifungal activity was assessed in terms of the percentage of inhibition of mycelial growth of the test fungi. The magnitude of inhibition was greater for *F. oxysporum* f. sp. *lycopersici* than *F. solani* f.sp. *Melonga* at 75% (v/v) dose (Table 4).

Rizki et al. reported that the ethanolic extract of *T. ammi* was effective against *A. niger*, *A. flavus*, *F. solani*, *Aspergillus alternata*, and *Helminthosporium* sp. Benzene and petroleum ether extracts also exhibited some activity (Rizki et al., 1997).

Pandey and Pant et al. (1997) reported antifungal activity of ajwain oil against *Pythium aphanidermatum*, *Macrophomina phaseolina,* and *Rhizoctonia solani*. The essential oils of the seeds exhibited strong fungitoxicity and completely controlled mycelial growth of all the tested fungi, even at very low concentrations. Sadik Tuzun et al. (2000) patented their invention of a natural preservative containing multiple plant extracts as a safe fungicide, bacteriocide, nematicide, and insecticide for plant protection against household pests. Thymol and carvacrol were components of their natural preservative compound.

Fermented sausages are considered to be good substrates for the growth of yeasts. Encinas et al. (2000) identified the presence of *Trichosporon ovoides* during the stage of manufacturing of Spanish fermented sausages. Saxena et al. (2012) reported on the antifungal action of ajwain oil against *T. ovoides*, with no inhibition (0 mm), 50 µl/mL (MIC), and 100 µl/mL (MFC) determined by an agar diffusion assay and broth dilution method, respectively. These findings suggest that higher concentrations of ajwain oil can protect the fermented sausages against *T. ovoides*.

Antifungal Mode of Action

The antifungal activity of ajwain oil can be collectively attributed to thymol and carvacrol. The mode of action of thymol against yeast and fungi has been sparsely investigated, but studies point to interactions with the cell envelope and intracellular targets. Thymol disrupts vesicles and cell membranes and impaired ergosterol biosynthesis in *Candida* strains, which consequently affected cell membrane integrity (Ahmad et al., 2011; Hyldgaard et al., 2012). Thymol also induces cell lysis in *S. cerevisiae*, thereby altering the cell structure and thus preventing the proliferation of cells (Bennis et al., 2004). Carvacrol exhibits antifungal action similar to that of thymol, causing disruption of Ca^{2+} and H^{+} homeostasis, upregulation and downregulation of gene transcription, disruption of membrane integrity, and impairment of ergosterol biosynthesis in *Candida* strains (Ahmad et al., 2011).

Ajwain Oil as an Antioxidant

Oxidation is one of the most important processes occurring in food systems. It affects many interactions among food constituents, leading to both desirable and undesirable products. Food lipids are very susceptible to oxidation; therefore, oxidation reactions are one of the major sources of deterioration that occurs during preparation, storage, and distribution of foods. Lipid oxidation products are ubiquitous in foods, although much variation exists in the kind and levels present. Although levels of these compounds are generally low, the problem of lipid oxidation severely compromises the quality and limits the shelf life of foods. All foods that contain lipids, even at a very low level (<1%), are susceptible to oxidation, leading to rancidity.

Changes in foods caused by lipid oxidation include loss of flavor, development of off-flavors, loss of color, loss of nutrient value, and the accumulation of compounds that may be detrimental to the health of consumers (W'sowicz et al., 2004). Oxidation affects the quality of food, but it also has an impact on the charge and conformation of the protein's three-dimensional structure, loss of enzyme activity, and changes in the nutritive value (Karel et al., 1975). Interactions between lipids and proteins have a significant effect on the progress of oxidative reactions in foods. Due to strong interactions, the oxidation reactions can easily transfer from lipids to proteins.

Gandomi et al. studied the antioxidant activity of ajwain oil by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and β-carotene-linoleic acid assay. Ajwain oil contributes as an antioxidant and the activity is confined to the concentration of the essential oil tested. The antioxidant potential of ajwain oil was less than the synthetic antioxidant butylated hydroxyl toluene. The activity of alcoholic extract oil is higher than that of aqueous extract. It seems that the high percentage of oxygenated monoterpenes, particularly thymol, and the high level of γ-terpinene and *p*-cymene of ajwain oil might be related to its high antiradical activity. The antioxidant properties of ajwain oil are attributed to oxygenated monoterpenes and monoterpene hydrocarbons such as thymol, carvacrol, γ-terpinene, and *p*-cymene (Gandomi et al., 2013).

Kamleshiya et al. reported a comparative study of the antioxidative activity of ajwain oil; they found that the reducing power of the aqueous and methanolic extracts was 26% and 36%, respectively, as compared to ascorbic acid. Similarly, the DPPH assay revealed antioxidant activity of 39% and 55% for aqueous and methanolic extract, respectively, as

compared with ascorbic acid (Kamleshiya et al., 2012). When compared with ascorbic acid, the H_2O_2 radical scavenging ability of the ajwain oil was higher than ascorbic acid at same concentration. The ability of the oil to quench hydroxyl radicals seems to be directly related to the process of preventing lipid peroxidation, thus indicating that they are good scavengers of active oxygen species (Chatterjee et al., 2013). Anti-inflammatory and antioxidant activity of *T. ammi* seeds were evaluated in collagen-induced arthritis in rats. It was concluded that seeds suppressed the induction of oxidative enzymes and markers in joint. It acts either by termination of cellular infiltration or reducing the generation of oxidants (Umar et al., 2012).

Ajwain Oil as an Antispoilage Agent

Almost all groups of microorganisms can contribute to the spoilage of foods under certain conditions. It is assumed that all microorganisms are initially present on a food product, but growth occurs based primarily on nutrient composition and on the chemical and physical parameters (Gram et al., 2002). Proteinaceous foods such as meat, poultry, fish, shellfish, milk, and some other dairy products undergo rapid spoilage. Because they are highly nutritious, with a neutral or slightly acid pH and have high moisture content, a wide range of microorganisms grow on them with similar pattern of microbial spoilage. Seafood spoilage organisms grow faster and produce the metabolites responsible for off-odors, off-flavors, and slime (Jos and Huis, 1996).

Ajwain oil has been demonstrated to have antibacterial, antifungal, antiparsitic, antigermicide, antifilarial, nematicidal, molluscicidal, fumigant, antitermitic, and anthelmintic activities. It has been used traditionally in India and other countries for this purpose. This exclusive property of ajwain oil accounts to be used in food preservation against spoilage. The antimicrobial activity of a given essential oil may depend on only one or two of the major constituents that make up the oil. However, increasing amounts of evidence indicate that the inherent activity of essential oils may not rely exclusively on the ratio in which the main active constituents are present, but also on interactions between these and minor constituents in the oils. Various synergistic antimicrobial activities have been reported for constituents or fractions of essential oils when tested in binary or ternary combinations. Henceforth, ajwain oil and its component can produce the best results as food preservative, when synergistic effects with other essential oils are taken into consideration.

The European Commission has registered thymol and carvacrol as flavoring agents, intended to be used in food products and considered to be safe for the consumer. The US-FDA has also classified these substances as generally recognized as safe. There are regulatory limitations on the accepted daily intake of essential oils or essential oil components; therefore, before they can be used in food products, a daily intake survey should be available for FDA evaluation.

SUMMARY POINTS

- *Trachyspermum ammi* L. (Ajwain) is widely grown in Egypt, Iraq, Iran, Afghanistan, Pakistan, and India, in arid to semi-arid regions where soils contain high levels of salts.
- Ajwain oil contains thymol as main active ingredient.
- Seeds are used traditionally in various bowel disorders, such as indigestion, flatulence, colic, and diarrhea.
- Ajwain oil exhibits a wide variety of pharmacological activities, including antibacterial, antifungal, and antioxidant activities.
- Ajwain oil is used by food industries to prevent the growth of molds and bacteria.
- Thymol and carvacrol have been considered safe by the European Commission and the US-FDA as a flavoring agent.

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Chapter 20

Amazon Rosewood (*Aniba rosaeodora* Ducke) Oils

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List of Abbreviations

DBH Diameter at breast height

INTRODUCTION

Rosewood is found in the Amazon region of Brazil, Guyana, Suriname, Peru, Colombia, and Venezuela. In Brazil, it occurs on both banks of the Amazon River, from the state of Amapá to northwest Peru, as well as from the south-central region of the state of Pará extending to the Purus River basin, in the southern state of Amazonas. Rosewood is found in moist upland forest, with its preferred habitat being the headwaters of small rivers (Ducke, 1938; SUDAM, 1972; Sampaio et al., 2003). In the Amazon, the distribution of adult trees (with a diameter at breast height of >20 cm) appears to be associated with groups of five to eight trees, spaced 50–100 m between trees and 300–400 m between groups (Alencar and Fernandes, 1978). Rosewood is a tall tree, reaching 30 m in height and 2 m in diameter; it has a straight cylindrical trunk and yellow-brown bark. The well-shaped crown occupies the upper canopy. Flowering occurs between October and March, with fruiting from February to June. The species changes its leaves during fruiting (Magalhães and Alencar, 1979). The low frequency of natural regeneration was reported to be only 35%, with trees having an average of 10 seedlings per tree (Mitja and Lescure, 1996). The fruits fall to the ground when ripe, but they can be predated by beetles still in the treetops or after dispersion, which makes the availability of seed the critical point for the propagation of the species.

In recent decades, the export of Brazilian rosewood oil has experienced a long decline. Many factors have contributed to trade reduction. The exhaustion of the source supplies, logistics and costs of production, strict Brazilian government regulation, and the synthetic linalool trade are the major contributors to this decline.

BOTANICAL ASPECTS

Two plant sources produce rosewood oil in the Amazon region: *Aniba rosaeodora* Ducke (syn. *A. rosaeodora* Ducke var. *amazonica* and *Aniba duckei* Kostermans) and *Aniba parviflora* (Meiss.) Mez (syn. *Aniba fragrans* Ducke) (Kubitzki and Renner, 1982; see Figures 1 and 2).

There are some disagreements related to the exact botanical status of these species. *Aniba rosaeodora* was identified by Adolph Ducke in 1927, but the commercial exploitation of rosewood oil was initiated in French Guiana with the species *Ocotea caudata* Koeller and *A. parviflora* (Meiss.) Mez, in 1883 (Ducke, 1930; SUDAM, 1972). Adolph Ducke believed that *A. rosaeodora* was confined to the Guianas, ranging from the Oyapoc River to Surinam. A rosewood specimen with smaller leaves found south of the Amazon River, between the cities of Juruti (Pará State, Brazil) and Maués (Amazonas state, Brazil), was interpreted by this botanist as a geographical variety; it was identified as *A. rosaeodora* var. *amazonica* (Ducke, 1930). Later, this variety was elevated to a specific rank as *A. duckei* Kostemans (Kostemans, 1938). Kubitzki and Renner (1982) analyzed plant material from *A. rosaeodora* and *A. duckei* and concluded that there is no basis for distinguishing these two entities. According to them, specimens with small leaves also occur in the Guianas, at the same time that plants with large leaves are seen in the north and south of Central Amazon. Previously, Gottlieb and Mors (1958a,b) isolated the flavanone pinocembrin from *A. rosaeodora* and the benzophenone cotoin from *A. duckei*. Based on this chemical evidence, these two scientists confirmed the occurrence of different rosewood sources.

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FIGURE 1 *Aniba rosaeodora* Ducke.

FIGURE 2 *Aniba parviflora* (Meiss.) Mez.

Aniba parviflora (syn. *A. fragrans*) is sometimes confused with *A. rosaeodora* (syn. *A. rosaeodora* var. *amazonica* and *A. duckei*), the real rosewood tree, by small farmers and oil producers. In this case, it is called "macacaporanga." The oil of this plant has less linalool content, ranging from 32% to 40% (Maia et al., 2001), whereas rosewood oil has linalool content between 78% and 93% (Maia et al., 2007). In the forest, the macacaporanga tree is very similar to young trees of rosewood, but its commercial exploitation is hampered by its low content of linalool and the distinct scent of rosewood oil.

USAGE AND APPLICATIONS

Rosewood oil has several medicinal uses: analgesic, anticonvulsant, antidepressant, antimicrobial, antiseptic, aphrodisiac, bactericidal, cellular stimulant, cephalic, stimulant, tissue regenerator, tonic, depressive sleeplessness, and grief (Lawless, 2002; Price and Price, 1999). The sedative and anticonvulsant properties of linalool in glutamate-related seizures, the inhibition of compound action potential in rodents, and the inhibition of adenylate cyclase in the retinas of chicks have been reported (Elisabetsky et al., 1995, 1999, Almeida et al., 2009; Sousa et al., 2010; Sampaio et al., 2012). It was also reported that linalool-rich rosewood oil induces vagovagal bradycardia and the depressor reflex in rats (Siqueira et al., 2013).

The effects of the essential oil from rosewood *A. rosaeodora* on the human epidermoid carcinoma cell line A431, immortal HaCaT cells representing an early stage of skin carcinogenesis, transformed normal HEK001 keratinocytes, and primary normal NHEK keratinocytes have been investigated. The identification of selective induction of apoptosis in pre-cancerous and cancerous skin cells highlights the potential anticancer activity of this essential oil (Soeur et al., 2011). The pharmacological effects attributed to linalool include anti-inflammatory activity and antinociception induction (Peana et al., 2002, 2003).

The antimicrobial activity of rosewood oil was investigated against *Acinetobacter baumanii*, *Aeromonas veronii* biogroup *sobria*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *P. vulgaris*, *Edwardsiela tarda*, *Salmonella enterica* subsp. *enterica* serotype *typhimurium*, *Serratia marcescens*, *Staphylococcus aureus*, and *Enterobacter aerogenes*. The oil inhibited these organisms at concentrations between 0.12% and 2.0% (Hammer et al., 1999) and 0.125 and 1.0 mg/mL (Lobato et al., 1989).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Brazilian rosewood oil has a characteristic aroma and is a long-established ingredient of fragrances, flavor, and food products. This colorless to pale-yellow oil is defined as a subnote of the family of woody scents and is considered as a high-impact note, presenting a balanced bouquet of floral, sweet, woody, and citric odors (Curtis and Williams, 2001). Zellner et al. (2006) performed olfactory analysis and used the notes floral, sweet, perfumed, citric, green, and linalool-like to describe samples of wood and leaf oils of the Amazon rosewood. The woody, citric, and green notes represented the main differences between the wood and leaf oils—the first being more significant for the wood-derived oil, as the others for the leaf-derived oil. Linalool is the major constituent (80–97%), which can be transformed into various derivatives of value to the flavor and cosmetic industries (Lawless, 2002).

The rosewood oil yield from commercial exploitation in the Amazon, which is sold in the international market, ranges from 0.7% to 1.2%. This is only half of the real oil yield existing in the plant because of the low efficiency of field distilleries (50%). The yield and quality of rosewood oil is also associated with other factors, as described in this section.

In seasonal study of a specimen of *A. rosaeodora*, the leaf oil varied from 1.6% in September and October to 2.2% in March (Maia et al., 2007). This leaf oil variation was previously observed in a specimen of *A. duckei* (Araújo et al., 1971) and is connected with the plant phenology. The higher rosewood oil content occurs between November and May, when the plant changes the old leaves. In these months, the average yield reaches 2.0%. The higher oil content in March (2.2%) coincides with the rainy season and thus increased precipitation in the Amazon (see Table 1).

Oil producers and wood collectors can distinguish some trivial rosewood types, according to their appearance and oil content. Regularly, they recognize the trees belonging to "rosewood-preciosa," "rosewood-tachi," "rosewood-itaúba," and "rosewood-imbaúba" types, based on morphological aspects such as wood and bark colors, leaf size, wood weight, and the leaf, bark and wood scents. However, in Herbaria of the Amazon Region, all of these morphological types are identical to that of *A. rosaeodora* Ducke. According to oil producers, to distill one drum of rosewood oil (180 L), they need 12–13 tons of wood chips from the rosewood-preciosa type, 15–16 tons from rosewood-tachi, and 18–20 tons from rosewood-itaúba and rosewood-imbaúba types (Maia et al., 2007). Different types of rosewood sources, based on their appearance and oil content, have been cited in other reports (Gottlieb et al., 1964; FAO, 1995; May and Barata, 2004).

The leaves of rosewood age and precipitation affects the quality of oil. Older leaves have greater percentages of α-terpineol, terpinen-4-ol, and linalool oxides, which result from the oxidative process in the plant. Younger leaves are richer in linalool. The low linalool content observed in March (68.0%) and April (74.8%) could be related to the higher water circulation in the oil-containing cells (see Table 1). Tertiary alcohols like linalol are more soluble and more easily eliminated.

With respect to wood, older trunks were observed to have greater amounts of α-terpineol, which is an oxidized product of linalool. In logging, the oil producer cuts the trunk wood near the ground, with a view to achieving more excellent scaling. However, this measure undermines the quality of oil because it increases the level of α-terpineol. The right thing would be to cut the trunk at breast height.

TABLE 1 Seasonal Study of Rosewood (*Aniba rosaeodora* Ducke)[a]: Oil Yield and Percentage of Linalool (Maia et al., 2007)

Sample	Collection Date	Oil (%)	Linalool (%)
PR01	September 2001	1.6	90.5
PR02	October 2001	1.6	88.1
PR03	November 2001	2.0	87.4
PR04	December 2001	2.0	96.1
PR05	January 2002	1.9	82.8
PR06	February 2002	1.9	84.2
PR07	March 2002	2.2	68.0
PR08	April 2002	2.0	74.8
PR09	May 2002	2.0	85.5
PR10	June 2002	1.7	91.2
PR11	July 2002	2.0	92.8
PR12	August 2002	1.9	86.6

[a]*The specimen planted in the Zoobotanical Park of Emilio Goeldi Museum, Belém, PA, Brazil, by Adolph Ducke, in 1946.*

TABLE 2 Morphological Types of Rosewood (Leaf and Wood): Oil Yield, Percentage of Linalool, and Physical Properties (Maia et al., 2007)

Samples	Plant Part	Oil (%)	Linalool (%)	Density	Refractive Index	Specific Rotation
Rosewood preciosa	Leaf Wood	3.1 3.4	83.9 75.3	0.9045 0.9070	1.4669 1.4674	+0.189 +0.110
Rosewood tachi	Leaf Wood	2.6 2.9	82.1 84.8	0.8920 0.8885	1.4679 1.4660	+0.138 +0.112
Rosewood itauba	Leaf Wood	2.6 1.8	79.7 78.9	0.9000 0.9090	1.4687 1.4610	+0.233 +0.056
Rosewood imbauba	Leaf Wood	2.4 2.0	79.8 76.9	0.9095 0.9035	1.4690 1.4703	+0.207 +0.100

Yields of oils from the leaves and wood of the morphological types of rosewood-preciosa, rosewood-tachi, rosewood-itaúba, and rosewood-imbaúba are very distinguishable. The oil yields of rosewood-preciosa had higher values (3.1% and 3.4%, respectively). The smaller content value for leaf oil has been observed for rosewood-imbaúba (2.4%); for wood oil, the smallest content was found in rosewood-itaúba (1.8%). These results confirmed the field observations of plant collectors with respect to the oil yield from popular types of Amazon rosewood. In the prospective work of mapping the trees that will be cut for commercial exploitation, rosewood collectors give preference to rosewood-preciosa over the others types existing in the forest (see Table 2).

The linalool content in wood oils of rosewood varieties differs from the oil yields. A higher content of linalool has been found in the oil of rosewood-tachi (84.8%), with the lowest content being observed in the oil of rosewood-preciosa (75.3%). In the leaf oil of the morphological types of rosewood, linalool content shows little variation between rosewood-preciosa (83.9%) and rosewood-itaúba (79.7%). In field work, plant collectors normally prefer rosewood-preciosa because of its higher oil yield, but they ignore the lower linalool content when compared with other morphological types. The international market requires rosewood oil to have at least 85% linalool; thus, buyers may refuse the purchase of many barrels of rosewood oil. The oil yield, percentage of linalool and values for density, refractive index, and specific rotation of the rosewood morphological types are listed in Table 2. These physical properties are important specifications used in

TABLE 3 Main Constituents of Leaf and Wood Oils from a Specimen of *Aniba rosaeodora* Collected by Adolph Ducke (Maia et al., 2007)

Constituents	RI	Leaves Oil (%)	Wood Oil (%)
α-Pinene	939	0.4	0.1
β-Pinene	953	0.4	0.3
Limonene	1029	0.1	0.7
1,8-Cineole	1031	0.5	0.3
trans-Linalool oxide (furanoid)	1074	0.8	0.7
cis-Linalool oxide (furanoid)	1088	0.7	0.6
Linalool	1098	85.5	84.8
trans-Linalool oxide (pyranoid)	1176	0.3	0.1
α-Terpineol	1187	0.1	2.9
α-Copaene	1376	0.5	0.4
β-Selinene	1488	0.9	0.7
α-Selinene	1496	0.4	0.6
Spathulenol	1576	0.7	0.1
Caryophyllene oxide	1581	0.8	0.1
Minor oxygenated sesquiterpenes	1655–1764	4.3	3.4
Benzyl benzoate	1762	0.2	0.6
Total		94.4	94.3

RI = retention index on DB-5ms (stationary phase) capillary column.

the international market to control the sale of rosewood oil, and they are in line with values that were previously reported (Gottlieb et al., 1964; SUDAM, 1972).

The quantitative variation observed in samples of the morphological types of rosewood can be due to varying environmental factors that occur in the areas where they were collected. The sample collection has been made from specimens growing wild in the left side of the Jatapu River, municipality of Novo Airão, Amazonas state, Brazil.

From the morphological point of view, as already mentioned, the species *A. parviflora* is much like *A. rosaeodora*, causing frequent confusion between these two plants (Ferraz et al., 2009). Despite the similarity, the oils of the wood and leaves of these species have very distinct scents. Olfactory and compositional analysis of both oils, using enantioselective gas chromatography/olfactometry and two-dimensional gas chromatography, allowed a clear distinction between the two species (Zellner et al., 2006; Tranchida et al., 2008).

The main components of the oils of leaves and wood from a specimen of *A. rosaeodora* Ducke, growing in the Zoobotanical Park of Emilio Goeldi Museum (city of Belém, Pará state, Brazil) are shown in Table 3. The botanist Adolph Ducke planted this specimen in 1946, from which a voucher (MG 17.710) is deposited in the Herbarium of Emilio Goeldi Museum.The main components identified in the oil of leaves and wood of a specimen of *A. parviflora*, growing in the region of Curuauna River, Municipality of Santarém, Pará state, Brazil, are shown in Table 4.

According to the literature, rosewood oil is widely used in most major food categories, such as soft drinks, alcoholic beverages, and confectioneries (Lawless, 2002; Rhind, 2014). Rosewood oil has shown acaricidal activity against *Thyrophagus putrescentiae* in an impregnated fabric disc bioassay, at concentration of 93 μg/cm^2, with similar value of benzyl benzoate and Deet (Ando, 1994; Kim et al., 2003). Similarly, the rosewood oil was tested for its insecticidal activity against *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), an economically important pest of various greenhouse vegetables, particularly tomatoes and cucumbers. The oil was highly effective against the adult, nymph, and egg insects at a concentration of 93 μL/mL (Choi et al., 2003). Rosewood oil (5–10 μL/mL) was also used in macro- and micro-dilution tests to

TABLE 4 Main Constituents of Leaf and Wood Oils From a Specimen of *Aniba parviflora*

Constituents	RI	Leaf Oil (%) (Pereira, 2012)	Wood oil (%) (Maia and Mourão, 2014)
α-Thujene	921	0.3	0.2
α-Pinene	939	2.1	
β-Pinene	953	1.6	
Myrcene	990	2.4	1.6
α-Phellandrene	1002	1.6	4.1
p-Cymene	1019	0.8	1.6
β-Phellandrene	1025	12.2	17.3
(*E*)-β-Ocimene	1039	2.7	1.4
γ-Terpinene	1051	0.1	0.1
cis-Linalool oxide (furanoid)	1064	0.1	0.1
Terpinolene	1072	0.2	0.2
Linalool	1097	40.1	44.9
Borneol	1165	0.2	0.2
Terpinen-4-ol	1172	0.4	0.2
α-Terpineol	1188	0.2	0.7
Geraniol	1244		0.1
α-Cubebene	1344	0.1	0.8
α-Ylangene	1366	0.1	0.1
α-Copaene	1372	0.2	0.9
β-Elemene	1386	0.3	0.3
β-Caryophyllene	1415	3.8	3.9
γ-Elemene	1426		0.3
α-Humulene	1450	0.5	0.5
β-Chamigrene	1468		0.5
Germacrene D	1476	1.5	0.4
β-Selinene	1483	0.1	2.0
α-Selinene	1491		2.1
Bicyclogermacrene	1500	0.8	
γ-Cadinene	1508		0.2
δ-Cadinene	1513	0.1	0.6
Elemol	1548	0.6	
Germacrene B	1552	0.2	0.3
(*E*)-Nerolidol	1557	1.5	0.2
Spathulenol	1570	1.2	0.8
Caryophyllene oxide	1575	2.7	1.0
Globulol	1579		0.2
Guaiol	1591	0.4	0.2

TABLE 4 Main Constituents of Leaf and Wood Oils From a Specimen of *Aniba parviflora*—cont'd

Constituents	RI	Leaf Oil (%) (Pereira, 2012)	Wood oil (%) (Maia and Mourão, 2014)
Humulene epoxide II	1602	0.4	0.2
10-*epi*-γ-Eudesmol	1624		0.6
γ-Eudesmol	1627	0.4	2.1
Agarospirol	1639	8.4	
Selina-3,11-dien-6-α-ol	1640		0.2
β-Eudesmol	1645	1.6	
α-Eudesmol	1648	1.6	1.6
Benzyl benzoate	1745		0.1
Unidentified sesquiterpenes		3.8	2.3
Total		95.3	95.1

RI = retention index on DB-5ms capillary column.

determine their fungistatic and fungicidal activities against some food poisoning and spoilage fungi. In this concentration range, the growth of all micromycetes was inhibited. The percentage of linalool in the oil was 81.3% (Simic et al., 2004).

Linalool—the main constituent of rosewood oil—is a natural compound that is water soluble at room temperature. It is a tertiary terpene alcohol that is biosynthesized as d-, l- or dl-linalool by many plants, especially in herbs, spices, fruits, and trees. Linalool has been produced in high amounts, either from natural precursors or through chemical synthesis. It is estimated that more than 95% of the total worldwide linalool production is used for vitamin E synthesis or is added to processed food and beverages, perfumes, cosmetics, soaps, household detergents, and waxes for its flavoring and fragrant properties. It is also used traditionally for stored-food pest control from natural sources as the rosewood oil. The worldwide linalool production was estimated to be 12,000 tons in 2000. Half of this amount was produced by chemical synthesis, while the rest was obtained from natural plant sources (OECD SIDS, 2002).

Approximately 70% of the terpenoids of floral scents are represented by linalool (Stashenko et al., 2008). Because of their chiral properties, two enantiomers of linalool occur in plants: (3S)-(+)-linalool (coriandrol) and (3R)-(−)-linalool (licareol). They have different fragrance profiles. Coriandrol is perceived as sweet, floral, herbaceous, and petitgrain-like with citrus and fruity notes, while licareol has a woody, lavender-like aroma. The International Joint Food and Agriculture Organization of the United Nations/ World Health Organization Expert Committee on Food Additives established an acceptable daily intake of 0–0.5 mg/kg body weight/day for linalool. Amazon rosewood oil and Chinese Ho oil (*Cinnamomum camphora* Nees) are the main sources of (R)-(−)-linalool (about 90%) while coriander oil (*Coriandrum sativum* L.) contains relevant levels of (S)-(+)-linalool (over 80%) (Aprotosoaie et al., 2014).

Linalool is a key contributor to the aroma of hops (*Humulus lupulus* L.) in beers. The hoppy flavor in beers is perceived at linalool concentrations above 20 μg/L, and it is responsible for a fruity-flowery flavor (Kautner et al., 2000; Kautner et al., 2003). Sweet basil oil (*Ocimum basilicum* L.) is widely used in the food industry and its European type, considered to be of the highest quality and producing the finest odor, contains linalool as the main component; fresh or dried aromatic leaves are used as a flavoring agent for foods, confectionery products, and beverages (Marotti et al., 1996; Radulovic et al., 2012). The antimicrobial potential of linalool can be strengthened combining appropriate concentrations with other terpenes, such as citral and β-pinene. Linalool and β-pinene, at concentrations of 40–60 μL/L, led to lower spoilage in an orange soft drink when a wild strain of *Saccharomyces cerevisiae* was used (Belletti et al., 2010).

The US Food and Drug Administration allows the use of numerous natural and artificial flavorings in beverages, candy, ice cream, and baked goods containing various amounts of linalool and its esters, due to its flavorful and fragrant properties (Colliver et al., 2007; Vilas and Venkatraj, 2009; Caputi and Aprea, 2011). The potential effectiveness of microcapsules and the effects of different types of matrices on the stability of linalool have been evaluated, such as controlled-release systems for its slow volatilization, when applied as an insecticide against stored food pests (Lopez et al., 2008, 2012). Cocoa beans (*Theobroma cacao* L.) contain linalool primarily as (S)-enantiomer. Model experiments and analyses of commercial products, such as cocoa powders and chocolates, revealed that the technological procedures employed in the manufacturing of cocoa products do not result in significant changes of the original enantiomeric distribution of linalool (Schmarr and Engel, 2012).

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Chapter 21

Angelica (*Angelica glauca* and *A. archangelica*) Oils

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List of Abbreviations

FDA Food and Drug Administration
FEMA Flavour and Extract Manufacturers Association
GRAS Generally recognized as safe
NDBR Nanda Devi Biosphere Reserve

INTRODUCTION

Essential oils are complex mixtures of volatile compounds, used as additives to protect food materials against microbial aggression and as a flavor to enhance taste. The oils from plant material can prevent unfavorable antioxidant–prooxidant balance from appearing in the food. High-altitude floras are known for their great medicinal value in curing various diseases and for their use as food supplements. Alpine floras have unique properties for producing secondary metabolites compared to temperate-region plants and vice versa.

A plant can be considered as a system where many biochemical reactions occur. The secondary metabolites are the product or byproduct of biosynthesis, where several factors alter the synthetic route, including soil, climate, rainfall, altitude, grazing frequency, and amount of sunlight, causing the plant to produce different metabolites. Based on the geoclimatic distribution of the flora, botanicals may be consumed by local residents to cure disease, and some plants are frequently used in food preparation.

The genus *Angelica* is an important medicinal and aromatic plant used in traditional medicine to cure various diseases. *Angelica glauca* (Figures 1 and 2) grows at high altitudes in the Himalayan region of India; it is locally known as *chora* or *gandrayan*. The plant is a prioritized medicinal plant of western Himalaya and ranks third on the list of 52 medicinal plants prioritized for consultation and conservation (Sastry and Chatterjee, 2000). *Angelica archangelica* (Figures 3 and 4) seed oil was granted generally recognized as safe (GRAS) status by the Flavor and Extract Manufacturers Association in 1965 and is approved by the US Food and Drug Administration for food use (GRAS). The Council of Europe (1970) included *A. archangelica* seed oil in the list of substances, spices, and seasonings deemed admissible for use with a possible limitation of the active principle in the final product. Both the plants are extensively used in traditional medicines for various treatments as well as in a variety of food recipes, but little scientific information is available on its biological activities, especially of essential oils.

BOTANICAL ASPECTS

Angelica glauca Edgew. (syn: *Angelica nuristanica*), (Figures 1 and 2) belonging to the family Apiaceae, is commonly known as Smooth Angelica. It is distributed in the north-temperate, arctic regions and New Zealand. In India, this plant is found from Kashmir to Simla at an altitude range of 8000–10,000 ft. This plant is considered to be crucially endangered in the Himalayan region and needs to be conserved. *Angelica glauca* is an aromatic, glabrous herb that grows up to 1–2.5 m high. The leaves are one-, two-, or three-pinnate, toothed, and usually large. The umbels are compound, with many rays and few, narrow, or absent brats. The bracteoles are small. The calyx-teeth are obsolete; petals are obovate, emarginated, white, or lurid-purple. The fruit is ovoid or ellipsoid, dorsal and intermediate are not prominent; carpels are complanate and broadly marginate; furrows are 1-2-vittate; and carpophores 2-partite. The seed is much compressed dorsally and the inner face is plane concave or almost grooved (Hooker, 1879).

Angelica archangelica Hoff. (syn: *Angelica officinalis*) (Figures 3 and 4) is commonly known as angel's herb, root of the holy spirit, and garden angelica. It is distributed in north temperate regions. In India, this plant is found from Kashmir at

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FIGURE 1 *Angelica glauca* inflorescence with seeds. *Source:* http://planttaxonomy.blogspot.in/2011/01/angelica-glauca.html

FIGURE 2 *Angelica glauca* aerial parts. *Source:* http://www.ayurvediccommunity.com/Botany.asp?Botname=Angelica%20glau

FIGURE 3 *Angelica archangelica* inflorescence. *Source:* https://farm2.staticflickr.com/1068/4724445244_39a193fc55_b.jpg

FIGURE 4 *Angelica archangelica* aerial parts with inflorescence. *Source: http://botany.csdl.tamu.edu/FLORA/schoepke/ang-ar-1.jpg*

an altitude range of 8000–13,000 ft, in Jogi at an altitude of 11,000 ft, and in Sikkim and Lachen at an altitude range of 10,000–11,000 ft. This plant is a tall and perennial herb. The leaves are two- to three-pinnate; the ultimate pinnae is toothed and usually large. The umbels are compound, with many ray and few, small, or absent bracts. The bracteoles are many, linear, or obsolete. The calyx-teeth is obsolete in Indian species. The petals are white and ovate. The fruits are ellipsoid, subquadrate, or oblong. The carpels are dorsally compressed and lateral ridges are winged or acute, dorsal and intermediate narrower. The seed is much compressed dorsally and the inner face is plane (Hooker, 1879).

USAGE AND APPLICATIONS

Traditionally, *A. glauca* is used in medicines, aromatic spices, and condiments. The roots of this plant are also used by the local people for giving the flavor of celery to their food. Local inhabitants of Tibet and Nanda Devi Biosphere Reserve consume large quantities of *Dongcha,* which is prepared with the powder of *A. glauca* and *Pleurospermum angelicoides* rhizomes mixed with *Allium* leaves and salt. They also use the decantant of root stock of *A. glauca* and *P. angelicoides* boiled in water to treat stomach pains and headaches (Kandari et al., 2007).

The roots of the *A. glauca* are also used for seasoning curry (Collet, 1921). In the Kumaun and Garhwal regions of Uttarakhand, as well as other parts of India, the roots are used in dal or curry flavoring to enhance taste. The majority of the roots are used for the preparation of horse gram recipes by the people of Uttarakhand during the winter season. This herb is given to women after the delivery of a baby for vitality and strength.

Angelica glauca has high global and domestic demand for the preparation of various drug formulations. *Angelica* has a long history of medical, magical, and culinary tales to its credit. In traditional medicine, *A. glauca* is used in the treatment of dyspepsia, constipation, infantile atrophy, dysentery, menorrhagia, and rinderpest. The roots of *A. glauca* in the powdered form are used traditionally in different remote areas of the Uttarakhand region, where a primary health care system is not available. The roots are used for the treatment of stomach trouble in children, for vomiting, as a cardiac stimulant, and for gastric disorders and constipation.

Angelica archangelica has been used primarily as a digestive, as a tonic, for flatulence (Boon and Smith, 1999), and especially for mild cramping in gastrointestinal disturbances (Blumenthal, 2000). In folk medicine, *A. archangelica* is also used as an antiseptic, expectorant, diuretic, and antiemetic, as well as for toothaches, wounds, rheumatism, skin rashes, fever, and headache (Bhat et al., 2011). This plant is a good remedy for cold, cough, pleurisy, wind, colic, and disease of the urinary organs, although it should not be given to the patients who have a tendency toward diabetes, as it causes an increase of sugar in the urine (Bhat et al., 2011).

Angelica archangelica is used extensively in the liquor industry for flavoring, such as in benedictine, boonekamp, and chartreuse (Blumenthal, 2000; Boon and Smith, 1999). *Angelica archangelica* is commonly used in Chinese medicine for

cerebral diseases and in traditional formulation/recipes for the treatment of typhus fever, typhoid, and chronic rheumatism. Both plants are extensively used in traditional medicines for various treatments, as well as in a variety of food recipes.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Antioxidant Activity

The essential oil of *A. glauca* exhibited good DPPH radical scavenging activity, showing 93.4% inhibition, as well as 45.05% inhibition of peroxidation (Irshad et al., 2011). The essential of *A. glauca* can be used for the preservation of food materials. The IC_{50} value of *A. archangelica* oil (3.89 μL/mL) has strong antioxidant potential (Prakash et al., 2014) and thus can help in the inhibition of oxidative deterioration in food products.

Antimicrobial Activity

Studies on disk diffusion and the minimal inhibitory concentration of *A. glauca* essential oil demonstrated inhibition of the growth of bacteria and fungi (Irshad et al., 2011). In one report, the essential oil of *A. glauca* exhibited in vitro antifungal activity (Irshad et al., 2012). The essential oil of *A. archangelica* showed activity against eight mold species, including some aflatoxigenic *Aspergillus flavus* strains. The oil of *A. archangelica* can be used to protect agri-food items during storage and processing in the food industry (Prakash et al., 2014).

The essential oil obtained from the roots of *A. archangelica* showed good antimicrobial activity against microorganisms such as *Clostridium difficile*, *Clostridium perfringens*, *Enterococcus faecalis*, *Eubacterium limosum*, *Peptostreptococcus anaerobius*, and *Candida albicans*, with minimum inhibitory concentration values of 0.25, 0.25, 0.13, 0.25, 2.25, and 0.50% v/v, respectively (Fraternale et al., 2014). The essential oil of *A. archangelica* showed moderate antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus* (Gram-positive), *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* (Gram-negative) bacterial strains. There was only a small difference between the power of activity against Gram-positive and Gram-negative bacteria, with minimal inhibitory concentrations ranging from 0.62 to 1.25 mg/ml and 1.25–2.5 mg/ml, respectively (Skalicka-Wozniak et al., 2011). Thus, the essential oils of *A. glauca* and *A. archangelica* can be used as food additives for food preservation.

Insecticidal Effects

The essential oil from *A. glauca* roots showed antifeedant activity against *Spodoptera litura,* a major lepidopteran pest, at concentrations of 0.01%, 0.1%, 1%, 2.5%, and 5%. The oil of *A. glauca* exhibited significant antifeedant activity (100% protection at 5% of either; 60% and 95% protection at 2.5% of the respective oils) (Sharma et al., 1990). The essential oil obtained from the seeds of *A. archangelica* showed chronic and acute toxicity, antifeedancy, and growth inhibition of *Spodoptera littoralis* larvae. Significant acute toxicity was caused only by the essential oil (LD_{50} 96 μg/larva) producing growth inhibition (Pavela and Vrchotova, 2013).

Major Chemical Compounds of the Essential Oil of *Angelica glauca*

The chemical constituents of *A. glauca* essential oil were analyzed by gas chromatography coupled with mass spectrometry (GC/MS) and gas chromatography equipped with flame ionization detector (GC-FID). The major essential oil components of α-phellandrene (1), *trans*-carveol (2), β-pinene (3), thujene (4), caryophyllene oxide (5), β-caryophyllene (6), γ-terpinene (7), nerolidol (8), β-bisabolene (9), and germacrene D (10) have been reported in aerial parts of *A. glauca* (Agnihotri et al., 2004). In the roots, the main compounds β-phellandrene (11), (*Z*)-ligustilide (12) (Kaul et al., 1996), methyl octane (13), limonene (14), β-phellandrene (11), β-pinene (3), (*Z*)-3-butylidene-phthalide (15), (*Z*)-ligustilide (12), (*E*)-ligustilide (16), and citronellyl acetate (17) (Thappa et al., 2005) have been reported (Figure 5).

Major Chemical Compounds of the Essential Oil of *Angelica archangelica*

The chemical constituents of *A. archangelica* essential oil were analyzed by GC/MS and GC-FID. The major components were α-pinene (18), octanal (19), β-myrcene (20), δ-3-carene (21), limonene (14), γ-terpinene (7), α-humulene (22), germacrene D (10) (Pasqua, 2003), β-phellandrene (11), *p*-cymene (23), and sabinene (24) (Wedge et al., 2009). The seed (fruit) oil contains β-phellandrene (11), α-pinene (18), germacrene D (10), α-phellandrene (1), sabinene (24) (Nivinskiene et al., 2005), tridecano-13-lactone (25), 12-methyltridecano-13-lactone (26), tetradecano-14-lactone (27),

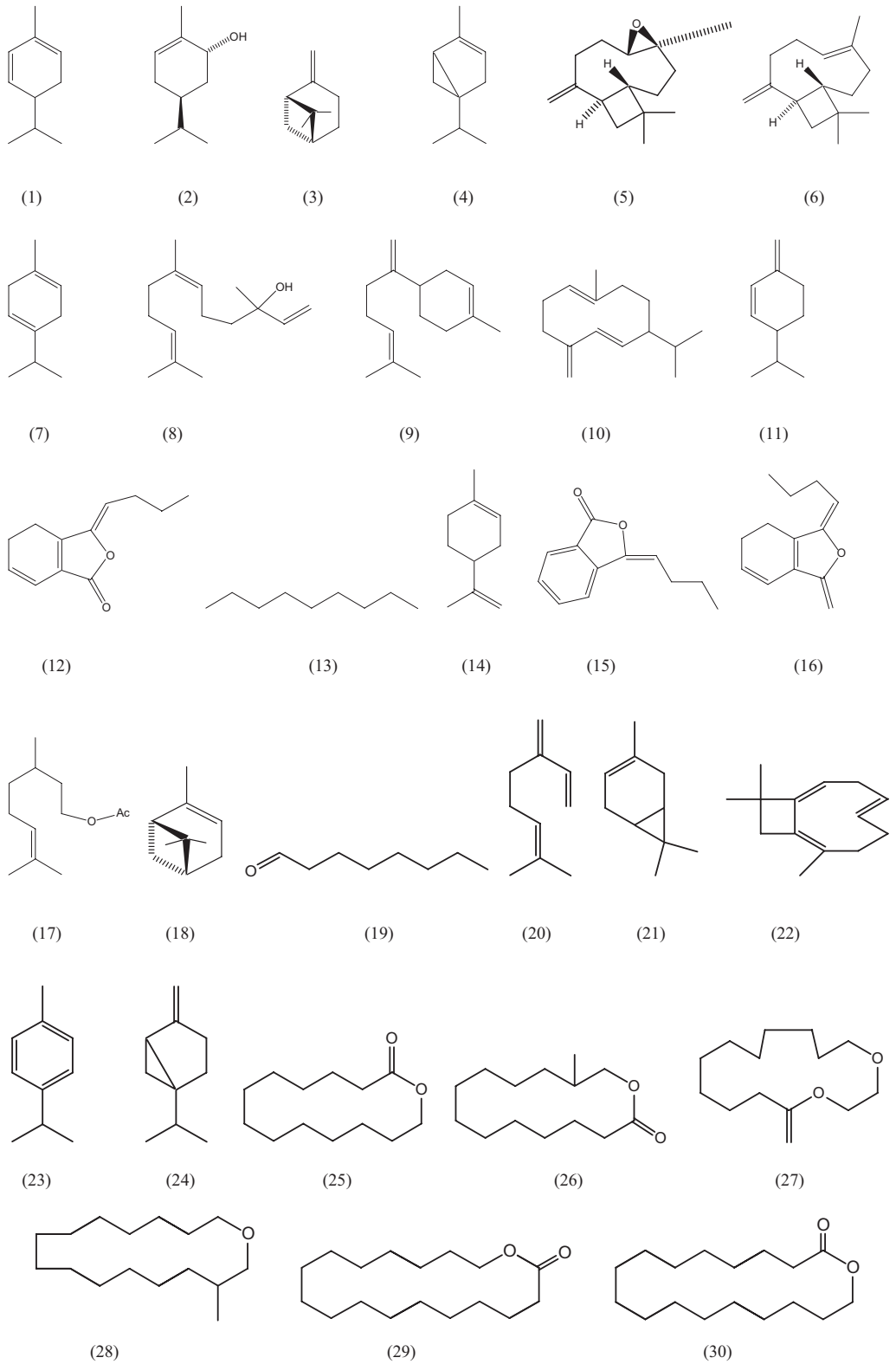

FIGURE 5 Structure of the major constituents of *Angelica glauca* and *Angelica archangelica* essential oils: (1) α-phellandrene; (2) *trans*-carveol; (3) β-pinene; (4) thujene; (5) caryophyllene oxide; (6) β-caryophyllene; (7) γ-terpinene; (8) nerolidol; (9) β-bisabolene (10) germacrene D; (11) β-phellandrene; (12) (*Z*)-ligustilide; (13) methyl octane; (14) limonene; (15) (*Z*)-3-butylidene-phthalide; (16) (*E*)-ligustilide; (17) citronellyl acetate; (18) α-pinene; (19) octanal; (20) β-myrcene; (21) δ-3-carene; α-humulene (22); *p*-cymene (23); sabinene (24); tridecano-13-lactone (25); 12-methyltridecano-13-lactone (26); tetradecano-14-lactone (27); 14-methylpentadecano-15-lactone (28); hexadecano-16-lactone (29); heptadecano-17-lactone (30).

14-methylpentadecano-15-lactone (28), hexadecano-16-lactone (29), and heptadecano-17-lactone (30) (Lopes et al., 2004) from the essential oil of *A. archangelica* (Figure 5).

SUMMARY POINTS

- *Angelica glauca* and *A. archangelica* are medicinal plants that grow at high altitudes.
- Both the plants are used as flavoring ingredients in a variety of foods.
- Secondary volatile compounds are present in the *Angelica* oils.
- *Angelica* oils possess antioxidant and antimicrobial activity.
- *Angelica* oil can use as a food additive and preservative.

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Chapter 22

Aniseed (*Pimpinella anisum*, Apiaceae) Oils

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INTRODUCTION

Essential oils are complex volatile mixtures of substances found in many vegetal species. They are considered one of the most important natural products from an economic standpoint, mainly due to their medicinal properties, fragrance, and flavor; they play an important role in pharmaceutical, cosmetic, and food industries. This special raw material is usually extracted by hydrodistillation or steam distillation methods, but essential oil also can be obtained directly by expression and centrifugation of citric fruits. Chemically, essential oils are mainly constituted of monoterpenes and sesquiterpenes, but they also can be constituted by aromatic substances, such as phenylpropanoids, and aliphatic substances (Bakkali et al., 2008).

One of the most important species that produces essential oil is *Pimpinella anisum*, commonly known as anise. This is one of the oldest species used by people; it has been cultivated since ancient times, initially in Egypt and later in Greece, Rome, and the Middle East. Anise essential oil is usually extracted from dried ripe fruits by hydrodistillation, but it also can be obtained by supercritical fluid extraction. It is mainly constituted by anethole, an aromatic substance that appears as the major compound of the oil, usually corresponding to more than 80% (w/w) of the oil (Brasil, 2010; Samojlik et al., 2012; Rodrigues et al., 2003; Özel, 2009; Ullah and Honermeier, 2013) (Table 1). Other substances, such as methyl chavicol, himachalene, linalool, and α-terpineol, are also found as important constituents of this oil (Figure 1) (Matos, 2004). Anise and its essential oil are important raw materials in the food industry in a wide range of applications. Anise can be used as a flavoring, antioxidant, antispoilage agent, and preservative in many products, such as candies, sweets, toffees, and beverages. Moreover, it can be used during harvesting, packing, and in integrative agriculture practices of pest control. The aim of this chapter is to provide relevant information regarding this species in the food industry context.

BOTANICAL ASPECTS

Pimpinella anisum L. is the accepted name for the species of the Apiaceae family that is commonly known as "anise". Many synonyms are used for this plant, including *Anisum odoratum* Raf., *Anisum officinale* DC., *Anisum officinarum Moench, Anisum vulgare Gaertn., Apium anisum* (L.) *Crantz, Carum anisum* (L.) Baill., *Ptychotis vargasiana* DC., *Selinum anisum* (L.) E.H.L. Krause, *Seseli gilliesii* Hook. & Arn., *Sison anisum* (L.) Spreng., and *Tragium anisum* (L.) *Link* (The Plant List, 2010).

Anise has white flowers and yellow-brown or green-brown fruits (Figure 2), which contain not less than 2% (w/w) of essential oil, related to dry material. The small size and form of its dried ripe fruits cause them to be erroneously referred to as "seeds," with the raw plant material often described as "aniseed."

Anise grows spontaneously in the Mediterranean region (Brasil, 2010; Besharati-Seidani et al., 2005; Hajou et al., 2004) and is often cultivated in order to obtain its fruits, which are primarily the plant part used for essential oil extraction, although extraction of roots also can be performed. It is well known that environmental conditions, such as temperature, luminosity, soil fertility, insect attacks, season, and precipitation, among other factors, have fundamental impacts in secondary metabolite production. In anise cultivation, it is especially important that the growing season occurs in warm climatic conditions, in order to obtain good fruits and essential oil yields (Ullah and Honermeier, 2013). The stage of plant maturity and, therefore, harvesting stage are also important factors that influence essential oil production. According to Özel (2009), the best yield of fruits and essential oil can be achieved when harvesting time starts after complete maturation of the main umbel, while the quality of essential oil is higher when harvesting at the beginning of waxy stage and the main stem has color changes. Moreover, data indicate that a plant density of about 40 plants/m^2 allows good yields for commercial fruit production (Rassam et al., 2007).

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TABLE 1 Major Anethole Content Expressed as Percentage of Total Oil Composition

Anethole Content (%)	Extraction	References
88.5	Hydrodistillation	Samojlik et al. (2012)
89.4	Supercritical fluid	Rodrigues et al. (2003)
92.1	Hydrodistillation	Özel (2009)
96.9	Hydrodistillation	Ullah and Honermeier (2013)

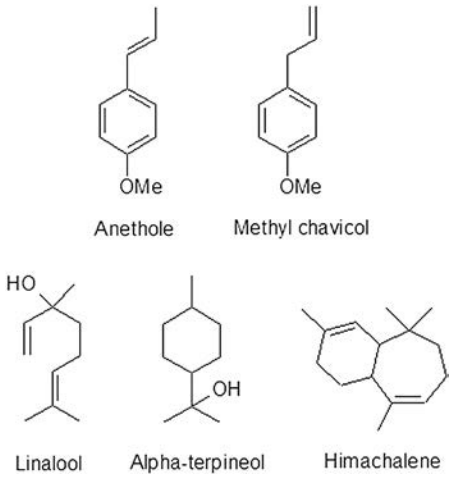

FIGURE 1 Chemical constituents of aniseed essential oil.

FIGURE 2 Dried ripe fruits of *Pimpinella anisum* L. (Apiaceae).

USAGE AND APPLICATIONS

Anise is one of the oldest species, used by ancient Greeks, Romans, and Arabs. It was also recommended by notable personalities, including Pliny, Pythagoras, and Hippocrates. The fruits from this species were also used as currency in the Middle Ages (Alonso, 2004). Anise has morphological similarities to another species from the family Apiaceae, resulting sometimes in serious problems. For example, misidentification can occur between *Pimpinella anisum* and *Conium maculatum*, causing intoxication and/or death (Colombo et al., 2009).

Aniseed and its essential oil have been used in folk medicine for a wide range of therapeutic uses, such as a diuretic, mild expectorant, tranquilizer, stomachic, antifungal, antibacterial, anticonvulsant, carminative, milk secretion inducer,

antispasmodic, expectorant, and intestinal purifier, among others (Samojlik et al., 2012; Janahmadi et al., 2008; Hajou et al., 2004). This species and the parameters of quality control for the herb are described in many official compendiums around the world, such as the British Pharmacopeia, United States Pharmacopeia, and Brazilian Pharmacopeia. Moreover, its use is regulated in Europe; aniseed is also listed as generally regarded as safe (GRAS) by the US Food and Drug Administration (FDA; Hajou et al., 2004).

The use of aniseed in folk medicine encouraged many researchers to investigate the medical properties of essential oil from this species, including its strong antimicrobial activity. Moreover, many studies of this essential oil include elucidation of the mechanism of action. For example, aniseed essential oil was able to induce bronchodilatory effects, which may be explained by the inhibition of muscarinic receptors (Boskabady and Ramazani-Assari, 2001). Despite the remarkable advantages and potential uses of essential oils, in the case of aniseed, a study performed by Janahmadi et al. (2008) indicated that patients suffering from epilepsy should use products that contain essential oil from aniseed with caution due to its capacity to induce hyperexcitability (Janahmadi et al., 2008).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Aniseed has been used as spice since ancient times, being one of the most important plant species used in the culinary field. Before the advent of freezers, many essential oil producers had a main role in food conservation; nowadays, we may attribute this property to antimicrobial effects. In this regard, the complex mixtures of secondary metabolites that constitute the essential oils have a major role on food preservation. The dry ripe fruits of anise are used in order to obtain its essential oil, which also plays an important role in food technology, being used as flavoring in many products, such as breads, cakes, candies, and beverages (Hajou et al., 2004). The maximum levels of the oil acceptable by FDA are 750 ppm for alcoholic beverages and 680 ppm for candies (Cáceres, 2009). Anise is especially important in food technology because many consumers prefer to use "green products" without synthetic preservatives (Gülçin et al., 2003). Essential oils are also used to avoid spoilage and contamination of food by microorganisms, exhibiting their potential in meat preservation, meat products, fish dishes, rice, and fruits. A high fat content usually reduces these activities (Burt, 2004).

The use of aniseed essential oil as an additive is also promising. According to Tepe et al. (2006), an essential oil with high anethole content (82.8%) obtained from *P. anisetum* showed great antioxidant activity in DPPH free radical-scavenging and β-carotene/linoleic acid assays. This result suggests that essential oil from *P. anisum*, which also has a high content of anethole, may have usefulness in this approach. This is especially important to the food industry because antioxidants can prevent food degradation by inhibiting the lipid peroxidation chain. Aniseed essential oil has great potential in the development of new products because it does not exhibit significant toxicity and has minimal hepatoxicity in normal therapeutic dosages (Albert-Puleo, 1980). Its main constituent, anethole, has higher LD_{50} than vanillin, another common flavoring agent. However, human ingestion of 1–5 mL of aniseed essential oil can induce nausea, vomiting, and pulmonary edema (Alonso, 2004). The ingestion of high quantities of liquor made with aniseed can induce effects quite similar to those observed for absinthe liquors (Alonso, 2004).

Aniseed has been extensively used as a raw material for beverages, including teas and infusions (Figure 3), due to its pleasant and sweet flavor, which is associated with its essential oil. Many countries around the Mediterranean region, such as Turkey, Greece, Italy, Spain, and France, have traditional alcoholic beverages produced with *P. anisum*, such as anis, arak, pastis, ouzo, sambuca, zivania, and raki. They are produced by distillation of ethyl alcohol with the fruits, affording a flavored beverage with high content of ethanol (Jurado et al., 2007; Yucesoy and Ozen, 2013).

As expected, the volatile substances that constitute the essential oil of *P. anisum*, with predominance of anethole, are responsible for the flavor of these products. These homogeneous beverages can be achieved due to the solubility of essential oils and its constituents in alcohol, such as ethanol. However, a problem remained in recent decades in the development of nonalcoholic beverages with high content of essential oils because these substances has an intrinsic water-insoluble nature. In this regard, nanotechnology may emerge as a new alternative to develop nonalcoholic beverages with considerable amounts of water-insoluble substances, including essential oils and its constituents. This could be achieved by nanoparticles, nanocapsules, nanodispersions, and nanoemulsions (Patel and Velikov, 2011), in addition to other classes of nanostructures that may enhance the solubility of lipophilic substances. Nanotechnology would also offer additional advantages to the food industry, such as development of secure products with remarkably low toxicity, enhanced bioavailability of functional food, and activity of preservatives (Silva et al., 2011; Varona et al., 2009).

Nanoemulsions, which have a small mean droplet size, have been achieved with many essential oils or their constituents, including oil in water formulations with essential oil of *Illicium verum*, which presented high content of anethole, such as *P. anisum* (Kourniatis et al., 2010; Donsi et al., 2012; Fernandes et al., 2013). In this context, aniseed essential oil, mainly anethole, would be incorporated through an esferic structure called micelle. The structural nature of these aggregates of

FIGURE 3 Preparation of a beverage obtained by infusion of aniseed.

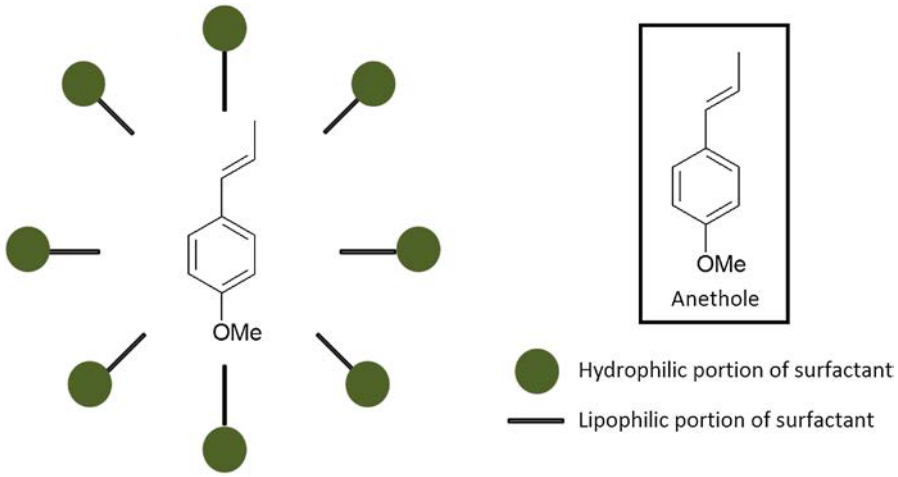

FIGURE 4 Schematic representation of the essential oil from aniseed (*Illicium verum*), mainly anethole, inside the micelle of an oil in water nanoemulsion.

surfactants allows the protection of the essential oil in the interior of the micelle, which has hydrophilic moiety exposed to the external phase, allowing the water-homogeneous dispersion oil in water formulation (Figure 4), and thus its utilization as a nonalcoholic beverage.

The utilization of products in the agriculture, storage, and packing is also an open field for research that can provide new products in few years. Many essential oils are recognized by their insecticidal activity and can be used in integrative practices for the control of insects (Rattan, 2010). The essential oil from aniseed exhibited strong fumigant activity against two stored-product insects, resulting in 100% of mortality of the eggs (Rodrigues et al., 2003). Thus, essential oils could be used in the field or incorporated during packaging to release controlled amounts of the biocide substances, therefore protecting the products for a longer time. Aniseed, mainly represented by its essential oil, has been used in culinary and food production contexts for centuries, and this raw material remains as an inexhaustible source of products to food industry.

SUMMARY POINTS

- *Pimpinella anisum* is one of the oldest species used by people.
- Anise has white flowers and yellow-brown or green-brown fruits.
- Anise fruits contain not less than 2% (w/w) of essential oil.
- This essential oil is widely used in the food industry as a flavoring, antioxidant, antispoilage agent, and preservative in food.

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Chapter 23

Anise Myrtle (*Syzygium anisatum*) Oils

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INTRODUCTION

Anise myrtle leaves have been used traditionally by the indigenous people of Australia for therapeutic applications. More recently, anise myrtle leaves and its essential oil (EO) have been used as a food ingredient in savoury and sweet dishes. When crushed, Anise myrtle leaves give an intense aroma of aniseed due to the high percentage of the aromatic volatile compound anethole. Anise myrtle EO has over 90% anethole (Blewitt and Southwell, 2000) and is comparable to other sources of anethole-rich EOs such as fennel (*Foeniculum vulgare* Mill.) with 69% (Diao et al., 2014), anise seed (*Pimpinella anisum* L.) with over 90% (Zheljazkov et al., 2013), and star anise (*Illicium verum)* with 81% (Gholivand et al., 2009).

Anise myrtle leaves are used as a fresh and dried herb in culinary applications and also as a herbal tea in both Australia and other countries. The EO is used in the alcoholic beverage industry, in mouth care products, in food flavors, pharmaceutical preparations, and soaps and perfumes (Blewitt and Southwell, 2000). The wide range of applications of the anise myrtle EO indicates the potential for expanding the product range in the food, cosmetic, and pharmaceutical sectors. The production volume of anise myrtle dried leaves and EO is lower than lemon myrtle (*Backhousia citriodora*); however, production capacity can be increased if there is a market for these products (Clarke, 2012).

This chapter reviews the application of anise myrtle essential oil in food and its general usage, botanical aspects, and chemical properties.

BOTANICAL ASPECTS

Syzygium anisatum (Vickery) (formerly *Backhousia anisata* and *Anetholea anisata*) (Craven and Biffin, 2005), known locally as ringwood, is a rainforest tree characterized by the distinct aniseed odor of its undulate leaves, creamy white eucalypt-type flower, and small, dry capsule-like fruiting bodies (Blewitt and Southwell, 2000). *Syzygium anisatum* is a large tree that can reach up to 50 m in height and occurs mainly in two small valleys of northeast New South Wales, namely Bellinger and Nambucca. The alluvial soils of these moist coastal valleys have allowed this species to survive and flourish in spite of an increasingly drying Australian landscape (Blewitt and Southwell, 2000; Brophy and Boland, 1991) (Figure 1).

Usage and Applications

Interest in distilling the anise myrtle essential oil was evident as early as 1946 as anethole had become unavailable from the traditional European sources such as anise seed, star anise, and fennel oils during the war (Blewitt and Southwell, 2000). Anethole content of the species was first documented in 1949 by MacKern (1950) and the next study only appeared in 1990 where gas chromatography–mass spectroscopy was used to identify two chemotypes with different concentrations of anethole and methyl chavicol (estragole) (Brophy et al., 1995; Brophy and Boland, 1991). The leaves of *S. anisatum* are currently used by the native food industry in Australia as a source of fresh and dried herb and the starting material for the extraction of the essential oil. The EO is used in the alcoholic beverage industry, in mouth care products, in pharmaceutical preparation, and in personal care products like soaps. It is also used as a flavor-masking agent in expectorants and sedatives in cough medicines. The oil has applications as a fragrance in the cosmetic industry (Blewitt and Southwell, 2000).

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FIGURE 1 Anise myrtle trees, picture kindly provided by Australian Rainforest Products.

TABLE 1 Chemical Composition of the Essential Oil of Anise Myrtle

Component	Percentage
Syzygium anisata EO: anethole type	
(E)-anethole	71.2–93.7
Methyl chavicol	5.0–15.3
Syzygium anisata EO: methyl chavicol type	
(E)-anethole	22.1–42.8
Methyl chavicol	55.8–75.0

Usage and Applications in Food Science

Anise myrtle is harvested all year round and the leaves are steam distilled to obtain the EO commercially. The leaf oil is used to provide an aniseed flavor for culinary use (Ahmed and Johnson, 2000). The food and beverage industry use this aniseed flavor in a number of products, for example, as herbal infusions in hot or iced tea and as syrup in soft drinks and cocktails. Sensory descriptions developed for the anise myrtle dried herb include the aroma of aniseed, menthol, and the herbal flavor of aniseed, some sweetness and slightly cooling on the palate (Smyth et al., 2012).

A study by Blewitt and Southwell (2000) identified two chemotypes, the first had a high concentration of (E)-anethole, or *trans*-anethole (93–95%), and the second was a rich source (66–77%) of methyl chavicol (estragole). In this study, *S. anisata* was surveyed within the Bellinger Valley of eastern Australia, and 26 sites were identified of which 10 were selected for further study and a total of 44 trees were assessed for EO quality. The chemical composition of anise myrtle EO is given in Table 1 and the physical properties assessed were relative density (0.984), refractive index (1.5572), and optical density (−1.6) for *S. anisata* EO: anethole type, similarly the relative density (0.971), refractive index (1.5329), and optical density (−1.7) for *S. anisata* EO: methyl chavicol type (Blewitt and Southwell, 2000).

TABLE 2 In Vitro Testing of Anise Myrtle Essential Oil and Extracts Against Different Food-Related Bacteria

Anise Myrtle	Inhibited Microorganism	References
Essential oil	*Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Candida albicans, Alcaligenes faecalis*	Wilkinson and Cavanagh (2005)
Essential oil	*Escherichia coli,* methicillin resistant *Staphylococcus aureus, Staphylococcus epidermis, Enterobacter aerogenes, Proteus vulgaris, Samonella typhimurium, Salmonella enteritidis, Enterococcus faecalis*	Hood et al. (2003)
Ethanol extracts	*Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus*	Dupont et al. (2006)
Methanol extracts	*Aeromonas hydrophila, Bacillus cereus, Vibrio cholera, Yersinia enterocolitica*	Zhao and Agboola (2007)

Syzygium anisata provides an excellent alternative source for anethole and is comparable to other anethole-rich sources like aniseed, star anise, and fennel EO. The yield of the pale yellow EOs from the fresh leaf is reported as 1.3–2.0% (Brophy and Boland, 1991).

ANTIMICROBIAL PROPERTIES

Anise myrtle EO and some extracts have shown broad spectrum antibacterial activity against food-related organisms as shown in Table 2 indicating its potential to be used as a natural preservative by the food industry.

Anethole in its *trans* form is a major aromatic compound found in the EO of several plants, for example, fennel, anise seed, and anise myrtle (Zafeiropoulou et al., 2010). Anethole shows broad spectrum antimicrobial activity; however, its antimicrobial properties are weaker than well-known antibiotics (Yukie et al., 2008). Anethole significantly enhanced the antifungal activity of polygodial and dodecanol against bakers' yeast *Saccharomyces cerevisiae* and the pathogenic yeast *Candida albicans* (Fujita et al., 2007; Himejima and Kubo, 1993). Antimicrobial activity of anethole was reported as the second most effective after eugenol in inhibiting the growth of *Salmonella typhimurium* at a concentration of 75 µg/mL when grown in culture media. However, the sensitivity of anethole to *Staphyloccus aureus* and *Vibrio parahaemolyticus* was less than for *S. typhimurium*. The order of sensitivity of these food pathogenic bacteria toward anethole was *S. typhimurium* > *S. aureus* > *V. Parahaemolyticus* (Karapinar and Esen Aktuğ, 1987). The bacteria used in this study were more sensitive to eugenol and anethole than fungal strains. Anethole at a concentration of 2 mg/mL completely inhibited the growth of three *Aspergillus* strains namely *Aspergillus flavus*, *Aspergillus ochraceus*, and *Aspergillus versicolor*, while eugenol completely inhibited these fungi at a concentration of 250 µg/mL when grown in culture media (Hitokoto et al., 1980). Another study reported on the inhibitory effects of anethole and eugenol on the growth and toxin production of *Aspergillus parasiticus*. Anethole at a concentration of 400 µg/mL completely inhibited the growth of *A. parasiticus* but at this concentration it showed a stimulative effect on the toxin production. A similar scenario was observed for eugenol, showing complete inhibition at 300 µg/mL; however, eugenol levels below 200 µg/mL enhanced production of aflatoxin by *A. parasiticus* (Karapinar, 1990).

Anethole has a low solubility in water. The benefit of increasing the solubility in water is the application of the anise myrtle EO as a functional ingredient, for example, as a flavoring or antimicrobial agent in different food systems. Nanoencapsulation of hydrophobic antimicrobial compounds has a huge potential for improving the effectiveness and efficiency of delivery in a food system. A study on the encapsulation of anethole by nanoprecipitation and emulsification solvent evaporation methods indicated a better inhibition of *S. typhimurium* with nanoprecipitation at a minimum inhibitory concentration of 227 µg/mL (Esfandyari-Manesh et al., 2013).

FLAVORING AGENT

Anethole from different plant sources has been used as a flavoring agent in the food industry. Table 3 gives the maximum levels of anethole used in different food categories (Taylor and Dormedy, 1998).

A study on the retention of *trans*-anethole by two biopolymers matrices, gelatin gels and starch solutions, indicated that the type and concentration of the biopolymer did not seem to affect aroma retention (Zafeiropoulou et al., 2010).

TABLE 3 Use of Anethole (Maximum Levels) Reported for Different Food Categories (in ppm [mg/kg])

Food Category	Levels (ppm)
Nonalcoholic beverages	42.5
Alcoholic beverages	723
Frozen dairy desserts	53.5
Candy	531
Baked goods	495
Gelatins and puddings	52.8
Meat and meat products	10

This information would be useful to food companies interested in developing products incorporating gelling agents with anethole as a flavoring agent.

SAFETY OF ANISE MYRTLE ESSENTIAL OILS

Anise myrtle is valued as an aromatic herb with many culinary applications for both the leaf and EO. Commercial applications of anise myrtle is now restricted to the species with high anethole content. This is due to the toxicity tests generated from laboratory trials with mice that have shown the administration of methyl chavicol (estragole) induces liver tumors in rodents because of metabolism to the hepatocarcinogen 1′-hydroy-estragole (Anthony et al., 1987; Chan and Caldwell, 1992). The methyl chavicol concentration generally consumed in the dried herb of anise myrtle fall into the low-dose category and, as such, is unlikely to pose a significant carcinogenic threat to humans. The same substance is also found in different amounts in basil, chervil, fennel, and tarragon and these are not currently restricted by law. Concerns about anethole is also not warranted as concentrations are less than 0.5%, and this level is not commonly consumed in anise seed, star anise, and fennel oils which are regarded as safe (Blewitt and Southwell, 2000). In addition, anethole can exist as either *cis* (Z) or *trans* (E) form, and even though the isomers have similar biological properties there is a significant difference is toxicity; *cis*-anethole is more toxic than *trans*-anethole. In plant sources from which anethole is extracted, for example, anise myrtle, the anethole is present in *trans* form (Tisserand and Young, 2014).

SUMMARY POINTS

- Leaves of anise myrtle (*Syzygium anisatum)* are steam distilled for the production of the EO.
- Anethole is the major chemical component contributing to the aniseed flavor of the EO.
- It has broad spectrum antimicrobial activity but the efficacy on bacteria is better than fungi.
- Anethole in combination with other compounds such as polygodiol enhances the antimicrobial activity.
- Anise myrtle EO is used in the food and beverage industry as a flavoring agent.

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Chapter 24

Annona Species (Annonaceae) Oils

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List of Abbreviations

DPPH 2,2-diphenyl-1-picrylhydrazil
EO Essential oil
FID Flame ionization detector
GC Gas chromatography
HRGC High-resolution gas chromatography
IC Inhibitory concentration
MIC Minimum inhibitory concentration
MS Mass spectrometry
ORAC Oxygen radical-absorbance capacity

INTRODUCTION

Annonaceae is one of the largest Magnoliidae families, with about 2400 species distributed in 108 genera, mostly pantropical (Chatrou et al., 2012). In Brazil, they are found in 385 species, of which 158 are endemic; the majority (~259 species) are found in the Amazonian region (Lobão et al., 2012). The members of this family play a significant role in the composition of Brazilian vegetation and have been a subject of great interest (Maas et al., 2001).

The Annonaceae family was cataloged in 1789 by Jussieu (Hutchinson, 1974). Chemical studies with species of this family have reported the isolation of terpenoids (mainly diterpenes), essential oils whose composition is predominantly of monoterpenes and sesquiterpenes, and alkaloids, especially isoquinoline alkaloids (Almeida et al., 2012). This family contains a considerable number of plant species with economic significance because of their edible fruits around the world, namely tropical America, Australia, Africa, India, Malaysia, and Mediterranean Europe (Shashirekha et al., 2008).

The genus *Annona* L. belongs to the Annonaceae family and comprises approximately 162 species of trees and shrubs (Chatrou et al., 2012). Economically, this genus is the most important of the Annonaceae family due to its edible fruits and medicinal properties. Several *Annona* species furnish edible fruits, some of which are very much appreciated in Brazil, such as *Annona crassiflora* ("araticum"), *Annona squamosa* ("fruta do conde"), and *Annona muricata* ("graviola"). The fruits are usually consumed "in natura" or used in juices, desserts, or ice-cream preparations (Dutra et al., 2012). Previous chemical and pharmacological investigations on some species of this genus have indicated the presence of important bioactive compounds, exhibiting various pharmacological activities including cytotoxicity against various tumor cell lines; in addition, they have antimicrobial, antioxidant, antiplatelet, and antiparasitic properties, in particular against *Leishmania* sp. and *Trypanosoma cruzi*. These activities are generally attributed to the presence of alkaloids, acetogenins, and terpenes. In Brazil, the genus *Annona* contains around 60 species, with the largest part occurring in forests and few representatives in open areas (Costa et al., 2011a).

Numerous Annonaceae species are odorous; these fragrances are the consequence of the presence of essential oils, mainly containing terpene compounds. Few volatile substances have been studied so far in comparison with alkaloids and acetogenins. Several reviews have been dedicated to these volatile substances—a general review published in 1989 (Ekundayo, 1989), and three further studies on *Cananga odorata* flower oil (Lawrence, 1986)—as a consequence of the economic importance of this species, which supplies Ylang–Ylang oil to the perfume industry. Most studies have been made on oils obtained by steam distillation (in three-quarters of cases); the other works concern volatile fractions extracted by organic solvents. These compounds are basically mono- and sesquiterpenoids. Typical constituents include α-pinene

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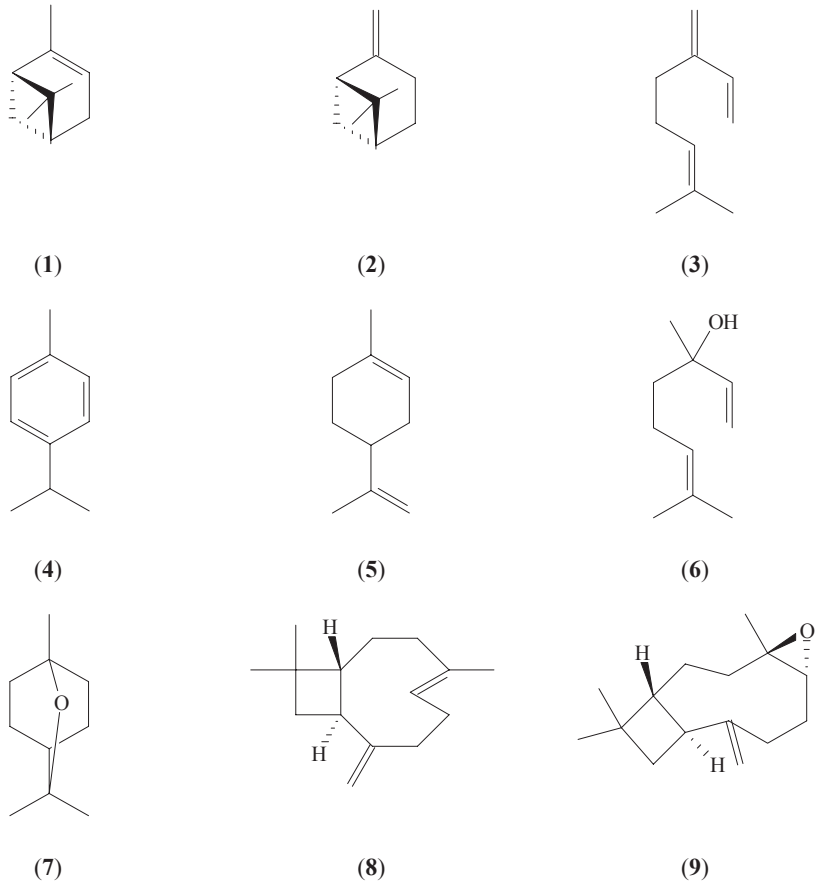

FIGURE 1 Representative constituents of essential oils from species of *Annona*.

(1), β-pinene (2), myrcene (3), *p*-cymene (4), limonene (5), linalool (6), and 1,8-cineole (7). The sesquiterpenoids represent another common group of components. Figure 1 shows some structures of representative constituents of the essential oils of *Annona* species.

Several studies have been dedicated to oils isolated from various parts of Annonaceae species, mainly those belonging to *Xylopia* and *Annona* genera, which are the most numerous (Fournier et al., 1999). Considering that this family has 108 genera and 2400 species, only the flavor compositions of the most common *Annona* species are summarized in this chapter, including *Annona atemoya*, *Annona cherimolia*, *Annona coriacea*, *Annona foetida*, *Annona glabra*, *A. muricata*, *Annona pickelii*, *Annona reticulata*, *Annona salzmannii*, *Annona senegalensis*, *A. squamosa*, and *Annona vepretorum*.

BOTANICAL ASPECTS

The name *Annona* is derived from the Latin word for "annual harvest." This genus is the most important source of edible fruits from Annonaceae family. These species are shrubs or small trees, with height from 5 to 11 m; they are erect or somewhat spreading and possess gray-brown bark, which is often rough and corrugated (Pino et al., 2001).

The botanical aspects of the genus *Annona* have been described by Pinto et al. (2005), as presented in this section. The genus has numerous unifying characteristics, especially relating to plant height, root system, bark, stem, floral biology, pollination, fruit set, and fruit type. There are important variations among *Annona* seedlings in the same species, affecting not only the mature foliage and productivity of the plants, but also the fruit size, form, color, quality, and number of seeds in the fruit. These variations are often pronounced enough to have resulted in several botanical names for the same species.

In general, the *Annona* are shrubs or small trees, whose height varies from 5 to 11 m depending on several factors, such as species, climate, soil, and crop management. They are erect or somewhat spreading in habit, with gray-brown bark, and are often rough and corrugated. Generally, *Annona* stems are ferruginous to greyish, and tomentose when young but later becoming glabrous. With few exceptions, *Annona* are deciduous (even tropical species), especially when cultivated in areas with dry or cool seasons and without irrigation.

The root system has abundant thin lateral roots and a taproot that is not as strong as in other tropical fruit trees, such as mango (*Mangifera indica* L.). Although the taproot is not generally pronounced, longer taproots will grow in lighter soil texture. The taproot of an adult soursop tree can reach approximately 1.5–1.8 m in depth in oxisols of the Cerrado ecosystem (Brazilian savannah) in Central Brazil.

Annona flowers are hermaphrodite, usually somewhat fragrant, solitary or in fascicles with two to four flowers, with three green sepals and six petals arranged into two verticils. The external verticil has three yellow-greenish petals and the internal one has three yellowish petals. The flowers have several conglomerated and spirally arranged stamens below and around an upper globose-shaped (conical) dome of numerous united carpels, which have one ovule each. After fertilization, the united carpels will form a syncarp or composite fruit.

Flowering starts when the plant is 3–4 years old, although it may occasionally occur earlier depending upon environmental conditions. Anthesis (flower opening) starts slowly, with the separation of the apex of the external petals and takes from 6 to 8 h to complete.

Pollination is mainly carried out by insects or sometimes by wind. The fact that flowers are protogynous (pistils are mature before pollen is liberated from anthers) suggests that self-pollination is not the rule for *Annona*. Due to the protogynous flowers, the fruit set may be poor when the pollinating insect populations are small; also, small, asymmetric fruits may be produced because fruit size and form depends on the number and position of fertilized ovules. As a result, the number of fruits per plant, as well as fruit size and shape, are highly dependent on abundant insect pollination and amenable to control via hand pollination where the pollinating insects are rare.

Leaves have an impressed primary vein on the upper side; indument of simple (to stellate) hairs; bracts are two per flower; flowers are solitary or in few- to many-flowered, terminal, leaf opposed, or infra-axillary inflorescences. Sepals are connate or free, petals are free and sometimes connate at the base, often thick and fleshy, white, yellow, rarely orange or red. Fruit is syncarpous and composed of many carpels, as well as many seeded (Maas et al., 2001).

USAGE AND APPLICATIONS

Essential oils are aromatic and volatile liquids are extracted from plants. The chemicals in essential oils are secondary metabolites, which play an important role in plant defense as they often possess antimicrobial properties. The interest in essential oils and their application in food preservation have been amplified in recent years by an increasingly negative consumer perception of synthetic preservatives. Furthermore, foodborne diseases are a growing public health problem worldwide, calling for more effective preservation strategies (Hyldgaard et al., 2012).

Although the food industry primarily uses essential oils as flavorings, they represent an interesting source of natural antimicrobials for food preservation. However, application of essential oils as food preservatives requires detailed knowledge about their properties, such as the minimum inhibitory concentration (MIC), the range of target organisms, the mode of action, and the effect of food matrix components on their antimicrobial properties (Hyldgaard et al., 2012). The purpose of this section is to provide an overview of chemical composition and bioactivities about the essential oils obtained from species of the genus *Annona*, and to identify research avenues that can facilitate the implementation of essential oil constituents as natural food preservatives.

The most frequent terpenoid constituents (those monoterpenes and sesquiterpenes whose yield is above 2% and that are mentioned in more than five different studies) are shown in Figure 1. The skeleton of each terpene is also given to all these constituents. These compounds are α-pinene (1), β-pinene (2), myrcene (3), *p*-cymene (4), limonene (5), linalool (6), 1,8-cineole (7), (*E*)-caryophyllene (8), and caryophyllene oxide (9). These terpenes are present in many oils; therefore, they have no special significance in the Annonaceae oils. From the data presented in the studies, fruits are the organs which are most studied because many Annonaceae fruits are edible. On the contrary, few studies have been performed on leaves and flowers.

USAGE AND APPLICATIONS IN FOOD SCIENCE

In general, *Annona* species are consumed as fresh fruits, but they are also widely used in semiprocessed and processed products, especially desserts. As world demand for exotic flavors and healthy foods expands, the use of *Annona* fruits is also likely to expand (Pinto et al., 2005).

The composition of the volatile fraction of foods is essential for the characterization and the identity of these materials. The flavor of a certain product, which is one of the main attributes for sensory analysis and consumer's acceptance, is given by the combination of (usually) many volatile molecules (Bicas et al., 2011).

Sometimes, one substance alone is able to reflect the approximate flavor of a product; in this case, it is called the "impact compound." In some circumstances, a combination of substances interacts with the receptors from the nasal mucosa and

is interpreted by the brain to create a sensory impression typical for each product. In all cases, aroma compounds are extremely important for industry and are used in a variety of products, including foods, beverages, cosmetics, and perfumes (Bicas et al., 2011).

In this section, the volatile constituents and bioactivity of most common species of the genus *Annona* are reviewed. These species present an increasing economic importance in tropical regions, especially in the market of eccentric juices.

Annona atemoya Aubl.

Annona atemoya Aubl. dominates the commercial market in Australia. The fruit volatiles of *A. atemoya* (the hybrid of *A. squamosa* L. and *A. cherimolia* Mill.) are identified. *A. squamosa* is a popular fruit in the tropical states of India, with a very sharp and short season, lasting for about 3 months a year. This fruit, which is popularly known as sweet sop, is heart-shaped, weighs about 150 g, and has a very bumpy skin. When ripe, its pulp is creamy, very sweet, and pleasantly flavored. It is usually eaten as a dessert fruit and finds immense applications in the preparations of beverages and ice creams (Bicas et al., 2011; Chikhalikar et al., 2000).

Annona cherimolia Mill.

Annona cherimolia Mill. is a subtropical fruit tree that originated from frost-free valleys of the Andes at an altitude between 700 and 2400 m. The common names include cherimoia (Brazil), cabeça-de-negro, anona do Chile (Portugal), chirimorrinon (Venezuela), cherimoya (Colombia, Ecuador, Cuba), anona (Costa Rica), chirimoyo (España, Chile), and others (Pino, 2010). Despite its unusual appearance, cherimoya is readily accepted by the Western consumers due to its fine taste and flavor (Pino, 2010). The pulp of this fruit is creamy, very sweet, and pleasantly flavored; it is well known as a dessert fruit and has a lot of applications in ice creams and beverages (Bicas et al., 2011; Chikhalikar et al., 2000).

The cherimoya fruit is consumed mostly fresh, generally chilled, and often with salt and lemon. Fruit pulp is often mixed with wine, milk (to make milkshakes), and yogurt, processed into ice cream and sherbet, and baked into cookies and pastries. Most of these preparations, and others, can be made at home (Cordeiro et al., 2005).

Cherimoya is heart-shaped with a smooth skin that becomes dull red when the fruit is ripe. When ripe, the pulp is creamy, very sweet, and delicately flavored. In the study of volatile components of Cuban *Annona* fruits, 47 volatile components were identified in *A. cherimolia*. The major volatiles in cherimoya were α-thujene, α-pinene, terpinen-4-ol, and germacrene D (Pino, 2000).

Annona coriacea Mart.

Annona coriacea Mart., popularly known as "marolo," "araticum," "araticum-liso" (Souza and Lorenzi, 2008), has been used as traditional medicine for the treatment of parasites, ulcers, and inflammatory processes. It is also reportedly used for rheumatism and as an antihelminthic (Siqueira et al., 2011).

The essential oil of the leaves from *A. coriacea* was extracted by hydrodistillation in a Clevenger apparatus and analyzed by gas chromatography (GC)/mass spectrometry (MS) and GC/flame ionization detector (FID). The oil yield was 0.05% m/m. Sixty compounds were identified, in a complex mixture of sesquiterpenes (76.7%), monoterpenes (20.0%), and other constituents (3.3%). Bicyclogermacrene was its major compound (39.8%), followed by other sesquiterpenes. Most of the monoterpenes were found in low concentration (<1%). Only β-pinene and pseudolimonene presented at the highest level of 1.6%. The volatile oil presented antileishmanial and trypanocidal activities against promastigotes of four species of *Leishmania* and trypomastigotes of *Trypanosoma cruzi*, which were shown to be more active against *Leishmania* (L.) *chagasi* (IC_{50} 39.93 μg/ml) (95% CI 28.00–56.95 μg/ml) (Siqueira et al., 2011).

Annona foetida Mart.

Annona foetida Mart.—a species commonly known as "araticum caatinga," "envira-ata," or "graviola do mato"—is a tropical tree found in the Brazilian and Peruvian Amazon region. Besides the antileishmanial activity demonstrated by the extracts, fractions, and pure compounds isolated from this source, this species is also reported to be used in folk medicine in Northeastern Brazil against rheumatism, intermittent fevers, and ulcers, particularly the leaves and the bark (Corrêa et al., 1984).

The essential oil from the leaves of *A. foetida* obtained by hydrodistillation was analyzed by GC/FID and GC/MS. The results showed that the major constituents were bicyclogermacrene (35.12%), (*E*)-caryophyllene (14.19%), and α-copaene (8.19%). The antimicrobial and antileishmanial activities were investigated. The oil showed potent antimicrobial activity against *Candida albicans* and *Rhodococcus equi*. The oil also showed significant antileishmanial activity, giving the best

results against *Leishmania guyanensis*. A preliminary cytotoxicity assay for this oil was carried out on hamster and mice (Balb/c) peritoneal macrophages. The results obtained were similar to pentamidine and considered not to be cytotoxic to macrophages (Costa et al., 2009).

Annona glabra L.

Annona glabra L. is an indigenous wild tree, common in all Cuban swamps, mainly along the coast line. It blossoms from April to June and bears a round or oblong fruit in August. The whitish pulp is sweet and somewhat aromatic (Pino et al., 2002).

The species *A. glabra* is a small tree called araticum-do-brejo, araticum-caca, araticum-cortiça, araticum-da-praia, araticum-de-boi, araticum-da-água, araticum-do-mangue, and araticum-panam, which grows in many places in Northern and Northeastern Brazil. The common names include pond apple, alligator apple, monkey apple, corkwood, bobwood (United States), and bagá (Cuba) (Pino, 2010). The fruit has an edible yellow pulp and is cultivated in Brazil and Central America. Santos et al. (1998) analyzed the volatile constituents of the fruits of pond apple (*A. glabra*) growing wild in North Brazil by GC/MS. The most abundant class of compounds found in the aromas was terpenoids, principally α-pinene, limonene, α-phellandrene, and (*E*)-β-ocimene. The concentrations of terpenoid compounds vary according to the different stages of ripening (Bicas et al., 2011).

Annona muricata L.

Annona muricata L. is a small tropical fruit tree named "guanabana" or "soursop." Originating in Central America, it is grown in many tropical and subtropical regions, including parts of South America, Africa, Asia, and Australia (Kossouoh et al., 2007). When the fruit is ripe, the pulp is creamy, acidic, and delicately flavored. It is usually eaten as a dessert fruit, but it can be made into drinks and ice creams (Pino et al., 2001).

Soursop fruits are occasionally consumed fresh or more commonly made into juices, ice creams, or sherbets. Most people consider them to be too acidic to eat fresh, but they are esteemed for making refreshing drinks, nectars, ice creams, and similar foods. Nectar (sweetened pulp) can be prepared and used after dilution with three parts of water. In Java, Indonesia, fruits of soursop are added to soup (sajoer) (Cordeiro et al., 2005).

An industrial application involves the extraction of the essential oils present in soursop pulp. These oils, such as esters of aliphatic acids, have the potential to improve the flavor of processed fruit products (Jirovetz et al., 1998a).

Two works were carried out on *A. muricata*. The first was reported by Pélissier et al. (1994) on leaves, parings, and fruit pulp. From the leaf oil, 59 compounds were identified, principally β-caryophyllene (31.4%), δ-cadinene (6.7%), α-muurolene (5.5%), T- and α-cadinol (4.3%). The second work was reported by Boyom et al. (1996) on the leaves and seeds; β-caryophyllene or (*E*)-caryophyllene (40%) were preponderant in leaves and α-phellandrene (25%) was preponderant in seeds. For comparison, the study of the chemical composition of the leaf oil of *A. muricata* growing in Benin was carried out. Essential oils from leaves of *A. muricata* were obtained by steam distillation with a very low percentage. Analysis made by GC and GC/MS showed the presence of 80 compounds. The most abundant constituents were β-caryophyllene (13.6%), δ-cadinene (9.1%), *epi*-α-cadinol (8.4%), and α-cadinol (8.3%) (Kossouoh et al., 2007).

The fruit contributed to the economic growth of several tropical countries in tropical America, Africa, Malaysia, and Australia (Shashirekha et al., 2008) and can be utilized in the development of exotic processed products. This fruit is cherished for its unique and pleasant flavor, and the white pulp is usually used for manufacturing various kinds of products, such as juice blends, puree, syrups, jams, and ice cream. Moreover, the soursop pulp has already been marketed in Europe, North America, and Brazil. Essential oil compounds of the *A. muricata* fresh fruit pulp from Cameroon were identified by Jirovetz et al. (1998a), using gas chromatographic/mass spectrometric (GC/FID and GC/MS) and olfactometric methods. More than 50 compounds were identified where esters of aliphatic acids are especially dominating, with methyl 2-hexenoate, ethyl 2-hexenoate, methyl 2-octenoate, and methyl 2-butenoate as the main compounds. Additional mono- and sesquiterpenes such as α-caryophyllene, 1,8-cineole, linalool, (*R*)-terpineol, linalyl propionate, and calarene are highly concentrated in the essential oil of these fresh fruits (Bicas et al., 2011).

Annona pickelii (Diels) H. Rainer

Annona pickelii (Diels) H. Rainer is a small tree (3–5 m tall) endemic to Brazil, which is popularly known as "araticum-do-mato," "araticum-da-mata," "jaquirinha-do-mato," and "jussara" (Pontes et al., 2004; Maas et al., 2011). It is found only in the States of Paraíba, Pernambuco, and Sergipe, occurring in dense forests of "restinga" (Pontes et al., 2004; Dutra et al., 2012).

A total of 21 compounds were identified in the essential oil of *A. pickelii*, with sesquiterpenes predominating in essential oil. Bicyclogermacrene (38.0%), (*E*)-caryophyllene (27.8%), α-copaene (6.9%), and α-humulene (4.0%) were the main components of *A. pickelii*. The essential oil showed potent trypanocidal and antitumor activities (Costa et al., 2013). The essential oils showed significant antioxidant capacity in the oxygen radical absorbance capacity (ORAC) and 2,2-diphenyl-1-picrylhydrazil (DPPH) assays. The antimicrobial activity of this essential oil was also evaluated against bacteria and fungi; its larvicidal activity against *Aedes aegypti* larvae was also studied (Costa et al., 2011b).

Annona reticulata L.

Annona reticulata L. is commonly referred to as custard apple or bullock's heart. The bullock's heart has a smooth skin fruit that becomes dull red when ripe. Its custard-like pulp is rather sugary and less flavored than the other familiar species, such as *A. squamosa* (sweetsop) and *A. muricata* (soursop). The fruits are eaten fresh and the pulp can be used to flavor ice cream. Less volatile substances such as alkaloids, diterpenoids, and acetogenins have been identified so far from the various parts of the plant (Ogunwande et al., 2006). The flavor of custard apple pulp is considered to be poor and hence of little commercial value, although it is a popular backyard fruit that attracts children. Chilling of this fruit, as well as other *Annona*, improves the flavor (Cordeiro et al., 2005).

Bullock's heart is very popular in Cuba, where it is commonly known as mamón. This fruit is also known as custard apple (United States), anona, anona colorada (México), corazón (Venezuela), anón pelón (Colombia), araticum-do-mato (Brazil), and others. The fruit has been introduced in diverse regions of the American tropics and Southeastern Asia, without achieving a level of importance comparable to that of *A. cherimolia* or *A. squamosa*.

Forty-nine volatile components were identified in *A. reticulata*. The major volatiles in bullock's hearts were α-pinene, β-pinene, and germacrene D (Pino, 2010). A review of the literature revealed that only a few papers deal with the volatiles from the leaves of the plant. The main components of the leaf oil from India (Jirovetz et al., 1998b) were the sesquiterpenes spathulenol (7.4%), δ-cadinene (6.5%), elemol (4.6%), α-copaene (4.0%), and α-eudesmol (3.0%) in combination with monoterpenes terpinen-4-ol (4.0%) and α-terpineol (3.8%). The root oil was found to be rich in spathulenol (11.9%), δ-cadinene (9.3%), α-muurolene (6.8%), β-bisabolene (5.2%), and α-bergamotene (3.1%).

The major compounds of the fruit oil from Cuba (Pino, 2000) were α-pinene (23.8%), β-pinene (17.7%), germacrene D (10.5%), and limonene (5.9%). In another investigation, the principal constituents of the fruit volatiles from four cultivars (Cenizo, Rojo, Verde, and De Ojo) of Cuban origin were α-pinene (50.0–100.5 mg/kg), β-pinene (71.5–97.1 mg/kg), myrcene (12.6–20.1 mg/kg), limonene (14.2–20.5 mg/kg), terpinen-4-ol (1.1–22.0 mg/kg), and germacrene D (10.0–27.3 mg/kg) (Pino et al., 2003). The fruit oil from Malaysia was found to have mainly terpinen-4-ol and α-terpineol as the major constituents, while the sample analyzed by Bartley from Australia (Bartley, 1987) was shown to be rich in alkanes (mainly methyl and ethyl esters of C_3 to C_6 aliphatic chain), in addition to some terpenoid compounds. Also, limonene and β-linalool were the two terpenoid compounds identified from the fruit sample collected from Brazil (Augusto et al., 2000).

Hydrodistilled oil obtained from the leaves of *A. reticulata* grown in Nigeria was analyzed by capillary GC and GC/MS. Thirty-nine components were characterized. These consisted of 18 monoterpenes amounting to 29.0%, 20 sesquiterpenes totaling 52.9%, and one aromatic ester making up 10.9%. The oil contained (*E*,*E*)-farnesyl acetate (19.0%), ar-turmerone (12.0%), benzyl benzoate (10.9%), and γ-terpinene (7.4%) as the major constituents (Ogunwande et al., 2006).

Volatile components of bullock's heart growing in Cuba were isolated by simultaneous steam distillation–solvent extraction analyzed by GC/MS. The major volatiles were β-pinene, α-pinene, and germacrene D from the 49 identified constituents. Furthermore, the volatile compounds were isolated from four cultivars of bullock's heart fruit from Cuba by simultaneous steam distillation–solvent extraction. In total, 180 volatile constituents were detected by high-resolution GC and capillary GC/MS in the aroma extracts. The authors concluded that the presence of many terpene compounds is thought to contribute to the unique flavor of the bullock's heart fruit (Bicas et al., 2011; Pino et al., 2003).

Annona salzmannii A. DC.

Annona salzmannii A. DC. is a 6–20-m tall tree that is popularly known as "araticum-da-mata" and "araticum-apé" (Pontes et al., 2004). It is very common in Brazil, mainly in the states of Bahia, Pernambuco, and Paraíba (Pontes et al., 2004). Some of its organs (leaves, roots, and seeds) are used in folk medicine to treat several human ailments, such as verminosis, dysentery, ulcers, and inflammatory conditions (Corrêa, 1984).

A total of 23 compounds were identified in the essential oil of *A. salzmannii*, with δ-cadinene (22.6%), (*E*)-caryophyllene (21.4%), α-copaene (13.3%), bicyclogermacrene (11.3%), and germacrene D (6.9%) being the main components. The essential oils showed potent trypanocidal and antitumor activities (Costa et al., 2013). The essential oil showed significant

antioxidant capacity in the ORAC and DPPH assays. The antimicrobial activity of these essential oils was also evaluated against bacteria and fungi, as well as the larvicidal activity against *Aedes aegypti* larvae (Costa et al., 2011b).

Annona senegalensis Pers.

Annona senegalensis Pers. is a shrub with a height range of 1–2 m. Its leaves are large, oval, and entire and they are fragrant when crushed. The flowers are yellowish, waxy, closely petalled, and very fragrant. The fruits are pulpy, solid, and orange at maturity. Fresh fruit contains many seeds in a sweet and fragrant pulp (Nébié et al., 2005). Wild soursop fruits are sold in local markets in Africa. The fruit has a pineapple-like odor and sweet taste. It keeps for only a few days. It is used in sherbets, ice creams, and for making drinks (Cordeiro et al., 2005).

Different parts of *A. senegalensis* are used in traditional medicinal to treat diseases such as respiratory, eye, and skin diseases. It is also used to treat diarrhea, elevated blood pressure, dysentery, and as antidote against snake and scorpion venom, while the bark is used as an insecticide. The essential oil, obtained by steam distillation of air-dried leaves of *A. senegalensis* growing in Burkina Faso, has been analyzed by GC and GC/MS. The oil was found to contain germacrene D (19.2%), β-caryophyllene (19.1%), γ-cadinene (11.1%), and α-humulene (9.7%) as its major components (Nébié et al., 2005).

Annona squamosa L.

Sugar apple (*A. squamosa* L.) seems to be native to southeastern Mexico, in dry areas and up to 1000 m in altitude, although it grows well in regions of medium humidity. The plant is a small tree that grows wild in many places in the north of South America, Central America, and the Caribbean region. The common names include anona, araticum, and fruta-do-conde (Brazil), sweetsop and sugar apple (English-language countries), kaneelapell (Suriname), sitaphal (India), and schuppenannone (Germany).

The pulp may be eaten raw and tastes aromatic sweet, with a custard-like flavor; it is used also to prepare juices, jellies, and compotes. The sugar apple is consumed as a fresh dessert fruit, or used for preparing juice and ice cream. In the latter case, it should not be pasteurized or cooked, but simply blended into a semi-solid cream just before freezing (Cordeiro et al., 2005). Leal (1990) reported that it can also be used to make wine, as can cherimoya.

Sweetsop or custard apple (*A. squamosa*), a native plant of the West Indies, is cultivated throughout India. Its medicinal properties are well known. Its root is a drastic purgative and is administered for acute dysentery in West Indies. Leaves are applied as poultice over boils and ulcers in Brazil.

The powdered seeds are used as insecticide and to treat dandruff by the native peoples of Northern Brazil. In Brazil, the fruits are highly appreciated; commercial plantations exist in the southeastern and northeastern regions. In the Amazon region, there are few plantations so far. In general, the fruits are sold at a high price (approximately US$ 0.50 for each fruit) there. Therefore, a growing economic importance of *A. squamosa* fruits in that region is to be predicted in the medium term (Bicas et al., 2011; Andrade et al., 2001).

Storage of the fresh fruits of *A. squamosa* has limitations because it is perishable. Cold storage is not promising because of the development of an unattractive brown color on the skin, which decreases the market value (Purohit, 1995).

The chemical composition of the fruit pulp of *A. squamosa* growing in the Brazilian Amazon was investigated and compared with data of specimens occurring in Southeastern Asia. In accordance with the sweet taste of the fruit pulp, the amounts of sugars were found to be quite high (58% of dry mass). The triglyceride concentration was found to be very low. The presence of the diterpenoid compound kaur-16-en-18-oic acid in a considerable amount (0.25% of dry mass) was detected in the lipid fraction. The essential oil of the fruit pulp was obtained and its volatile constituents were identified by GC–MS. The major compounds were α-pinene (25.3%), sabinene (22.7%), and limonene (10.1%) (Andrade et al., 2001).

Several reports have been published covering the nonvolatiles of *A. squamosa*. The composition of custard apple leaf oil has also been the subject of several studies. In 1954, the leaf oil was reported to contain α-pinene (7%), caryophyllene (50%), and a cadinane sesquiterpene. In 1977, carvone (24.9%), linalool (7.7%), and diacetyl (9.3%) were tentatively identified. However, more recent studies reported the presence of germacrene D (17.5%) and β-elemene (12%) as major constituents along with α-pinene, β-pinene, sabinene, bicyclogermacrene, T-cadinol, and T-muurolol. The oil from the leaves of *A. squamosa* has been analyzed by GC and GC/MS. β-Cedrene (23%) and β-caryophyllene (14%) were found to be the major compounds (Joy and Rao, 1997).

Annona vepretorum Mart.

Annona vepretorum Mart. is a shrub or tree that is 2.5–10 m tall (Maas et al., 2001), popularly known as “araticum” and “araticum-bruteiro.” It is endemic to the northeast area of Brazil, particularly the states of Bahia, Pernambuco, and Sergipe (Maas et al., 2001; Costa et al., 2012).

The essential oil of the leaves of *A. vepretorum* Mart. is constituted predominantly by monoterpenes (29.2%) and sesquiterpenes (68.9%). The major compounds identified were bicyclogermacrene (43.7%), spathulenol (11.4%), α-felandrene (10.0%), α-pinene (7.1%), (*E*)-β-ocimene (6.8%), germacrene D (5.8%), and *p*-cymene (4.2%). The trypanocidal activity against *Trypanosoma cruzi* epimastigote forms, as well as the antimicrobial and antioxidant properties, were investigated. The essential oil showed a potent trypanocidal activity with an IC_{50} value of 31.9 ± 1.3 μg/ml. For antimicrobial activity, the best result was observed against *Candida tropicalis*, with an MIC value of 100 μg/ml. The essential oil showed weak antioxidant activity (Costa et al., 2012).

SUMMARY POINTS

- The genus *Annona* L. is one of the most important of the Annonaceae family due to its edible fruits and medicinal properties.
- Typical constituents found in *Annona* essential oils include α-pinene, β-pinene, myrcene, *p*-cymene, limonene, linalool, and 1,8-cineole.
- Terpenes present in Annonaceae fruits can be used in cosmetics and pharmaceuticals for the production of manufactured products.
- *Annona* essential oils possess several biological activities, such as anticancer, analgesic, antiinflammatory, and cytotoxicity profile.

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Chapter 25

Basil (*Ocimum basilicum* L.) Oils

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List of Abbreviations

GRAS Generally recognized as safe
IC_{50} Half-maximal inhibitory concentrations
LC_{50} Median lethal concentration
LD_{50} Median lethal dose

INTRODUCTION

Basil belongs to the genus *Ocimum*. Basil is also a common name for several plants in the genera *Acinos* (e.g., *Acinos arvensis*, basil thyme) and *Clinopodium* (e.g., *Clinopodium vulgare*, wild basil). In addition to cultural and traditional rituals, basil is one of the most popular herbs in the cooking and food network with its wide range of applications, especially in food flavoring and preservation. Sweet basil is a common name for the culinary herb *Ocimum basilicum* of the family Lamiaceae or Labiatae (the mint family) and is the most economically important among all *Ocimum* spp. (Figure 1). This chapter focuses on *Ocimum basilicum*, although other *Ocimum* basils are briefly covered. An essential oil is a plant oil containing volatile aromatic chemicals. Basil oil is an essential oil obtained from basil plants. Basil oil (also sweet basil oil) described in this chapter is referred to as *O. basilicum* oil. There are different chemotypes of basil oil on the market. Each possesses more or less a different aroma and personality for varying applications because they are derived from different cultivars of basil.

There are many excellent reviews, books, and websites of basil and basil oil. This chapter primarily focuses on basil oil with regard to its botanical aspects and its applications in food science, especially food production, food preservation, and culinary flavoring. A literature search with "basil oil" as a keyword gave more than 3000 publications in the Google Scholar database and 168 using the keyword of *Ocimum* oil (accessed on March 18, 2014). References cited were mainly those in Google Scholar between 2010 and March 2014, while early references were cited to provide the continuity and coverage of the information on the subject. Little information was available about any potential toxicity of basil oil and its components to humans. Medicinal application, aromatherapy, and toxicology of basil oil are out of the scope of this chapter.

BOTANICAL ASPECTS OF BASIL

The name basil is derived from the Greek word, basileus, which means "royal or king-like" because of the royal fragrance of this herb. Basil is commonly referred to the genus *Ocimum*. The Plant List (2010) accepted 66 of a total of 327 species of herbs and shrubs recorded in the genus *Ocimum*. There are many basil varieties, with new varieties being added every year. The taxonomy of basil has been greatly complicated by interspecific hybridization and polyploidy, as well as large morphological diversity and chemotype variation within basil species.

Sweet basil or basil is a common name for the culinary herb *O. basilicum* (Figure 1). Basil is probably native to Asia and Africa, but is cultivated as a popular garden annual herb worldwide. It is thought to have been brought to ancient Greece by Alexander the Great (356–323 BCE.), to England from India in the mid-1500s, and to the United States in the early 1600s. Basil requires a low maintenance and is easy to grow. This plant usually blooms in early summer and can be harvested in midsummer. Basil plants can grow well indoors and outdoors under sunny and moist conditions. The plant is produced commercially in many countries such as Egypt, India, Indonesia, Mexico, and the United States. In 1999, the United States imported 3574 tons of dried basil leaf.

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FIGURE 1 Basil is a common name for the culinary herb *Ocimum basilicum*. The right photo shows attraction of basil to tephritid fruit flies.

EXTRACTION, COMPOSITIONS AND USAGE OF BASIL OIL

Basil Oil Extraction

Essential oil can be extracted from plants through hydrodistillation, steam distillation, and other methods. Hydrodistillation and steam distillation have some drawbacks such as thermal decomposition of labile components, high energy consumption, and being time-consuming. New green techniques, such as supercritical or pressurized fluid extraction and microwave- or ultrasound-assisted techniques are potential solutions to overcome these disadvantages. On a commercial production scale, basil oil is traditionally extracted via distillation of basil leaves, stem, or other parts of basil plant. Clevenger hydrodistillation is typically used for laboratory-scale production. Hydrodistillation is the official standard method for extracting essential oils for quality control.

Chemical Composition and Usage of Basil Oil

Different basil varieties have different scents because the herb contains aroma chemicals that are at varying proportions for various breeds. For example, the strong clove scent of sweet basil is derived from eugenol. Licorice basil contains anethole. Sweet basil oil that contains primarily linalool has a sweet fresh and herbaceous aroma. Composition and content of basil oil varies largely with cultivars, geographic regions, seasons, tissue materials, growth stages, growth regulation, cultivation conditions, fertilization and soil amendment, and harvest and postharvest conditions. The oil yield of sweet basil was approximately 0.1–0.7%.

More than 200 chemicals in basil oil have been reported. The chemical constituents include monoterpenes, sesquiterpenes, triterpenes, flavonoids, and aromatic compounds. Major components in basil oil include linalool, estragole (methyl chavicol), anethole, eugenol, and methyl eugenol, varying by chemotype (Figure 2). Three major components in sweet basil commonly cultivated in the United States are linalool (7–59%), estragole (5–29%), and eugenol (2–12%). Linalool, a monoterpenol, showed a wide range of biological activities such as sedative, stress relief, and neurological effects. Estragole is sweet, herbaceous anise–fennel type odor. It is used in fragrance compositions and gives a nice fruity and anise aromatic note. Eugenol is used in perfumeries, flavorings, and as a local antiseptic medicine.

Food authentication and adulteration are an international issue. The desire to make a profit is the driving force for adulteration and mislabeling of food. A literature search of the Google Scholar database did not find reports concerning problems about basil oil authentication and adulteration, although the current price of basil oil varies largely between US $1/L and US $150/L. α-Linalool could be used as a marker compound of *O. basilicum* oils. Analysis of basil oil has been primarily relied on gas chromatography (GC) and GC–mass spectrometry.

There are many types of basil oil in the market. Each possesses a more or less different aroma and personality for varying applications because they were derived from different cultivars of sweet basil. Basil oil productivity is ecophysiologically and environmentally responsive. Uses of basil oils are mainly based on their biographical origin, chemotype, and the purpose. Basil oils have been commonly used in aromatherapy, cosmetics and perfumery, folk medicine, catering, hygiene and cleaning products, and pest control. The type of basil oil typically used in aromatherapy is the linalool chemotype, commonly referred to as a basil linalool essential oil or linalool chemotype.

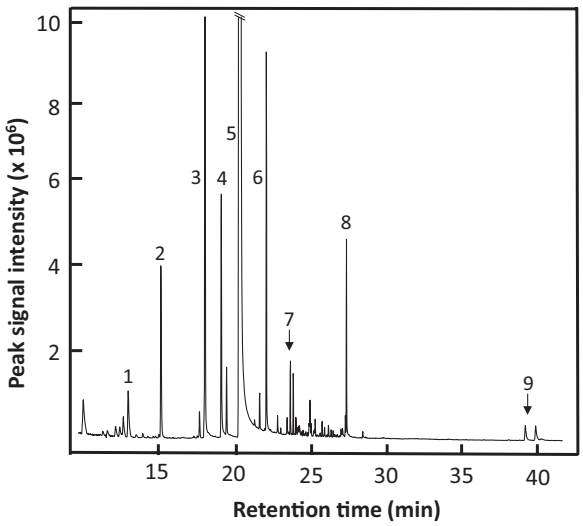

FIGURE 2 Gas chromatography–mass spectrometry total ion current chromatogram of volatile components of basil oil (1000 μg/mL in acetone). Peak identification: 1, α-pinene; 2, linalool; 3, *trans*-anethole; 4, 4-methoxy benzaldehyde; 5, estragole; 6, 1-methoxy-4-(1-methoxypropyl)-benzene; 7, *trans*-caryophyllene; 8, 2,3-dihydro-1*H*-indene-5-ol; 9, 1-(1,1-dimethyl)-2-methoxy-4-methyl-3,5-dinitrobenzene.

APPLICATIONS OF BASIL OIL IN FOOD SCIENCE

Two characteristics of basil oil are a nice flavor and a wide spectrum of biological activity. In addition to antimicrobial and insecticidal activity, basil oil exhibited high antioxidant activity, antiprotozoa activity, and anticancer effects. Therefore, basil oil has been widely used in culinary flavoring, food preservation and safety, and food production.

Culinary Flavoring

Basil is one of the most popular culinary herbs. Among more than 12 culinary basil herb varieties, sweet basil is one of the most popular varieties used in cooking. Dried sweet basil is usually used for Italian food, whereas fresh Thai (*O. basilicum* var. *thyrsiflora*), lemon (*Ocimum X citriodorum*), or holy basil (*Ocimum tenuiflorum*) are used in Asian food such as Vietnamese and Thai food. The aroma found in basil leaves and stem was mainly from three predominant constituents, linalool, estragole, and eugenol. Basil is usually added to food in the last because the flavor can be destroyed or blanched if it is cooked for too long. Dried basil is good for roast meat, fish, and chicken.

Due to its pleasant fragrance, basil oil is an additive in many foodstuffs. Basil oil has been used in sausages and other meat products. Basil oil has been added to cheeses and other dairy products. Basil oil is also an additive in alcoholic and nonalcoholic beverages. In addition, basil oils are widely used in combination with other spices and herbs in confectionary products, sweets, bakery products, puddings, condiments, vinegars, ice creams, mustard, and in pickled vegetables.

Food Preservation and Food Safety

Food spoilage is due to physical, chemical, enzymatic, microbial activities. Some spoiled foods are harmless to consume, whereas some are harmful. The toxic effects from consuming spoiled or contaminated food are referred to as food-borne illness. Food-borne illness is a growing public health concern worldwide. It is estimated that 31 species of pathogens cause 9.4 million cases of food-borne illnesses each year in the United States (CDC, 2011). The top five pathogens contributing to domestically acquired food-borne illnesses are Norovirus, *Salmonella* (nontyphoidal), *Clostridium perfringes*, *Campylobacter* spp., and *Staphylococcus aureus*. The top five pathogens resulting in hospitalization are *Salmonella* (nontyphoidal), Norovirus, *Camphylobacter* spp., *Toxoplasma gondii*, and *Escherichia coli* O157 (CDC, 2011). Consumers want fresh and minimally processed food without synthetic preservatives. Natural and effective food preservatives become important for a successful control of food-borne pathogens and mycotoxin biosynthesis in food. Basil oil or its component has been used to increase the shelf life of many foodstuffs because of its antimicrobial properties and antioxidative effects.

The United States Food and Drug Administration and European Commission have accepted and classified the components from basil oil as generally recognized as safe (GRAS). That is, no risk to the health of the consumer. There are still regulatory limitations on the accepted daily intake of three major components (linalool, estragole, and eugenol) in basil oil

because of large variation of the chemical composition of basil essential oil and basil extracts. Samples of basil oil may have the same common name even they are composed of different subspecies of *Ocimum basilicum* or other species. Test microorganisms, methods, and evaluation criteria all vary among different publications. Therefore, before being added to food products as preservatives a quick interaction test between essential oil and food should be performed.

Antimicrobial Activities

Many studies investigated antifungal, antibacterial, and antioxidant activities of essential oils from *Ocimum* spp. including sweet basil. Food-borne fungi can cause grain and food spoilage and may produce mycotoxins. Of the tests that have been run on many different types of natural chemicals, basil oil is one of the most favorable. Among the seven fungi isolated from plants *Alternaria* sp., *Aspergillus flavus*, *Botrytis cinerea*, *Cladosporium herbarum*, *Eurotium amstelodami*, and *Eurotium chevalieri*, *E. chevalieri* was the most sensitive to basil oil, whereas *A. flavus* was the most resistant (Jakowienko et al., 2011). Sweet basil oil was potent against the fungi *Penicillium aurantiogriseum*, *Penicillium glabrum*, *Penicillium chrysogenum*, and *Penicillium brevicompactum* (Kocić-Tanackov et al., 2012). The highest sensitivity was *P. chrysogenum*, with complete inhibition of growth with 1.5% basil extract. Basil oil at 0.6% v/v exhibited 100% inhibition of mycelium growth to the rice pathogenic fungi *Fusarium moniliforme* and *Pyricularia arisea* and 50% inhibition to *Fusarium proliferratum*, but was not effective against *Rhizoctonia solani*. Basil oil at 0.8% v/v could inhibit spore germination of *F. monoliform* (91%) and *Alternaria brassicicola* (100%) (Piyo et al., 2009). Basil oil at 3000 ppm exhibited complete inhibition against the toxigenic fungi *A. flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, and *F. moniliforme* (Soliman and Badeaa, 2002). Eugenol exhibited very strong antifungal activity against *Absida glauca*, *Aspergillus nidulans*, *Aspergillus niger*, *Colletotrichum capsici*, *F. monoliforme*, *Pestolotia psidi*, and *Rhizopus nadssus* (Soković et al., 2013). Linalool and anethole exhibited good antifungal activities at 0.03–0.3 mg/mL and 1.3–2.8 µL/mL, respectively, in microdilution tests (Soković et al., 2013).

Minimal inhibitory concentrations of sweet basil oil were 145–160, 40–45, and 80–95 µg/mL against the Gram-negative bacteria *Salmonella typhi* and *E. coli*, Gram-positive bacteria *S. aureus* and *Bacillus subtilis*, and fungi *A. niger* and *Candida albicans*, respectively (Shirazi et al., 2014). Sweet basil oil exhibited strong activity against *Streptococcus pneumoniae*, *Hemophilus influenzae*, *C. albicans*, and *A. niger*, but not *Pseudomonas putida* and *Pseudomonas aeruginosa* (Srivastava et al., 2014). Ouibrahim et al. (2013) reported bacteriostatic effects of sweet basil oil on 20 Gram-positive and Gram-negative bacterial strains. Basil oil exhibited no activity against the Gram-positive bacteria *Brochotrix thermosphacta*, *Enterococcus faecalis*, *Lactobacillus delbrueckii*, *Lactococcus lactis*, and *Lactobacillus plantarum*. *Vibrio* species such as *Vibrio parahaemolyticus* showed a high sensitivity to basil oil.

Preservatives for Processed Foods

Agar plate tests of sweet basil oil at a concentration of 1.5% showed complete inhibition against *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium subglutinans*, and *Fusarium verticillioides* isolated from cakes (Kocić-Tanackov et al., 2013). Saggiorato et al. (2012) evaluated the antifungal activity of basil oil in vitro and on the surface of an Italian-type sausage. No significant differences were found in the product's taste at different oil concentrations. Sweet basil oil in vitro presented an antioxidant activity of half maximal inhibitory concentration, IC_{50}, of 12 mg/mL, while sweet basil oil formulated in the Italian sausage salami at 0.75 mg/g presented antioxidant activity in relation to the lipids but not to the proteins during processing and storage (Chichoski et al., 2011). The bacterial level of *Salmonella enteritidis* inoculated in ham, a fermented pork sausage, was unmeasurable after 2-day storage when 100 ppm of sweet basil oil was added (Rattanachaikunsopon and Phumkhachorn, 2010). The addition of 100 but not of 150 ppm to ham did not change the taste. A combination of modified atmosphere or vacuum packaging with basil oil (0.4% v/w) extended the shelf life of Anthotyros, a Greek whey cheese, to 6–12 days at 4 °C and maintained the freshness and the sensorial characteristics of the product (Tsiraki and Savvaidis, 2013). Clove basil (*Ocimum gratissimum*) oil showed a minimum inhibitory concentration ranging from 600 to 800 mg/L against spoilage and pathogenic molds *Aspergillus terreus*, *Aspergillus ustus*, *A. niger*, *Aspergillus aculeatus*, *P. brevicompactum*, and *Scopulariopsis brevicaulis* isolated from wagashi. Clove basil oil could be a very promising preservative for wagashi to prevent its deterioration (Philippe et al., 2012).

Stored Grain Protection against Insect Pests

Basil oil is a potential biopesticide (green pesticide). Basil oil is classed as GRAS and is exempt from the United States Environmental Protection Agency registration under section 25 (b) of the Federal Insecticide Fungicide and Rodenticide Act. Basil oil is volatile and therefore is a good biofumigant candidate for grain storage. *Ocimum* oils including basil oil

exerted strong fumigant and contact activities against stored product insect pests such as cowpea weevil (*Callosobruchus maculatus*) and adzuki bean seed weevil (*Callosobruchus chinensis*), maize weevil (*Sitophilus zeamais*), red flour beetle (*Tribolium castaneum*), sawtoothed grain beetle (*Oryzaephilus surinamensis*), and cereal storage pest bruchid beetle (*C. maculatus*) (Kim and Lee, 2014). Basil oil was also toxic to rice weevil (*Sitophilus oryzae*) and two other stored rice insect pests (*Rhyzopertha dominica* and *Cryptolestes pusillus*) (López et al., 2008; Follett et al., 2014). Vapor toxicity of a mixture of methyl eugenol, estragole, and linalool was higher than one alone to the three stored rice pests (López et al., 2008). Sweet basil oil at 2% showed significant mortality, repellency, and antireproductive effects to rice weevil, and the most striking effect was in reduction of the F1 progeny (Popović et al., 2006).

Postharvest Preservation of Fresh Fruits and Vegetables

Microbial contamination and growth is one of the most important causes of postharvest fruit and vegetable losses. Basil oil has been studied as an alternative to synthetic preservatives. Basil oil exerted significant postharvest protection of mango fruits against anthracnose diseases caused by the fungus *Colletotrichum gleosporioides* (Abd-Alla and Haggag, 2013). Postharvest spraying of sweet basil oil on table grape stored at 0 °C inhibited development of fungal decay for 60 days. In addition, it reduced weight loss, berry and rachis browning, and had no considerable adverse effect on the flavor of the fruits (Abdollahi et al., 2012). Dipping ready-to-eat seedless table grapes into a 20% eugenol solution in combination with modified atmosphere packaging increased shelf life by more than three weeks (Cristina et al., 2013). Sweet basil oil and eugenol exhibited satisfactory inhibition against the banana crown rot fungi *Colletotrichum musae* and *F. proliferatum* in vitro (Herath and Abeywickrama, 2008). Linalool vapors showed a moderate activity against peach fruit decay fungi *Rhizopus stolonifer*, *Sclerotinia sclerotiorum*, and *Mucor* sp. in a closed system, while eugenol exhibited no activity at all. Mixing the two in a ratio similar to their concentrations in the original basil oil enhanced the antifungal properties of basil oil, indicating a synergistic effect (Edris and Farrag, 2003). Synergistic preservative effects of sweet basil oils against acid-tolerant food microflora were also reported.

Basil oil showed significant activities against postharvest diseases caused by *Fusarium* fungi (Kocić-Tanackov et al., 2013). In vitro tests showed significant inhibition of sweet basil oil against pathogenic molds *B. cinerea* and *Mucor piriformis* when it was incorporated in medium or in vapor (Abdollahi et al., 2012). Basil and thyme oils, carvacrol, thymol, estragole, linalool, and *p*-cymene showed inhibitory effects toward the Gram-negative bacteria *Shigella sonnei* and *Shigella flexneri* on lettuce. Washing fresh lettuce with 0.1 or 1% (v/v) of estragole chemotype basil oil exhibited comparable effectiveness to natural microbial flora as 125 ppm chlorine (Wan et al., 1998). The plant pathogenic fungus *Botrytis fabae* and the rust fungus *Uromyces fabae* were controlled in vivo, with basil oil, as well as estragole and linalool, reducing infection of broad bean leaves significantly (Oxenham et al., 2005). Mint and sweet basil oils at 0.08% mL/L were effective against survival of *E. coli* and *Salmonella typhimurium* inoculated into fresh-cut lettuce and purslane samples during refrigerated storage (Karagözlü et al., 2011).

Being different from lettuce and spinach, basil has rarely been a source of food-borne illness outbreaks; however, an outbreak occurred in 2007 when basil was contaminated with *Salmonella enterica* serovar Senftenberg. Kisluk et al. (2013) studied whether *S. Senftenberg* developed possible resistance to basil oil or not. The results showed *Salmonella* can survive on the basil plants for at least 100 days. Compared with *S. enterica* serovar Typhimurium, *S.* Senftenberg was able to grow on stored harvested basil leaves. *S.* Senftenberg is more resistant to basil oil, linalool, estragole, and eugenol than *S.* Typhimurium. This may indicate that *S.* Senftenberg had adapted to the basil environment by developing resistance to basil oil.

Postharvest Preservation of Grains

Basil oil at 5% was fungistatic on *A. parasiticus* in vitro and aflatoxin production. Addition of whole and ground dry basil leaves at 5–10% markedly reduced aflatoxin contamination in sorghum, groundnut, maize, and melon seeds (Atanda et al., 2007).

Usages of Basil Oil as Preservatives in Food Packaging and Processing

Although many studies demonstrated potential of essential oils and their constituents as a preservative in vitro, there are limitations of using essential oils for food preservation. One is high cost and high concentrations that are often required for a sufficient efficacy. Another limitation is organoleptic property change, for example, browning and strong odor of treated lettuce. The third is that antimicrobial potency of essential oil constituents depends on food ingredients such as fat and starch and other parameters such as pH and temperature. Finally, the antimicrobial potency rapidly decreases on the subsequent samples in the same decontamination solution.

Different methods have been studied to overcome the limitations. One way is to encapsulate basil oil in polymers of edible and biodegradable package that provide its slow release to the food surface. Another way is to encapsulate essential oils into nanoemulsions. Such an approach increases the stability of volatile components, protecting them from interacting with the food matrix, and increasing the antimicrobial activity due to increased passive cellular uptake. Lowering the concentration of essential oils without compromising their antimicrobial activity can also be obtained by applying them in combination with other antimicrobial compounds that provide a synergistic effect. Synergies are known to occur for essential oil combinations. It is therefore a field with opportunities to find potent antimicrobial blends and lower concentrations while maintaining the antimicrobial efficacy, which may be a key to implementing essential oils in food preservation without simultaneous organoleptic effects.

Applications of Basil Oil and its Main Components to Livestock, Aquaculture, and Seafood

Day-of-hatch chicks were fed with linalool-supplemented diets for three weeks. Linalool had dramatic negative dose-dependent effects on feed conversion at concentrations in the feed exceeding 2% linalool, but not on gross pathology. Liver weights were significantly increased in the 5% linalool-treated birds (Beier et al., 2014). Matan et al. (2014) reported that the antibacterial activity of low concentration clove oil, sweet basil oil, and lime oil (5–20 μL/mL) to effectively control the growth of *E. coli*, *S. typhimurium*, and *S. aureus* on chicken eggs. Clove oil (10 μL/mL) or eugenol (5 μL/mL) in combination with atmospheric radiofrequency plasma treatment completely inhibited growth of *E. coli*, *S. thyphimurium*, and *S. aureus*. de Almedia et al. (2007) observed a potent activity of sweet basil oil and linalool against the protozoan parasite *Giardia lamblia* that can infect cattle, sheep, dogs, and cats as well as humans.

Sweet and clove basil oils showed an inhibitory activity against *Morganella morganii*, which can produce histidine decarboxylase to form histamine in fish during storage, and thus can reduce the incidence of histamine fish poisoning (Lomarat et al., 2013). Basil oil exhibited a moderate extension of shelf life of modified atmosphere packed cod fillets through inhibiting the specific spoilage organism *Photobacterium phosphoreum*. Soaking raw peeled shrimps in holy basil oil (1:2, w/v) for 15 min followed by modified atmosphere packaging extended shrimp shelf-life by 18 days compared with the untreated samples packaged under atmospheric conditions (4 days) (Chinnasri et al., 2013).

Applications of Basil Oil as Insecticides in Food Production

Pest management is facing challenges of safety and effectiveness due to human and environmental hazards caused by wide use of synthetic insecticides. Botanical insecticides are, therefore, of great interest of development. Potent insect juvenile hormone mimics were isolated from sweet basil. Sweet basil oil exerted high toxicity to grasshopper (*Acrida exaltata*) and tephritid fruit flies (Chang et al., 2009). The median lethal concentration, LC_{50}, values of basil oil to the vine mealybug (*Planococcus ficus*) ranged from 44 to 47 mg/mL (Karamaouna et al., 2013). Basil oil is a potential control agent against mite (*Tetranychus urticae*) and whitefly (*Bemisla tabaci*) under greenhouse conditions. Basil plants attract many insects such as butterflies, bees, and fruit flies (Figure 1). Basil oil is an attractant to many insects (Chang et al., 2013). Addition of basil oil on sticky traps increased approximately three and fivefold the number of trapped pea leafminer (*Liriomyza huidobrensis*) and greenhouse whitefly (*Trialeurodes vaporariorum*) (Górski, 2005), respectively. Sweet basil oil showed antifeedant and insecticidal activity against the cowpea aphid *Aphis craccivora* Koch. Successful adoption of basil oil in the protection of food commodities promises an eco-friendly option compatible with international biosafety regulations.

Disinfection of Seeds with Basil Oil

Basil oil was tested to disinfect seed-borne fungi and bacteria. Coating maize seeds with basil oil (1:1) effectively controlled seed-borne fungi, including *A. flavus*, *A. niger*, *Rhizopus* sp., *Penicillium* sp., and *Fusarium* sp. with no negative effects on seed vigor and viability. Laboratory, greenhouse, and field experiments showed that the basil oil had a promising alternative for the control of root rot disease of cumin caused by *Fusarium* spp. (Hashem et al., 2010). Basil oil (50 μL/g grain) inhibited mycelial growth of *A. flavus*, *Curvularia pallescens*, and *Chaetomium indicum* as well as prevented infection by *A. flavus*, *Aspergillus glaucus*, *A. niger*, and *Aspergillus sydowi* on maize grains.

Safety of Basil Oil Components

Rat oral median lethal dose, LD_{50}, values of eugenol, linalool, and estragole were approximately 2680, >1000, and 1820 mg/kg. Linalool gradually breaks down when it is exposed to oxygen, forming an oxidized by-product that may cause allergic reactions such as eczema in susceptible individuals. Estragole is suspected to be carcinogenic and genotoxic as indicated by a report

of the European Union Committee on Herbal Medicinal Products. Several studies have clearly established that the profiles of metabolism, metabolic activation, and covalent binding are dose-dependent and that the relative importance diminishes markedly at low levels of exposure (i.e., these events are not linear with respect to dose). In particular, rodent studies showed that these events are minimal probably in the dose range of 1–10 mg/kg body weight, which is approximately 100–1000 times the anticipated human exposure to this substance. The ex vivo urine drug screen test indicated clear elevation of DNA repair by estragole and estragole chemotype sweet basil oil in rats treated orally with doses up to 2 g/kg body weight. Because of these reasons, it is concluded that the present exposure to estragole resulting from consumption of herbal medicinal products (short time use in adults at recommended posology) does not pose a significant cancer risk. In the meantime exposure of estragole to sensitive groups such as young children and pregnant and breastfeeding women should be minimized. The Scientific Committee on Food from the Health and Consumer Protection Directorate-General took a more concerned position and concluded that "Estragole has been demonstrated to be genotoxic and carcinogenic". Therefore, the existence of a threshold cannot be assumed and the committee could not establish a safe exposure limit. Consequently, reductions in exposure and restrictions in use levels are indicated. Eugenol is hepatotoxic and its overdose may cause a wide range of symptoms from blood in the patient's urine, to convulsions, diarrhea, nausea, unconsciousness, dizziness, or rapid heartbeat. According to a published 1993 report, a 2-year-old boy nearly died after taking between 5 and 10 mL. Eugenol is subject to restrictions on its use in perfumery as some people may become sensitized to it; however, the degree to which eugenol can cause an allergic reaction in humans is disputed.

In summary, basil is a popular culinary herb and its oil content and component profiles are responsive to the environment and agronomical inputs. Basil oil has pleasant aroma and a wide range of biological activities. However, mechanism of action of basil oil and its components remains unclear. In addition to aroma therapeutic and medicinal applications, basil oil has been used as culinary flavors, food preservatives, and pest control agents. Good research opportunities are in two areas for food preservation: synergistic effects of mixing basil oil with other natural biocides and integration of basil oil with food processing and packing technologies.

SUMMARY POINTS

- Sweet basil (*O. basilicum*) is a popular culinary herb.
- Basil oil is used for culinary flavoring.
- Among more than 200 chemicals in basil oil, anethole, estragole, and linalool are major components. Its oil content and chemical profiles are responsive to cultivation conditions and the environment.
- Basil oil has good antimicrobial, protozoan, and antioxidant activities.
- Basil oil is a good food preservative.
- Basil oil is a potent attractant to many insects.
- Basil oil is a potential green insecticide for insect pest control.

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Chapter 26

Bay Laurel (*Laurus nobilis*) Oils

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List of Abbreviations

EO Essential oil
TBARS Thiobarbituric acid reactive substances

INTRODUCTION

Worldwide, the food industry relies on synthetic compounds that are used as additives to improve or maintain nutritional value, quality, safety, and freshness, to aid in the processing and preparation of food, and to make food products more appealing. Preservatives, colors, and flavors are the most common food additives, but other categories are also in use. Each group is tailored for a specific purpose, such as aroma enhancers, emulsifiers, gelling agents, sweeteners, anticaking agents, antioxidants, and acidulants. In some particular foods, additives are so essential that they are even used in organic products.

Studies raised suspicions about the negative health effects of some synthetic additives, thus restricting their applications in food and increasing consumer confidence about natural products (Santoyo et al., 2006). However, the idea that a chemical compound is "safe" because it is natural is not correct. The safety of both natural and synthetic chemicals always needs to be studied in detail to make sure that consumers are on the safe side.

Aromatic plants have been used for centuries with several purposes, including flavoring, medicine, nutrition, pharmaceutical, cosmetics, and the food industry. These plant essential oils (EOs) are described as excellent sources of natural preservatives, being assumed that this property is associated with the oil richness in naturally occurring phenolic compounds (Dudonné et al., 2009).

The present chapter aims to revise the information available about the composition and potential food applications of bay laurel (*Laurus nobilis*) EO that has been investigated in recent years.

BOTANICAL ASPECTS

The bay laurel, *L. nobilis* (Linnaeus), is a plant belonging to the Lauraceae family, also known as laurel, sweet bay, true laurel, Grecian laurel, Roman laurel, noble laurel, daphne, bay tree, or laurel tree.

Laurus nobilis is a multibranched shrub or tree with up to 10 m high (Ross, 2001). The bark is smooth with an olive green to black color. This species is an evergreen plant with leaves that are dark green, lanceolate, acuminate at both ends, about 10 cm long, and alternated in the branch (Ross, 2001). The leaves of bay laurel are shown in Figure 1.

The female and male flowers of *L. nobilis* are formed during spring in separate plants. The flowers are whitish-green with four petals fused at the base and located in axillary bushy umbels or short racemous panicles (Ross, 2001). The male flower usually has 10–12 stamens, while the female flower has four staminoids and the ovary is short-stemmed with one chamber with a hanging ovule, a short style, and a triangular obtuse stigma (Ross, 2001). The fruit develops on the stem into deep black 2 cm long ovate berries (Ross, 2001).

Laurus nobilis is native of the Mediterranean area and grows spontaneously in Europe and California (Figure 2; Kumar et al., 2003). It is widely cultivated in Europe, America, and in Arabian countries from Libya to Morocco (Kumar et al., 2003). The optimal conditions for *L. nobilis* growth are sunny and well-drained soils, and protecting cultivated plants from winter winds. This plant is propagated by seeds; however, even in warm climates seeds may take several months to a year to germinate. Stem cutting is also common practice; better roots are produced if cutting is done in July–August under Mediterranean conditions (Kumar et al., 2003).

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FIGURE 1 Leaves of bay laurel, *Laurus nobilis*.

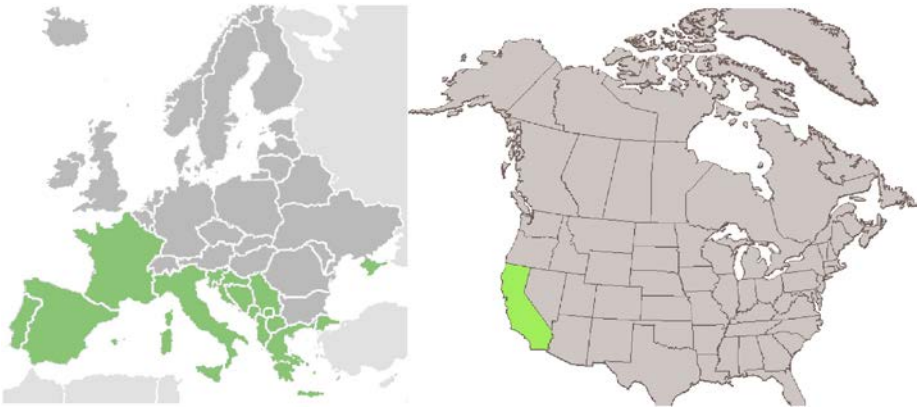

FIGURE 2 Geographic distribution of native bay laurel, *Laurus nobilis*, in Europe and North America. *Adapted from http://luirig.altervista.org/flora/taxa/index1.php?scientific-name=laurus+nobilis.*

USAGE AND APPLICATIONS

Bay laurel has symbolic meanings in many religions and cultures, being considered as a symbol for prosperity and fame in the Bible, the resurrection of Christ in Christian tradition, and is used by Greeks and Romans in specific events as a symbol of victory or highest status, such as in Pythian Games (precursor of Olympic Games) where a wreath of laurels was presented to winners. Romans and Greeks also made extensive use of bay laurel leaves as a spice in food, decoration, and in medicine (Nayak et al., 2006).

Bay laurel EO has been recognized to possess many interesting properties that have wide potential applications in many areas, including health. Table 1 presents the most relevant properties attributed to bay laurel EO in the literature, as well as the potential applications (Panza et al., 2011; Guelsen, 2012; Qnais et al., 2012; Saab et al., 2012). Such properties vary according to the oil chemical composition. Table 2 shows the different constituents of bay laurel EO that can vary according to different parameters, including temperature, season, habitat, and soil composition. Bay laurel EO can be extracted mainly from the plant leaves and fruits, showing a light yellow color. An example of application of bay laurel EO from fruits is the manufacture of some types of soaps, such as Aleppo soap.

USAGE AND APPLICATIONS IN FOOD SCIENCE

EO from plants have been extensively studied for different purposes in the seafood science field, including direct application to preserve during storage; incorporation in edible films prepared with fish proteins and gelatin assess the potential to

TABLE 1 Bay Laurel EO Properties and Potential Applications Already Validated by Several Scientific Studies

Properties		
Antiseptic	Antidiarrheal	Antimycotic
Antimicrobial	Antiinflammatory	Antioxidant
Anticarcinogenic	Anticorrosion	
Potential applications		
Inhibit human melanoma	Treat disorders in the digestive tract and skin	Massage therapy to relieve arthritic aches and pains
Stimulate appetite	Assist the breakdown of heavy foods	Promote the onset of menstruation
Decrease pain in muscles and joints	Aromatherapy to treat earaches and high blood pressure	Insect repellent
Preservative in cosmetic products soap industry	Neuroprotective paint industry	Food preservative

TABLE 2 Examples of Chemical Composition of Bay Laurel EO from Several Origins

Compounds (% Volatile EO)	Mediterranean	Tunisia	Turkey	Italy	Croatia	Argentina	Portugal
α-Thujene	–	0.5	0.3–0.3	0.4	0.2	0.5	0.5
α-Pinene	3.8–6.5	7.8	3.9–5.0	3.2	2.3	4.8	2.3
Sabinene	4.5–8.8	5.4	7.1–7.6	6.5	5.8	8.4	4.0
β-Pinene	2.9–4.6	5.9	3.0–3.8	2.9	2.2	3.5	1.8
α-Terpinene	0.2–1.0	0.6	–	0.2	–	0.4	0.8
Eucalyptol	37.2–44.1	42.3	24.2–32.1	35.7	38.2	43.8	27.2
γ-Terpinene	0.1–0.9	0.6	0.1–0.2	0.4	–	0.8	1.4
Linalool	8.7–11.2	2.5	0.7–1.5	–	2.8	12.7	8.4
δ-Terpineol	2.4–4.6	2.5	1.3–1.8	1.4	3.2	1.9	2.6
α-Terpineol	1.6–4.6	2.1	0.3–0.7	0.2	0.5	2.8	2.7
Carvacrol	–	–	–	–	–	1.8	3.2
α-Terpinenyl acetate	6.1–10.0	11.2	4.8–6.5	9.3	16.1	7.9	10.2
Eugenol	0.1–4.5	–	0.1–1.6	4.8	2.1	0.7	1.2
Methyl eugenol	2.1–5.1	3.5	0.2–1.2	6.8	12.5	3.6	5.4

Aadapted from literature available in Ramos et al. (2012).

preserve seafood; incorporation in ice to preserve seafood; and incorporation in farmed fish diets to study nonspecific fish immune responses.

Despite bay laurel leaves being the source of several popular spices used in a wide variety of recipes, particularly among Mediterranean cuisines, the usage of its EO in food science has been rather limited.

Meat Products

Modern improvements have been introduced in meat slaughtering hygiene and meat production techniques. Even so, food safety is an increasingly important public health issue. There is therefore still a need for new methods making meat products

TABLE 3 Treatment Conditions and Improvements of Meat Products Preserved with Bay Laurel EO Reported in the Literature

Meat Specifications	Bay laurel EO Specifications	Application of Bay Laurel EO	Conditions	Improvements
Djenane et al. (2012)				
Chicken meat	EO was extracted from plants of Tizi Ouzou province (Algeria)	1.2% (v/w) EO volume per flesh weight	Modified atmosphere packaging stored at 2–3 °C for 8 days	>2 days shelf-life
Alçiçek et al. (2003) and Çabuk et al. (2006a)				
Broiler chicks and chicken broilers	EO was extracted from different herbs from Turkey	0.0048% (v/w) EO volume per bird feed weight	Pellets were prepared and fed to birds daily for 21 days	Better growth performance and reduced mortalities in 21 days
Çabuk et al. (2006b)				
Brown nick hens	EO was extracted from different herbs from Turkey	0.0024% (v/w) EO volume per chicken feed weight	Pellets were prepared and fed to birds daily for 20 weeks	Increased egg production and chicken performance in 20 weeks

safe, especially those having a natural or "green" image. One such possibility is the use of EO as antibacterial and antioxidant additives, possibly in combination with existing methods. Ramos et al. (2012) highlighted the strong in vitro antibacterial and antioxidant properties of bay laurel EO rich in oxygenated monoterpene compounds and phenolic components that are considered as largely responsible for the antibacterial properties of EO in meat applications. Despite few studies reporting the use of bay laurel to preserve meat products, one study refers to its application in retail chicken meat packaged in a microaerobic atmosphere (Djenane et al., 2012, Table 3). The main results indicate that the presence of laurel oil significantly decreases meat oxidation and extends fresh meat odor. In addition, the results of the bioassays, together with the chemical profile, support the possibility of using bay laurel oil as potent natural preservatives to contribute to the reduction of experimentally inoculated *Campylobacter jejuni* in chicken meat. Burt (2004) published a review of studies testing the antibacterial activity of EOs (not including bay laurel) or their components in several meat products, including, among others, raw minced beef, mutton, and pork; chicken and pork cooked products, and sausages made from chicken and pork. One of the main conclusions of this review was that certain oils or components stand out as better antibacterials than others for meat applications: eugenol and coriander, clove, oregano, and thyme oils were found to be effective at levels of 5–20 μL/g, inhibiting *Listeria monocytogenes*, *Aeromonas hydrophila*, and autochthonous spoilage flora in meat products, sometimes causing a marked initial reduction in the number of recoverable cells. Mustard, cilantro, mint, and sage oils were less effective or ineffective. Based on such results and the composition of bay laurel EO, it is expected that this EO will also have a similar role. The high fat content in some meat products clearly reduces the action of EOs. For example, mint and cilantro EOs were not effective in products with a high level of fat, such as pâté (about 30–45% fat) and a coating for ham containing canola oil (Gill et al., 2002). The antimicrobial potency of EO constituents also depends on pH, temperature, and the level of microbial contamination of meat products, and these parameters will have to be taken into consideration with the application of bay laurel EO.

Other studies have considered the use of bay laurel EO as a chicken growth promoter. Alçiçek et al. (2003) revealed that a combination of different EOs including bay laurel is a potential growth promoter in broiler production (Table 3). Çabuk et al. (2006a) highlighted lower feed intake from young breeders, improvements in feed conversion ratio, and decreases in mortality of chicken broilers when supplied with feed containing a combination of different EOs including bay laurel (Table 3). Finally, Çabuk et al. (2006b) revealed that a combination of different EOs including bay laurel increased laying hens' egg production, decreased cracked and broken eggs, and increased hens' feed conversion ratio compared with control and antibiotic groups (Table 3).

The effect of most EOs on 2,2-diphenyl-1-picrylhydrazyl radical formation, lipid peroxidation, and sarcoplasmic protein deterioration has also been studied in meat products, though not with bay laurel. For instance, thyme EO was considered promising in this regard when applied to fresh chicken breast meat (Fratianni et al., 2010).

Another important aspect is the possible color change induced by the application of EO. Results obtained by Chouliara et al. (2007) on fresh chicken breast meat stored at 4 °C showed that the color parameters L*, a*, and b* were not considerably affected by oregano oil (0.1% and 1% w/w). A high number of studies have demonstrated synergism and antagonism

TABLE 4 Treatment Conditions and Shelf-Life of Seafood Preserved with Bay Laurel EO Reported in the Literature

Seafood Specifications	Bay Laurel EO Specifications	Application of Bay Laurel EO	Package and Storage Conditions	Extended Shelf Life
Erkan and Bilen (2010)				
Chub mackerel *Scomber japonicus*; fillets	EO was purchased from the Ecem Natural Company (Istanbul, Turkey)	1% (v/w) EO volume per fish flesh weight	High barrier plastic film bags; stored at −20 °C.; analyzed once a month for 11 months	1 month
Erkan et al. (2011a)				
Bluefish (*Pomatomus saltatrix*); ungutted	EO was purchased from the Ecem Natural Company (Istanbul, Turkey)	1% (v/w) EO volume per fish flesh weight	High barrier plastic film bags; stored in ice at 2 °C; analyzed each 2 days during 13 days	3–4 days
Erkan et al. (2011b)				
Hot smoked rainbow trout *Oncorhynchus mykiss*; fillets; farmed in Bağcı, Turkey	EO was purchased from the Ecem Natural Company (Istanbul, Turkey)	Added onto the surface (two sides) of each fillet to achieve a final 1% (v/w) EO concentration	High barrier plastic film bags; under vacuum; stored at 2 ± 1 °C; analyzed once a week for 7 weeks	3 weeks
Attouchi and Sadok (2012)				
Wild and farmed sea bream *Sparus aurata*; fillets; Tunisia	Dried leaves obtained from a retail spice market in Tunisia; EO prepared by hydrodistillation (3 h) using a Clevenger-type apparatus	Added onto the surface (two sides) of each fillet to achieve a final 0.5% (v/w) EO concentration	Polyethylene/polyamide pouches; under vacuum; fish:ice (ratio of 1:1 w/w) into polystyrene boxes provided with holes for drainage; boxes covered; stored at 2–4 °C; analyzed each 5 days during 20 days	5 days

between EOs or their components and food preservatives or preservation methods. For example, the presence of food additives such as sodium chloride, sodium nitrite, and nisin and the application of heat treatments, high hydrostatic pressure, and anaerobic packaging have to be carefully considered.

In view of EO organoleptic properties, its application requires a careful selection according to the type of meat. In general, they could be incorporated in manufactured meat products that are associated with herbs by tradition.

Seafood Products

Studies with bay laurel EO focused mostly on seafood preservation during storage, including chub mackerel, rainbow trout, bluefish, and sea bream. Detailed information in treatment conditions and shelf life is shown in Table 4.

Chub mackerel is a fatty fish with high unsaturated lipid composition, important endogenous prooxidant activity, and fast quality loss during frozen storage. Chub mackerel fillets treated with bay laurel EO reveal extended shelf life, mostly due to lower levels of thiobarbituric acid reactive substances and free fatty acids compared with fillets without bay laurel EO (Erkan and Bilen, 2010).

A bacteriostatic effect is observed in hot smoked rainbow trout fillets treated with bay laurel EO, contributing to improvements of the microbiological quality and shelf-life extension (Erkan et al., 2011b).

Bay laurel EO is also able to decrease spoilage bacterial growth and lipid oxidation in fresh bluefish and sea bream thus extending their shelf life (Attouchi and Sadok, 2012; Erkan et al., 2011a). However, bay laurel EO also decreases liquid holding capacity in sea bream meat due to an early proteolysis initiation (Attouchi and Sadok, 2012).

Agriculture Products

The application of bay laurel EO in fruits, pulses, cereals, vegetables, and other related products has focused on preservation during storage and as a tool to control diseases and pests in cultures. Figure 3 illustrates the studied applications of bay laurel EO in these products, and the EO properties.

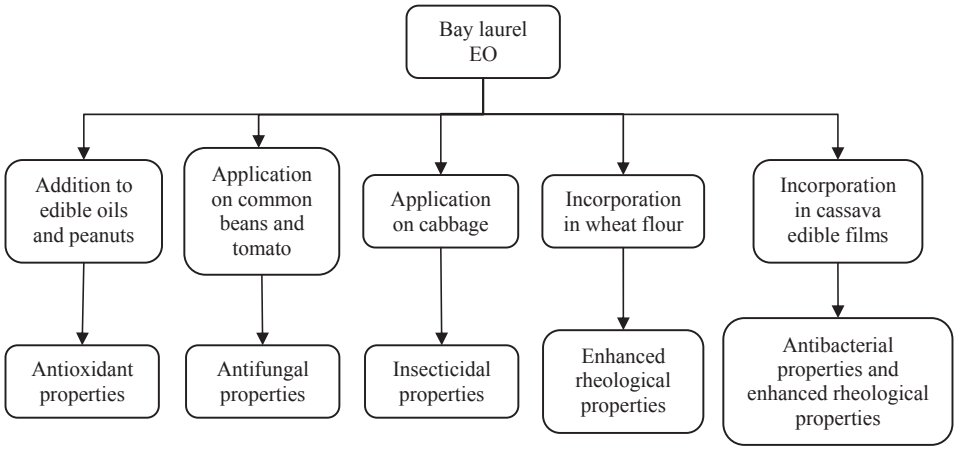

FIGURE 3 Summary of studied applications of bay laurel EO in food products based on fruits, pulses, cereals, and vegetables and highlighted properties of the EO.

Bay laurel EO extracted from leaves was tested for oxidative stability in edible oils and peanuts. Bay laurel EO (0.01–0.05%) improved the oxidative stability of edible oils of pomegranate kernel, poppy, grape, and linseed after 6 weeks at 60 °C, and prevented the increase of edible oils viscosity (İnan et al., 2012). Bay laurel EO (0.005–0.01% w/w) also prevented lipid oxidation and the development of rancid flavors in peanuts (*Arachis hypogaea*), thus extending the shelf life of coated/roasted and fried salted peanuts (Olmedo et al., 2008, 2012).

Concerning the control of cultures diseases and pests, Hoyos et al. (2012) showed that bay laurel EO (0.5%) inhibited the germination of the fungus *Pseudocercospora griseola*, the causal agent of angular leaf spot in several plants including the common bean (*Phaseolus vulgaris*). The volatile phase of bay laurel leaf EO was more effective to inhibit the growth of *Phytophthora infestans* (causal agent of the late blight disease on tomato) than the contact phase, showing a complete growth inhibition with 0.4–2.0 µg bay laurel EO per mL air (Soylu et al., 2006). This study also demonstrated that bay laurel EO caused morphological alterations in *P. infestans* hyphae, such as cytoplasmic coagulation, vacuolations, hyphal shriveling, and protoplast leakage.

Bay laurel EO can also be used to control insect infestations due to the insecticidal properties. Indeed, the cabbage aphid *Brevicoryne brassicae* (that feeds on a vast variety of plants from the Brassicaceae family, such as cabbage and broccoli) mortality increased up to 53% with 1–3% of bay laurel leaf EO independent of the application method used (impregnated papers or direct pulverization) (Kahan et al., 2008).

Another approach is to study the effect of bay laurel EO on wheat flour dough. The results of rheological properties showed that the addition of bay laurel EO allowed higher extensibility and energy of wheat flour dough (Özcan, 2009).

The incorporation of substances with bioactive properties in films is also a promising tool to create active packaging. The effects of bay laurel EO incorporated in edible films prepared with cassava on antimicrobial activity, mechanical, and barrier properties were investigated. The incorporation of 1.5% bay laurel EO in cassava films was responsible for the growth inhibition of several pathogenic bacteria and enhanced mechanical properties and oxygen barrier properties (Taqi et al., 2012).

SUMMARY POINTS

- Several studies have demonstrated that shelf life and food quality can be improved by using EO in some stages of food production.
- The main effects of aromatic plant EO are preventing or reducing the microbial growth and lipid oxidation during storage.
- Nevertheless, more research is needed to determine consistently their antimicrobial activity on food during storage to identify the main metabolic pathways of these compounds and their effects on other food quality parameters.
- Bay laurel EO has many interesting properties, such as antifungal, insecticidal, and antibacterial, which has several potential applications.
- In general, studies performed with seafood and meat products show that the application of bay laurel EO contributes to reduce the growth of spoilage and pathogenic bacteria populations and lipid oxidation, which results in extended shelf life of products.

- Antioxidant effects are also observed in other food products prone to lipid oxidation, like peanuts, in the presence of bay laurel EO.
- Bay laurel EO can also be used as to control diseases and pests in agriculture, acting as an antifungal and insecticide.
- Bay laurel EO can also be used to improve rheological properties of products such as dough and films.
- Bay laurel EO can also improve the growth performance of chicken and the quality of eggs in laying birds.
- Nonetheless, several limitations still need to be overcome before wider use of bay laurel EO by the food industry: (1) the high costs associated with oil extraction; (2) the diversity of oil chemical composition according to environmental conditions; (3) the reduced amount of oil extracted contrary to the needs of high volumes by the food industry; and (4) the intense oil sensorial properties leading to consumer rejection of food products.

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Chapter 27

Bergamot (*Citrus bergamia*) Oils

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INTRODUCTION

Bergamot is a citrus classified as *Citrus bergamia* Risso which belongs to genus Citrus, family Rutaceae and is defined as a hybrid of *Citrus aurantium* and *Citrus limon* L. It grows almost exclusively in Calabria, Italy, along the southern east coast in the area of Reggio; however, several authors also report bergamot production in East Africa, Côte d'Ivoire, Argentina, Brazil, Turkey, and some other Mediterranean countries (Bagetta et al., 2010; Bölükbaşı et al., 2010).

Bergamot is a fruit with a greenish yellow skin, very acid, with a fresh and elegant aroma. Its most important product is bergamot essential oil that is extracted from the pericarp and part of the mesocarp of the fresh fruit by cold processing or steam distillation. Bergamot essential oil is mainly used in the international perfumery industry, as it has not only the function of fixating the aromatic bouquets of perfumes, but is also utilized in combination with other essences. In the food industry, bergamot essential oil is used as a flavoring ingredient in many products such as teas, confectionary products, and soft drinks. Bergamot essential oil and/or its extracts are applied in Earl Grey tea preparations to give a pleasant and refreshing scent (Finsterer, 2002; Belsito et al., 2007). The quality and flavor of an Earl Grey tea depends on the teas used in the blend as well as the quality and amount of bergamot essential oil utilized. Cosmetic and pharmaceutical applications that include dermatology, gynecology, dentistry, and ophthalmology uses are reported; moreover, bergamot essential oil is used in aromatherapy controlling stress-induced symptoms (Verzera et al., 2003; Bagetta et al., 2010). Bergamot essential oil produced in Italy is ranked as of the highest quality at the international trading market. Chemical composition is strongly influenced by harvesting time (Sawamura et al., 2006; Bölükbaşı et al., 2010). The aim of this chapter is to describe and discuss the chemical composition of bergamot essential oil and its derivatives, as well as several applications that have been reported that make bergamot a potential additive for food preservation.

BOTANICAL ASPECTS

Bergamot (*C. bergamia* Risso & Poiteau) is named for the first time by Pierre Antoine Poiteau and Joseph Antoine Risso in 1818. According to the Integrated Taxonomic Information System, *C. aurantium* L. comprises two subspecies: *C. aurantium* ssp. *aurantium* L. or sour orange and *C. aurantium* ssp. *bergamia* (Risso & Poiteau) Wight & Arn. Ex Engler or orange bergamot. It has been suggested that bergamot is a hybrid between sour orange and lemon (*C. limon* L.), or a mutation of the latter. Others consider it a hybrid between sour orange and lime (*C. aurantiifolia*) or a cross between citron (*Citrus medica*) and orange, which is itself a cross between grapefruit (*Citrus*×*paradisi*) and mandarin (*Citrus reticulata*). The bergamot plant is an evergreen small tree that can grow up to 12 m tall, with an erect, cylindrical, dark grayish-brown stem and very thin irregular branches, with or without spines. It is propagated by grafting, and the best results are obtained by using bitter orange, which produces sturdy long-lived trees that are particularly resistant to the inclement weather. The bergamot plant comes into flower in April and May and the fruits ripen from November to March. The fruit is a slightly flattened subglobose to pyriform berry or hesperidium and the peel is 3–6 mm thick with numerous glands; this tissue contains the essential oil cavities. Changes in rind color are mainly due to degradation of chlorophyll and an increase of carotenoids. Carotenes and xanthophylls are the main pigments responsible for the yellow predominant content in flavonoids. Aromatic compounds provide the final aroma of the pulp and juice (Rapisarda and Germanò, 2010).

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USAGE AND APPLICATIONS

Several authors have reported the chemical composition of bergamot essential oil from selected geographical points (Italy, North Africa, and Turkey), different types of extraction methods, and have also characterized other fruit components such as bergamot juice, peel, and pericarp (Table 1).

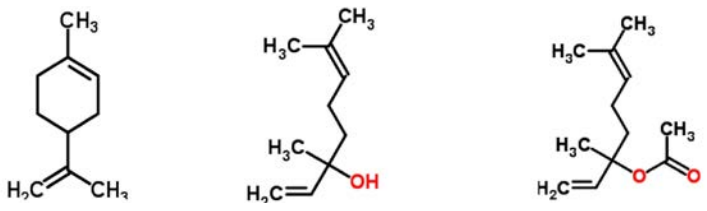
Bergamot essential oil comprises volatile (93–96% of total) and nonvolatile (4–7% of total) fractions; containing coumarins, psoralens, and bergamottine (5-geranyloxypsoralen). The major compounds of Italian bergamot essential oil are limonene (37.2%), linalyl acetate (30.1%), linalool (8.8%), γ-terpinene (6.8%), and β-pinene (6.2%), and another 55 compounds (Figure 1). Bergamot-like odor components include (Z)-limonene oxide, decanal, linalyl acetate, and geraniol. The proportion of limonene in bergamot essential oil is low, while those of linalyl acetate and linalool are relatively high compared with other citrus oils. The ratio of linalool/linalyl acetate (also called the "essence degree") is one of the quality indices of bergamot essential oil as it affects the aroma of bergamot essential oil (Sawamura et al., 2006; Bagetta et al., 2010; Nabiha et al., 2010).

Chemical compositions of Tunisian and Greek bergamot essential oil are different; 15 compounds accounting for 98.52% of the oils were identified. Tunisian bergamot essential oil is characterized by its high content of limonene (59.21%), linalool (9.51%), and linalyl acetate (16.83%); with a high ratio of linalool/linalyl acetate (0.6), which is higher than in bergamot essential oil from Italy (0.3) (Nabiha et al., 2010). Melliou et al. (2009) analyzed bergamot essential oil from Greece, although the morphology of the fruit is different to that of the common bergamot varieties, and the composition of the obtained oil clearly shows a similarity to oil obtained from Calabrian bergamots. Each of the main constituents showed significant variations in content depending on harvesting time.

TABLE 1 Chemical Composition of Bergamot (*Citrus bergamia*) Essential Oil According to Selected Authors

Component	Moufida and Marzouk (2003)	Sawamura et al. (2006)	Melliou et al. (2009)	Kirbaslar et al. (2009)	Nabiha et al. (2010)	Stević et al. (2014)
Limonene	73	37	25.28	36	59	37
Linalool	10	9	15.33	14	9.5	13
Linalool acetate	–	30	40.51	33	16.8	37
β-pirene	–	6	–	3.4	4.4	3
γ-teripene	–	7	10	5.6	1.1	3
Mycene	–	–	–	13	1.2	–
β-bisabolene	–	–	–	0.6	–	–
trans-α-bergamotene	–	–	–	0.4	–	–
Neryl acetate	–	–	–	0.9	–	–
p-cymene	5.6	–	–	–	–	–
α-pinene	1.4	–	–	–	–	–

–, Not reported.



Limonene Linalool Linalyl acetate

FIGURE 1 Chemical structures of the major compounds present in bergamot (*Citrus bergamia*) essential oil.

Verzera et al. (2003) reported that different rootstocks can change the chemical composition of bergamot essential oil. Seventy-eight components were identified and their results were in agreement with those reported in the literature for the Calabrian bergamot oils obtained industrially (limolene, β-pinene, linalool, and linalyl acetate). Because of the quality of their essential oils, Alemow and Volkamerian lemons can be considered as substitutes for sour orange rootstocks.

Other parts of bergamot have been also studied in order to identify other chemical components that may be present in bergamot essential oil composition. Kirbaslar and Kirbaslar (2006) analyzed Turkish bergamot leaf oil obtained by steam distillation. In total, 50 components were identified showing a high content of oxygenated compounds (90.4%), of which linalyl acetate (49.6%) and linalool (22.4%) were the major components.

On the other hand, bergamot juice has linalool, terpinen-4-ol, and *p*-cymene as main volatile components, and also a high content of palmitic acid. This is related to the important role of lipids in the formation of aroma compounds, the oxidation of unsaturated fatty acids released from lipids during thermal treatment and storage, which cause noteworthy alteration of flavor due to the formation of unpleasant aroma compounds (Moufida and Marzouk, 2003). Nevertheless, industrial bergamot juice has been produced for years but as a mere by-product of the essential oils industry. It has been found to be extremely rich in flavonoids. Neoeriocitrin, naringin, and neohesperidin are the most abundant flavanones, whereas apigenin di-C-glucoside, diosmetin di-C-glucoside, rhoifolin, and chrysoeriol 7-O-neohesperidoside are the most plentiful glycosyl flavones. Moreover, ethanol extracts had a significant amount of flavanone glycosides and low amounts of flavone di-C-glucosides and flavone mono- and di-O-glucosides. Bergamot flavonoids (found in lower levels in other citrus peels) have a potential for phytopharmaceutical applications and as natural additives for functional foods (Mandalari et al., 2006; Gatusso et al., 2007).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Several in vitro studies have been reported with regard to the potential use of bergamot essential oil as an effective antimicrobial (Table 2) as well as selected applications in food systems (Table 3). Kirbaslar et al. (2009) reported the antimicrobial activities of Turkish bergamot essential oil against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Kluyveromyces fragilis*, *Rhodotorula rubra*, *Candida albicans*, *Hanseniaspora guilliermondii*, and *Debaryomyces hansenii*; results of bergamot essential oil inhibitory activity were similar to those obtained with common synthetic antimicrobials (nystatin, ketoconazole, and clotrimazole). Bergamot essential oil exhibited higher activity (zone inhibition higher than 15 ± 0.5 mm by disk diffusion assay) than the other studied citrus peel oils. Stević et al. (2014) evaluated the antifungal effect of bergamot essential oil against 21 ubiquitous fungal species, some of them with potential

TABLE 2 In vitro Applications of Bergamot (*Citrus bergamia*) Essential Oil (EO) against Food-Borne and Deteriorative Microorganisms

Bergamot Extracts	Microorganism Inhibited	Reference
EO in vapor phase	*Listeria monocytogenes, S. aureus, Bacillus cereus*	Fisher and Phillips (2006)
Ethanol fractions from bergamot peel	*E. coli, Salmonella enterica, Pseudomonas putida*	Mandalari et al. (2007)
EO in vapor phase	*Arcobacter butzleri*	Fisher et al. (2007)
EO in vapor phase	*Enterococcus faecium, Enterococcus faecalis*	Fisher and Phillips (2009)
EO	*E. coli, S.aureus, B. cereus, L. monocytogenes, Candida albicans, Rhodotorula rubra, Debaryomyces hansenii*	Kirbaslar et al. (2009)
EO added to chitosan and HPMC edible films	*E. coli, Listeria monocytogenes, S. aureus*	Sánchez-González et al. (2011a)
EO added to gelatin edible films	*S. aureus, Listeria monocytogenes*	Ahmad et al. (2012)
EO added to chitosan and locust bean gum edible films	*Aspergillus flavus*	Aloui et al. (2014)
EO	8 different strains of *Fusarium* spp., *Aspergillus flavus, Aspergillus niger*	Stevic et al. (2014)
EO added to chitosan nanoemulsions	*E. coli* O157:H7, *Samonella* Typhimurium	Severino et al. (2015)

HPMC, hydroxypropylmethyl cellulose.

TABLE 3 Bergamot (*Citrus bergamia*) Essential Oil (EO) Applications in Food Matrices

Bergamot Application	Microorganism Inhibited	Food Matrix	References
EO in vapor phase	*Listeria monocytogenes*, *S. aureus*, *B. cereus*	Cabbage leaf and chicken skin	Fisher and Phillips (2006)
EO in vapor phase	*Arcobacter butzleri*	Cabbage leaf and chicken skin	Fisher et al. (2007)
Chitosan and HPMC coatings	Molds, yeasts, and mesophiles	Grapes	Sánchez-González et al. (2011b)
Chitosan coatings	*Penicillium italicum*	Oranges	Cháfer et al. (2012)
Chitosan and locust bean gum coatings	*Aspergillus flavus*	Dates	Aloui et al. (2014)
Modified chitosan coating nanoemulsion	*E. coli* O157:H7, *Samonella* Typhimurium	Green beans	Severino et al. (2015)

production of toxic metabolites, mycotoxins, with powerful mutagenic and carcinogenic effects. Bergamot essential oil exhibited antifungal activity against every tested fungi, particularly effective against *Aspergillus niger*, *Fusarium solani*, *Fusarium sporotrichioides*, *Fusarium oxysporum*, *Curvularia lunata*, V*erticillium dahliae*, *Phomopsis* sp., *Phoma* sp., and *Myrothechium verrucaria*. Other bergamot extracts with antimicrobial activity have been also studied; Mandalari et al. (2007) evaluated the antibacterial effects of ethanolic fractions with a high content of flavonoids, obtained as a bergamot peel by-product after an enzymatic treatment (pectinase) on Gram-positive and Gram-negative bacteria and yeasts. These fractions were more effective against *E. coli*, *Salmonella enterica*, and *Pseudomonas putida*. Furthermore, they found that pectinase converts common glycosides to aglycones with Gram-negative antibacterial activity.

Another interesting area of application is the use of bergamot essential oil by vapor contact, an alternative when essential oil and microorganisms are placed separately in a sealed environment, achieving microbial inhibition from a distance without direct contact with the antimicrobial agent. Few studies have been published regarding inhibition of microorganisms by the vapors generated by bergamot essential oil. Fisher and Phillips (2006) and Fisher et al. (2007) evaluated in vitro the effects of bergamot essential oil by vapor contact against *L. monocytogenes*, *S. aureus*, *B. cereus*, *E. coli* O157:H7, *Campylobacter jejuni*, and three *Arcobacter butzleri* strains, as well as on cabbage leaf and chicken skin. Bergamot essential oil vapors produced six log reductions of *L. monocytogenes*, *S. aureus*, and *B. cereus* populations on cabbage leaf. Gram-positive bacteria were more susceptible than Gram-negative bacteria in vitro, although *C. jejuni* and *E. coli* O157:H7 were inhibited. For *Arcobacter butzleri* on cabbage leaf, exposure to bergamot essential oil resulted in two log reductions, while these microbial strains in chicken were the most susceptible (six to eight log reductions). Tested bacteria were less susceptible in food systems than in vitro. They also evaluated the antibacterial effect of bergamot essential oil by vapor contact against *Enterococcus faecium* and *Enterococcus faecalis*. Bergamot essential oil vapor increased the permeability of cell membrane by 32–40 times. They observed vacuoles within the treated cells that were not present in the control cells. The lipophilic characteristic of bergamot essential oil allows the preferential partitioning from the aqueous phase into the membrane structure of the cell and thus could result in the uptake of bergamot essential oil into the cell, reducing ATP synthesis. Therefore, bergamot essential oil applied in vapor phase is effective against food-borne pathogens and spoilage microorganisms at relatively lower concentrations than when applied in liquid phase, thereby causing less effect on sensory attributes (Fisher and Phillips, 2009).

Several investigations have focused on the incorporation of natural antimicrobials to edible films as a matrix that can release these compounds onto food surfaces avoiding the volatilization of some active compounds. Bergamot essential oil was incorporated to different kinds of polymers and tested in food systems and in vitro conditions. Sánchez-González et al. (2011a) incorporated bergamot essential oil to chitosan and hydroxypropylmethyl cellulose (HPMC) edible films and evaluated their antibacterial effect against *E. coli*, *S. aureus* and *L. monocytogenes*. They reported a four log reduction of *E. coli* and a bacteriostatic effect on *L. monocytogenes* and *S. aureus*. Nevertheless, antimicrobial activity of bergamot essential oil varied with the type of bacteria, the nature of the essential oil, and the characteristics of the film matrix into which they were incorporated. Although bergamot essential oil was effective against the three pathogens, it was more effective at the same concentration against *L. monocytogenes* than *E. coli* when incorporated into HPMC films. Moreover, bergamot essential oil incorporated on chitosan coatings and applied to grapes had an antifungal effect on the surface growth of *Penicillium italicum*, improving weight loss and fruit firmness and exhibiting the greatest inhibition of respiration rates in terms of both O_2 consumption, and CO_2 generation (Sánchez-González et al., 2011b).

Bergamot essential oil was added to gelatin edible films inhibiting *S. aureus* and *L. monocytogenes* (Ahmad et al., 2012). Inhibition zones increased significantly ($p<0.05$) with increasing bergamot essential oil concentration. Important changes on molecular organization, higher intermolecular interactions among gelatin molecules, and enhanced heat stability with higher degradation temperature were found in the film structure when bergamot essential oil was incorporated; bergamot essential oil contributed to enhance several physical properties of the edible films.

Cháfer et al. (2012) applied chitosan coatings containing bergamot essential oil to oranges. Antifungal effects (*P. italicum*) were evaluated with a reduction of the microbial growth (expressed as the percentage of infected samples) of 50% compared with the uncoated samples. Furthermore, coatings did not lead to any relevant changes in quality parameters (acidity, pH, soluble solids, juice percentage, weight loss, firmness, color parameters, and respiration rate) throughout storage. Preventive coating applications were more effective as the disease incidence reached lower levels that when coatings were applied after infection. Aloui et al. (2014) reported the application of chitosan and locust bean gum in combination with bergamot essential oil to inhibit *Aspergillus flavus* both in vitro and on artificially infected dates for a storage period of 12 days. Bergamot essential oil was effective in reducing mycelial growth, conidial germination (87–90% inhibition compared with the control), and complete inhibition was obtained using bergamot essential oil concentrations higher than 2%. In fruit decay assays, coatings based on chitosan and bergamot essential oil were able to reduce fungal decay in the range of 52–62% at day 12.

Severino et al. (2015) incorporated bergamot essential oil to nanoemulsions on modified chitosan edible films and coatings. Edible films were capable to inhibit *E. coli* O157:H7 and *Salmonella* Typhimurium when bergamot essential oil was incorporated to chitosan coatings applied to green beans, exhibiting a bactericidal effect against the two tested pathogens during storage. They reported that in combination with other preservative treatments like γ-irradiation and modified atmosphere packaging the results were extremely effective against bacterial growth during storage, not only in controlling microbial growth, but also in reducing inoculated population.

SUMMARY POINTS

- Bergamot (*C. bergamia*) essential oil is the major product obtained from this citrus fruit.
- Major compounds found in bergamot essential oil are limonene, linalyl acetate, linalool, γ–terpinene, and β–pinene.
- The ratio of linalool/linalyl acetate is an important quality index for bergamot essential oil as it affects its aroma.
- Bergamot essential oil is an effective antimicrobial agent especially when applied by vapor contact.

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Chapter 28

Bitter Gourd (*Momordica charantia*) Oils

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List of Abbreviations

AMPK Adenosine 5′-monophosphate (AMP)-activated protein kinase
BGJ Bitter gourd juice
LDL Low-density lipoprotein
RIP Ribosome inactivating protein
RNase Ribonuclease

INTRODUCTION

Multiple lines of evidence support the health benefits of fruits. It is recommended to consume three to five kinds of fruits per day in order to ensure adequate nutrition and minimize cancer risk. Bitter gourd is a fruit/vegetable with high nutritional values: although eliciting a somewhat bitter flavor to our palate, it is "sweet" for our body (Fang and Ng, 2011a, 2012, 2013). Investigations have focused on different parts of the bitter gourd plant and their potential uses in tumor therapy and treatment of diabetes. Bitter gourd seeds, which are abundant in oils containing dozens of small compounds, manifest medicinal activities.

BOTANICAL ASPECTS

Around 47 *Momordica* species grow in Africa and another 12 *Momordica* species have been found in Asia and Australia (Schaefer and Renner, 2010). Bitter gourd is a member of the family of Cucurbitaceae. It is a herbaceous tropical and subtropical vine with the binomial nomenclature of *Momordica charantia* L. It is also vernacularly designated as bitter melon, bitter squash, balsam pear, karela, bitter apple, or wild cucumber in different countries and regions (Fang and Ng, 2011a). Bitter gourd is extensively cultivated in numerous Asian countries (especially China, India, Japan, and Malaysia), East Africa, the Caribbean, the Amazon, and also some parts of South America. In the Yangtze River Delta of China, it is sown in late March, and the tendril-bearing vine grows to 4–6 m; flower is initiated in May and extends to November.[1]

Because bitter gourd is monoecious, both male and female flowers are borne on the same plant. The oblong fruit, which is also named bitter gourd, matures 30–50 days after the onset of flowering as depicted by changes in the color of the warty exterior from green to orange. At the time the fruit has fully matured, the tender pericarp splits into segments and curls back to expose the seeds. Figure 1 depicts its main morphological characteristics. Because bitter gourd has many varieties and cultivars, the fruits in the market are also seen in different shapes (cylindrical and plump) and colors (e.g., white, light green, and dark green).

USAGE AND APPLICATIONS OF BITTER GOURD

Bitter gourd is a favorite culinary ingredient in many Asian kitchens. The internal sweet red pulp of the ripe fruit can be eaten directly. In Taiwan, white bitter gourd juice (BGJ) mixed with honey is a welcomed beverage. In addition to being a nutritious and healthy food item, bitter gourd is also widely exploited for medicinal use (Fang and Ng, 2011a, 2012). By the application of transcriptomic analysis, cell biology, epidemiological, and preclinical investigations, the different medicinal activities of bitter gourd have been unveiled, such as its antitumor, antidiabetic, antiobesity, and anti-HIV potential (Fang and Ng, 2013).

1 Before entering universities, the author Evandro Fei Fang, with his younger sister Season Jing Fang and under the guidance of their mother, had planted bitter gourd in the garden for six consecutive years.

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FIGURE 1 A biosketch illustrating the morphological characteristics of bitter gourd. Because bitter gourd displays monoecism, flowers of both sexes are located on the same plant. The oblong fruit matures 30–50 days after the onset of flowering and the color of the warty fruit turns progressively orange from green. When the fruit has fully matured, the pericarp breaks into segments and curls back to expose the seeds.

The antitumor activity of bitter gourd is the most promising. For example, *M. charantia* lectin, a type II ribosome inactivating protein (RIP), demonstrated apoptosis-inducing activity in human nasopharyngeal carcinoma cells in both in vitro and in vivo studies (Fang et al., 2012b). The type I RIP MAP30 from bitter gourd seeds evinced antitumor therapeutic potential in liver cancers in vitro and in vivo by activation of both caspase-8 regulated extrinsic and caspase-9 regulated intrinsic caspase cascades (Fang et al., 2012c). A new bitter gourd ribonuclease (RNase), designated as RNase MC2, manifested antitumor potential in both human liver cancer cells and human breast cancer cells (Fang et al., 2012a,d). RNases are ubiquitously distributed nucleases that cleave RNA into smaller fragments and are good candidates for development into antitumor drugs (Fang and Ng, 2011b).

The efficacy and associated molecular mechanisms of BGJ on tumor cells also have been investigated in a series of studies. Ray and coworkers showed that BGJ inhibited proliferation of breast cancer cells by attenuation of cell cycle progress and exacerbating cell apoptosis (Ray et al., 2010). Kaur and colleagues observed inhibition of human pancreatic carcinoma cells by BGJ, which was probably associated with the activation of cellular energy sensor adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) (Kaur et al., 2013). Activation of AMPK, a master regulator of energy metabolism, modulates lipid metabolism, mitochondrial biogenesis, mimicking exercise effects, and may finally lead to insulin sensitivity and a reduction in blood glucose level (Zhang et al., 2009). Effective compounds in BGJ comprise (but are not limited to) momordicine I, momordicine II, kuguaglycoside G, and cucurbitacin I (Kaur et al., 2013). Because bitter gourd oils constitute part of the juice, the antitumor efficacy of BGJ may at least be partially attributed to bitter gourd oils.

USAGE AND APPLICATIONS OF BITTER GOURD OILS IN FOOD SCIENCE

Composition

Most plant parts of bitter gourd have oils, and the preferred part is the seed. Different extraction methods have been established, such as using acetone hexane solvent or acetone benzene solvent (Khanna, 2005). US patent no. US6964786

TABLE 1 Composition of Bitter Gourd Seed Oils

Classification	Contents and Percentage	Functions	References
Fatty acids[a]	Butyric acid (C4-0, ND[a]) Caprioc acid (C6-0, ND) Caprylic acid (C8-0, ND) Capric acid (C10-0, 0.5%[b]) Lauric acid (C12-0, 0.5%) Myristic acid (C14-0, ND) Myristoliec acid (C14-1, ND) Palmitic acid (C16-0, 4.7%) Palmitoleic acid (C16-1, ND) Stearic acid (C18-0, 62.3%)[c] Oleic acid (C18-1, 12.5%) Linoleic acid (C18-2, 10.4%) Arachidic acid (C20-0, 5.3%) Others (2.8%)	Antidiabetic	Ahmad et al. (2012)
	α-Eleostearic acid (ESA) Punicic acid[d]	Antioxidant	Grossmann et al. (2009) and Saha and Ghosh (2012)
		Anti-inflammatory	
		Hypocholesterolemic	
		Antitumor (ESA)	
Essential oil[e]	*Trans*-nerolidol (61.6%) Apiole (8.9%) Cis-dihydrocarveol (5.0%)	Antimicrobial: *E. coli*; *C. albicans*; *S. aureus*	Braca et al. (2008)
Others	Vegetable oils (15–25%) Fragrance oil (3%) Perfume components (2%)		Khanna (2005)

[a]*ND: not detectable.*
[b]*% denotes percentage by weight of its own group, not compared to total bitter gourd seed oils.*
[c]*Constituents of fatty acids such as linoleic acid (C18-2, 10.4%) with (C18-2, 10.4%) denoting that linoleic acid consists of 18 carbon atoms, and that it is a polyunsaturated fatty acid having two double bonds, and contributes to 10.4% of total fatty acid in bitter gourd seed oil.*
[d]*They are isomers of conjugated linolenic acid (C18-2).*
[e]*Detailed information of essential oils is shown in Table 2.*

discloses that the purified oil is water immiscible, possesses a thick reddish orange color and a bitter taste, and that it is recommended to be used with diluents such as common food-grade oils.

The chemical composition of bitter gourd seed oil was unraveled by gas chromatography/mass spectrometry (GC/MS) and other ways. It should be noted that the chemical composition of bitter gourd seed oil changes with different cultivars and the method of preparation. Bitter gourd oil is composed of predominantly fatty acids, vegetable oils, fragrance oil, essential oil, and perfume components. Analysis of a commercial bitter gourd seed oil showed that it has a number of fatty acids, predominantly long-chain fatty acids of the type C18-0 (62.3%), then C18-1 (12.5%) and C18-2 (10.4%), but only very low levels of short-chain fatty acids are present (Ahmad et al., 2012). The results are comparable to the previously mentioned US patent data because the same purification method was employed in both studies (Khanna, 2005). See Table 1 for details.

Some other authors have reported that the dominant component is 9*cis*, 11*trans*, 13*trans*-conjugated linolenic acid (punicic acid, 60.2%), which has potent antitumor potential (Kohno et al., 2004). Bitter gourd seeds also contain trace amounts of essential oil. For instance, hydrodistillation of dried bitter gourd seeds (a voucher specimen no. 8591/1) using the Clevenger-type apparatus yielded 0.012% essential oil (Braca et al., 2008). The essential oil mainly consists of *trans*-nerolidol (61.6%), apiole (8.9%), and *cis*-dihydrocarveol (5.0%), and exhibits antimicrobial activity against some common microorganisms, including *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus* (Table 2) (Braca et al., 2008).

A Candidate in Solid Fat Application

It is generally believed that *trans* fatty acid (TFA) intake has deleterious effects on health because of its adverse effects on lipids and lipoproteins, and that TFA consumption should be minimized. Soybean oils and sunflower oils are nutritious

TABLE 2 Chemical Composition of Essential Oil in a Voucher Specimen (No. 8591/1) of Bitter Gourd[a]

Constituents	%
Trans-nerolidol	61.6
Apiole	8.9
cis-Dihydrocarveol	4.9
Germacrene D	4.4
Spathulenol	1.7
β-Selinene	1.5
α-Selinene	1.3
Carvone	1.2
Safrole	0.9
1,8-Cineole	0.6
β-Bisabolol	0.5
(E)-Anethole	0.5
Myristecin	0.4
δ-Cadinene	0.4
A-Pinene	0.4
Methyl eugenol	0.3
β-Phellandrene	0.3
Cedrol	0.3
β-Pinene; Octanal; *p*-Cymene; Limonene; Linalool	All <0.1

[a]*Data were from Braca et al. (2008) with minor modifications.*

and possess a high content of poly- and mono-unsaturated fatty acids; thus, they have been suggested as replacements for TFAs for use as ingredients and in frying, but they cannot meet the requirements of usages that specifically need solid fats (Hunter et al., 2010).

High stearic acid oil is a promising candidate. It should be pointed out that stearic acid is not hypercholesterolemic in humans because it is a *trans*-free, oxidatively stable, non-low-density lipoprotein (LDL)-cholesterol-raising oil (Hunter et al., 2010). This property may be partially associated with its rapid conversion into oleic acid (C18-1). An early clinical trial on 11 men (59–72 years of age) indicated that stearic acid suppressed plasma levels of total and LDL cholesterol (Bonanome and Grundy, 1988). A systematic review indicated that high-stearic acid soybean oil is a good candidate for a TFA substitute based on a diminution of LDL-cholesterol level and the ratio of total cholesterol to high-density lipoprotein (Hunter et al., 2010).

Stearic acid (C18-0) is the predominant fatty acid in bitter gourd seed oils. In view of the finding that stearic acid (C18-0, 62.3%) and oleic acid (C18-1, 12.5%) are the top two ingredients in the bitter gourd seed oil—both of which are completely absorbed and have anticholesterol activity—bitter gourd seed oil seems to be a good candidate for replacing TFAs after further detailed toxicological and nutrition studies. In particular, the effects of bitter gourd seed oil on emerging cardiovascular disease risk markers and its differential responses in different populations are scare and need further investigation.

CONCLUSIONS AND FUTURE PERSPECTIVES

On the basis of long-term biochemical, epidemiological, and clinical studies, we reason that bitter gourd seed oil is beneficial to human health due to its potential antidiabetic, antitumor, and other activities. We therefore suggest that the clinical community initiates clinical trials with bitter gourd oil to carefully address safety and health issues.

DISCLAIMER

This article was written in a personal capacity (E.F.F.) and does not represent the opinions of the US Food and Drug Administration, the US Department of Health and Human Services, or the US Federal Government.

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Chapter 29

Bitter Orange (*Citrus aurantium* L.) Oils

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List of Abbreviations

ATCC American type culture collection
***Ca*EO** *Citrus aurantium* essential oil
DPPH 2,2-diphenylpicrylhydrazyl
EO Essential oil
TBARS Thiobarbituric acid reactive species

INTRODUCTION

Herbs, either in the form of crude or purified products, have been used since ancient times for their inherent pharmacological properties in the secondary metabolites they possess. Essential oils (EOs) produced by plants are biologically active natural products having antimicrobial, allopathic, antioxidant, and bioregulatory properties.

Food-borne diseases are a growing public health problem worldwide. It is estimated that each year in the United States, 31 species of pathogens cause 9.4 million cases of food-borne illnesses (Scallan et al., 2011). Successful control of food-borne pathogens requires the use of multiple preservation techniques in the manufacturing and storage of food products. A consumer trend toward preference for products with lower salt and sugar content presents an increased need for efficient food preservatives, as lowering the salt and sugar content would otherwise compromise the product's shelf life (Zink, 1997). A wide range of preservatives are used to extend the shelf life of a product by inhibiting microbial growth. However, an increasingly negative consumer perception of synthetic food additives has spurred an interest in finding natural alternatives to the traditional solutions (Zink, 1997). Although originally added to change or improve taste, the antimicrobial activity of EOs makes them an attractive choice for substituting synthetic preservatives.

The genus *Citrus* includes many important fruits such as oranges, mandarins, limes, lemons, and grapefruits, which are sources of different EOs known for their antimicrobial and antioxidant activities and thus employed in various fields including food industry. This chapter is focused on bitter orange oils obtained from fruits, leaves, and flowers of *Citrus aurantium* spp. *amara*, which, due to their bioactivities, may be successfully employed in different steps of food production.

The major producing countries of bitter orange oils are Spain, Italy, France, Egypt, Tunisia, Morocco, Uganda, Ivory Coast, Paraguay, Brazil, Argentina, and China. The composition of these EOs varies depending on the geographical origin as well as fruit ripening, being richest when fruit is fully matured (Bourgou et al., 2012).

BOTANICAL ASPECTS

All citrus trees belong to the genus *Citrus*, family Rutaceae. Bitter ("sour") orange refers to *C. aurantium* L. and is hybrid between *Citrus maxima* (Pomelo) and *Citrus reticulata* (Mandarin) (USDA, 2013). This plant is native to south eastern Asia. Natives of the South Sea Islands like Guam, Samoa, and Fiji believe this tree was brought to their acreage in prehistoric times. This plant is now cultivated widely throughout the Mediterranean region, Guinea, West Indies, and Brazil as well as California and Florida.

The bitter orange tree grows in subtropical, near-tropical climates and can easily tolerate adverse conditions. It adopts a wide range of soil conditions and survives in drastic conditions. The height of bitter orange tree ranges from 2 to 9 m and has a more compact pinnacle than that of the sweet orange; it has smooth, brown bark, green twigs, and is angular and flexible. The plant produces leaves throughout the year. The flowers are highly fragrant, singly or in small clusters in the

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leaf axils and are about 3.7 cm wide. The fruit is round, oblate or oblong-oval, 7–8 cm wide, rough-surfaced, with a thick, aromatic bitter peel which turns to bright reddish-orange on maturation. The peel of fruits has a large number of small, sunken oil glands. The center of the fruit becomes hollow when the fruit is fully grown.

Various varieties of bitter orange are found in different parts of the world and are used for extraction of EOs. Bitter orange oils are obtained from different parts (peels, leaves, and flowers) of *C. aurantium* spp. *amara* Engler (family Rutaceae). Within the sour ("bitter") oranges the following groups exist: "standard" or "Seville" sour orange, grown for production of fruits; "Bouquet de Fleurs" grown for production of flowers from which the "neroli oil" is extracted, and "Granito" or "Abers" much used for extraction of EOs from the leaves. Chinoto and bergamot also belong to the species *C. arantium* L. but represent different varieties (*myrtifolia* Ker-Gawl and *bergamia* Wight & Arn, respectively). The most important oils obtained from the tree of bitter orange are:

- *Cold-pressed EOs*: Prepared from the peel of the fruits. It is a yellowish brown liquid having fresh floral odor. Because cold-pressed extraction method is used to prepare this oil, limonene (up to 90%) is the main component along with β-myrcene and α-pinene.
- *Petitgrain bigarade oil*: Prepared by steam distillation of the leaves and twigs from the pruning of the trees collected at different times throughout the year. It has a pale yellow color with woody odor and it consists mainly of esters linalyl acetate (45%), geranyl acetate (3%), along with linalool (28%), and geraniol, etc.
- *Neroli oil*: Prepared from the steam distillation or hydrodistillation of the flowers. It is a pale yellow with a light bitter–sweet floral odor. Linalool, linalyl acetate, and limonene are major components.

Furthermore, it is possible to extract the bitter orange flower absolute from both bitter orange flower water absolute, obtained by extraction with hexane from the water solution (i.e., hydrolate or floral water) after separation of neroli, and the concrete of bitter orange flowers, obtained by solvent extraction (usually hexane or light petroleum), using alcohol, winterization, filtration, and concentration. The countries producing EOs from the bitter orange tree are Spain, Italy, France, Egypt, Tunisia, Morocco, Uganda, Ivory Coast, Paraguay, Brazil, Argentina, and China.

Different advanced techniques in the field of chromatography have been applied to the analysis of EOs in gas chromatography (GC) as enantio GC–mass spectrometry (MS), fast GC–MS, and fast GC–flame ionization detector, multidimensional GC–GC, GC×GC, and GC–combustion isotope ratio mass spectrometry, while in liquid chromatography (LC), different approaches were reported using LC–MS and LC×LC. The compositions of the volatile fraction and of the oxygen heterocyclic fraction of bitter orange oil, petitgrain, and neroli have been exhaustively revised in Dugo and Di Giacomo (2002) and Dugo and Mondello (2011).

The volatile fraction of cold-pressed and distilled bitter orange oils contain monoterpenic and (at trace levels) sesquiterpenic and aliphatic hydrocarbons, monoterpenic and aliphatic aldehydes, monoterpenic and sesquiterpenic ketones, monoterpenic, sesquiterpenic and aliphatic alcohols, monoterpenic and aliphatic esters, oxides, and ethers. The most abundant component is the monoterpene limonene that represents 65–97% of the oil, depending on several factors, mainly the extraction method, harvesting time of the plant material, and the geographic origin of the oil. Cold-pressed citrus EO consists of a volatile fraction representing 85–99% of the oil and a nonvolatile fraction which represents 1–15%. This nonvolatile fraction contains an oxygen heterocyclic fraction characterized by the presence of coumarins, psoralens, and polymethoxflavones (including meranzin hydrate, meranzin, isomeranzin, bergapten, nobiletin, heptamethoxyflavone, epoxybergamottin hydrate, tangeretin, osthol, and epoxybergamottin).

The main compounds found in the volatile fraction of neroli and petitgrain bigarade oils are monoterpenic, sesquiterpenic and aliphatic hydrocarbons, monoterpenic and sesquiterpenic aldehydes, monoterpene and aliphatic ketones, monoterpenic, sesquiterpenic and aliphatic alcohols and esters, oxides, and ethers. Unlike cold-pressed and distilled bitter orange oils, hydrocarbons represent only the 8–25% of the of neroli and petitgrain bigarade oils, the most abundant components being slinalool (25–55%), limonene (2–12%), α-terpineol (3–7%), and linalyl acetate (3–55%), while alcohols and esters represent 30–70% and 7–60%, respectively. The variability among the different oil samples is explained by the same factors taken into consideration for the cold-pressed and distilled oils.

USAGES AND APPLICATIONS

In addition to being used by the beverage, food, and perfume industries (Dugo et al., 2011), oils and extracts from the bitter orange tree are of commercial interest for their pharmacological properties. They are used traditionally for the treatment of disorders related to digestive system such as constipation, flatulence, slow digestion, gastric problems, etc. It has also strong relaxing properties and is used as a mild sedative and an antidepressant calming oil for states of anxiety; its odor creates a sense of well-being. Finally, it is also used for colds, dull skin, flu, gums and mouth, and chronic bronchitis (Lawless, 1995).

The peel oil has shown potent antimicrobial activity against a variety of microorganisms including various Gram-positive and Gram-negative bacteria (Caccioni et al., 1998) and good antioxidant activity due to presence of flavonoids. The oil is used topically for the treatment of skin fungal infections like ringworm, jock itch, and athlete's foot (Ramadan et al., 1996). However, care should be taken as oil contains high concentration of limonene, and thus it may cause contact dermatitis when concentrated oil is applied on skin.

Dilute solution of bitter orange oils is used to treat pimples, acne, and muscle spasm. Its flower and oil are used by people for general feebleness, anemia, hair loss, cancer, frostbite, and as a tonic and for regulating fat levels in the blood, lowering blood sugar in patients with diabetes, stimulating the heart and circulation, disorders of the liver and gallbladder, and kidney and bladder diseases. However, there is insufficient evidence for these properties.

USAGE AND APPLICATION IN FOOD SCIENCES

Rationale and Limits

The chemico-physical and composite characteristics of food and soft drinks make these products susceptible to microbial spoilage. The growth of specific microbial groups such as acetic and lactic acid bacteria, molds, and yeasts is allowed due to high C:N ratios and low pH. Furthermore, the addition of CO_2 in beverages reduces growth possibilities, mainly favoring yeasts. Thermal treatments, to which ingredients and intermediate and final products can be subjected, affect the stability of these products. Because polyethylene terephthalate packaging cannot be thermally treated due the susceptibility of plastic material to heating, food and beverage stability relies upon the addition of preservatives, generally weak acids such as sorbic and benzoic acids. New strategies for the stabilization of food free from traditional preservatives are constantly being investigated by manufacturers (Rangan and Barceloux, 2009). In fact, consumers are inclined to consider these preservatives as extraneous and unsafe because they have no connection with the food matrix. Furthermore, preservatives could undergo chemical transformations giving origin to toxic compounds (e.g., benzoic acid may be partially transformed in benzene) (Meadows, 2006).

In this scenario, the search for new strategies and new antimicrobials for stabilization of food and beverages has become a central goal for producers. Aromatic compounds and EOs can be an interesting alternative. However, their organoleptic impact and the variable composition of the EOs (which can be reflected in their antimicrobial activity) limit the industrial use of these substances as preservatives (Burt, 2004). Furthermore, a stabilization strategy without the addition of excessive concentrations of the flavoring agent seems to be difficult to be realized.

However, active compounds and toxic effects should also be clearly evaluated before being used within the food industry. In fact, Dusan et al. (2006) reported that high doses of some EOs can have negative effects on intestinal cells and therefore the effect to the whole intestinal tract needs to be better evaluated in order to consider them as safe. Other studies showed that higher concentrations of EOs are needed to be effective when being used on food compared with in vitro studies (Fisher and Phillips, 2006). Furthermore, the use of EOs vapors may be a potential way of fighting the organoleptic effects brought about by direct contact between the food and oil. However, longer exposure to the vapor is required in order to produce a similar inhibitory effect (18 h compared with 60 s), with higher cost implications for the food industry (Fisher and Phillips, 2006).

BITTER OIL AND ANTIBACTERIAL ACTIVITY FOR FOOD PRESERVATION

Citrus EOs and their derivatives are mainly terpene mixtures and an antimicrobial activity has been reported for many of these compounds. In fact, such molecules inhibit cell growth or induce cell death by interacting with some cellular structures. A preliminary condition for these activities is the contact between the antimicrobial molecule and the target cells and this requires the molecules to be in their most hydrophobic state (i.e., in their vapor phase) because this makes their solubilization in the cell membrane possible. As each methodology for antimicrobial activity can measure different aspects of cell damage and the screening conditions can considerably affect the bioactivity of these substances, collected data depend on the used method (Sharma and Tripathi, 2008; Belletti et al., 2004). Therefore, such discrepancies could be attributed to the different assays employed.

Belletti et al. (2004) studied citrus EO antimicrobial activity by evaluating yeast growth kinetics in solid medium in relation to oil concentration using a gas chromatography method. This technique is an indirect index of microbial growth based on the detection of metabolic CO_2 released from the microbial cells in the headspace, containing the oils, of sealed vials. However, the headspace composition does not correspond to the whole oil components, and it gives a measure of the volatile composition of the oil depending on the vapor pressure of the molecules. Although this method has been extensively used to monitor microbial dynamics in food and beverages, as well as in model systems, many authors reported lower

antimicrobial activity for the most effective essences when added to the headspace; furthermore, the higher activity was observed when EOs were added directly in to the medium (Belletti et al., 2004; Sharma and Tripathi, 2008). In order to explain the higher efficacy of citrus oils in a liquid medium, several hypotheses had been proposed: (a) the solubilization of the active molecules in the aqueous phase of the solid medium does not allow their contact with the microbial cells located on the surface of the medium; (b) the molecules released faster into the headspace could not be the most effective; and (c) the surface exposure to the active molecules of cells superficially inoculated on the solid medium is lower as compared to that of cells suspended in a liquid medium. This latter effect may be enhanced by the inoculum procedure which favors the clumping of cells, further reducing the amount of exposed surface.

These backgrounds suggest that a standardization of the methods used to test the antimicrobial efficacy of EOs needs to be established. Then, appropriate screening procedures are needed to relate to the potential future applications (Suhr and Nielsen, 2003).

Hsouna et al. (2013) studied EO of fresh *C. aurantium* L. (*Ca*EO) flowers cultivated in north east Tunisia (Nabeul). The monoterpenes components were limonene and α-terpineol (27.5% and 14%, respectively), along with E-β-ocimene (4.3%) and δ-3-carene (2.4%). The sesquiterpene fraction represented 30.2% of the oil and among these (E)-nerolidol was the most abundant (17.5%). The antimicrobial activity of *Ca*EO was evaluated against a panel of 13 bacteria strains using agar diffusion and broth microdilution methods. They found that *Ca*EO exhibited moderate-to-strong antimicrobial activity against the tested species.

An investigation of the mode of action of *Ca*EO by the time–kill curve showed a drastic bactericidal effect after 5 min using a concentration of 624 μg/mL. As shown in Table 1, *Ca*EO exhibited varying degrees of antibacterial activity against all tested strains. The inhibition zones were in the range 1.4–22 mm (Iturriaga et al., 2012). *Citrus aurantium* essential oil showed an antibacterial activity against Gram-positive and Gram-negative bacteria with minimum inhibitory concentration (MIC) values of 0.312–2.5 mg/mL and 0.625–2.5 mg/mL, respectively (Hsouna et al., 2013). The Gram-positive bacteria were more susceptible than Gram-negative, and these differences could be attributed in part to the great complexity of the double membrane-containing cell envelope in Gram-negative bacteria. The ability of EOs to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for its lethal action. *Ca*EO was highly active against *Staphylococcus aureus* ATCC 6536, *S. aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, and *Listeria monocytogenes* (food isolate) showing an important growth inhibition at lower concentrations. *Ca*EO was also found to inhibit the growth of clinically important Gram-negative bacteria, such as *Klebsiella pneumonia* ATCC 10031, *Escherichia coli* ATCC 25922, and *E. coli* ATCC 8739, as well as food contaminants such as *Salmonella enterica* (food isolate) (Table 1).

The strong antimicrobial activity of *Ca*EO against the tested microorganisms could be attributed to the presence of high concentration of hydrocarbon, monoterpene, and oxygenated monoterpene appreciated for their antibacterial potentials. α-Terpineol, which was in appreciable amounts in this oil (14%), has been reported to inhibit the growth of a number of bacteria and fungi including *E. coli*, *Staphylococcus epidermis*, and *Candida albicans*.

Some studies have concluded that whole EOs have a greater antibacterial activity than the major components mixed, suggesting that the minor components could also be critical to the bioactivity and may have a synergistic effect or a potentiating influence (Burt, 2004). Thus, the synergistic effects of the diversity of major and minor constituents present in the EOs should be taken into consideration to account for their biological activity. From these results, *Ca*EO may be considered as a natural preservative against food-borne pathogens.

Friedman et al. (2004) studied bitter oil and other EOs for antibacterial activity against the food-borne pathogens *E. coli* O157:H7 and *S. enterica* in apple juices. They evaluated several parameters that influence bactericidal activities against *E. coli* O157:H7 and *S. enterica* of a selected group of active test substances in commercial clear Mott's apple juice with ascorbic acid and in commercial cloudy Mott's apple juice without ascorbic acid. The bactericidal assay was in terms of percentage of sample required to decrease 50% of bacteria (BA_{50}). The less percent sample required, the more effective the bactericidal activity. They found that bitter oil was active against *E. coli* (BA_{50} range in clear juice was 0.11% and 0.19% with and without ascorbic acid) and against *S. enterica* (BA_{50} range, 0.037% and 0.019%). Activity was greater for *S. enterica* than for *E. coli*, increased with incubation temperature and storage time, and was not affected by the acidity of the juices.

Dabbah et al. (1970) studied the activity of some citrus EOs, their deterpenated fractions, and of some of their components ((+)-limonene, geraniol, and terpineol) on food-contaminating bacteria. (+)-Limonene, the main component of citrus EO's volatile fraction, proved to have antibacterial, antifungal, and insecticidal activity. Enantiomers have been observed to have a different activity. Lis-Balchin et al. (1996) carried out a study on 25 strains; (+)-limonene was active against 22 strains, whereas (−)-limonene was active against 16 strains. The growth of *S. aureus* was inhibited by all the assayed citrus oils and their derivatives, ranging between a reduction of the initial inoculum of 100% for the terpene-less

TABLE 1 Antibacterial Activity of Bitter Orange (*Citrus aurantium* L.) Oil

Activity against Bacterial Strain		
Bacterial Strains	**MIC (mg/mL)**	**References**
Gram-positive		Hsouna et al. (2013)
Bacillus subtilis ATCC 6633	2.5 ± 0.5	
Bacillus cereus ATCC 14579	0.625 ± 0.3	
Staphylococcus aureus ATCC 25923	0.312 ± 0.2	
Staphylococcus aureus ATCC 6536	0.625 ± 0.3	
Staphylococcus epidermis ATCC 12228	1.25 ± 0.4	
Enterococcus faecalis ATCC 29212	1.25 ± 0.3	
Micrococcus luteus ATCC 1880	2.5 ± 0.6	
Listeria monocytogenes (food isolate 2132)	0.312 ± 0.1	
Gram-negative		
Salmonella enteritidis (food isolate)	0.625 ± 0.8	
Escherichia coli ATCC 25922	1.25 ± 0.7	
Escherichia coli ATCC 8739	1.25 ± 0.5	
Pseudomonas aeruginosa ATCC 9027	2.5 ± 1.0	
Klebsiella pneumoniae ATCC 10031	2.5 ± 0.9	
Bactericidal activities against	**BA_{50}**	Friedman et al. (2004)
In apple juice with ascorbic acid:		
Escherichia coli O157:H7	0.11%	
Salmonella enterica	0.19%	
In apple juice without ascorbic acid:		
Escherichia coli O157:H7	0.037%	
Salmonella enterica	0.019%	
	Growth inhibition zone (mm)	Iturriaga et al. (2012)
Listeria innocua	2.7 ± 1.2	
Pseudomonas fluorescens	1.3 ± 0.6	
Aeromonas hydrophila/caviae	22.7 ± 2.9	

The above values shows minimum inhibitory concentration (MIC), bactericidal activity (BA_{50}), and minimum inhibition zone (mm) of bitter orange (*Citrus aurantium* L.) oil against bacterial strains.

citrus oils and 67% for the lemon EO. Inhibition of *Pseudomonas* spp. (18 strains) ranged between 87% for lemon EO and 100% for terpineol. d-Limonene stored for over four years showed a considerable activity against *Salmonella senftenberg*; this confirms the statement by Zukerman (1951) reporting that oxidized d-limonene is more inhibitory than the freshly distilled product. Orange EO acted on *Salmonella* spp. by inhibiting growth in a range between 84% for *S. enteriditis* and 99% for *Salmonella gallinarum* and *Salmonella norwich*. Terpineol completely inhibited all 24 serotypes of *Salmonella* tested. Bitter orange EO inhibited the growth of *Pseudomonas* and of other Gram-negative bacteria (10–100%), as well as terpineol (94–100%).

Penicillium digitatum and *Penicillium italicum*, the pathogens responsible for the worst damage to fruits of *Citrus* spp. after harvesting, infect the fruit through microinjuries produced in the flavedo during harvesting and processing. These microinjuries can also involve the EO-containing glands, causing the oil to overflow; the infected fruits release a more oil

constituents than the noninfected. Caccioni et al. (1998) evaluated the effects on the growth of these pathogens of the EOs of *C. aurantium, Citrus sinensis, Citrus deliciosa, Citrus paradisii*, and citrange, a hybrid of *C. sinensis* × *Poncirus trifoliata*. Citrange EO was the most active. Bitter orange, grapefruit, tangerine, and lemon oils were less efficacious.

Frassinetti et al. (2011) studied the antibacterial EOs from bitter orange and others. The antimicrobial capability of these oils was determined against 10 strains of Gram-negative and Gram-positive bacteria, including some phytopathogenic strains. All oils showed good antibacterial activity against both Gram-negative and Gram-positive bacteria. The MICs for selected oils ranged from 15 to 250 μg/mL. The lowest MICs were 15 μg/mL and 20 μg/mL against *Xanthomonas citri* strains, respectively.

Iturriaga et al. (2012) tested *C. aurantium* EO for its antimicrobial activity against two fish spoilage bacteria (*Pseudomonas fluorescens* and *Aeromonas hydrophila/caviae*) and *Listeria innocua*, in order to assess their potential utilization in the preservation and safety of minimally processed fish products. The MIC of the selected extracts was determined by disc diffusion method against target bacteria and at two temperatures: bacteria's optimal growth temperature (30 or 37 °C) and refrigeration temperature (4 °C). They found that bitter EO was effective against *P. fluorescens*, *A. hydrophila/caviae*, and *L. innocua* with varying degree inhibition. Then they selected *Citrus* extract and incorporated with different biopolymer film forming solutions (gelatin, methyl cellulose, and their blend 50:50 w/w) and evaluated the developed films for their antimicrobial activity, just after preparation of the films and after one month of storage at 43% ± 3% relative humidity and 24 ± 3 °C. The most sensitive bacterium toward active films was found to be *L. innocua*, while *P. fluorescens* appeared as the most resistant one, in accordance with the previously performed antimicrobial tests for pure extracts. The inhibitory effectiveness of the films against the tested strains was maintained regardless of the biopolymer matrix for at least one month. Therefore, these edible films show potential for their future use in fresh fish fillets preservation.

Antimicrobial Action Mechanisms of Citrus Essential Oils

The mechanisms by which EOs bring about their antibacterial effect are incompletely understood, but there are a number of proposed mechanisms. Some studies have found Gram-positive bacteria to be more sensitive to EOs than Gram-negative bacteria, which, it has been suggested, may be due to the relatively impermeable outer membrane that surrounds Gram-negative bacteria (Smith-Palmer et al., 2001). Other studies suggest that over a longer time period the EOs would have the same effect on both bacteria (Tassou et al., 2000). However, this differential sensitivity has been observed when using citrus oils/components in vitro, but not in food studies (Fisher and Phillips, 2006). Little research has been carried out on the mechanism of action of EOs including citrus oil or vapors.

As *Citrus* fruits are characterized by a high percentage of neral and geranial acetic esters, many authors have postulated a significant relationship between their presence in the peel of fruits and inhibition of *P. digitatum*, *P. italicum*, and *Geotrichum candidum*, the major fungi responsible for postharvest spoilage of citrus (Wuryatmo et al., 2003). Belletti et al. (2004) reported that citral (the mixture of the isomers neral and geranial) was in a remarkable concentration (more than 7%) in citron oil, whose antimicrobial activity against *Saccharomyces cerevisiae* was the highest among their tested mixtures. The inhibitory effects of citral against microorganisms are due to the presence of a carbonyl group adjacent to the α- and β-carbons in the α and β-unsaturated aldehydes neral and geranial. This makes the β-carbon positively polarized and the aldehyde can act as a direct alkylating agent able to bind cellular nucleophilic groups.

β-Pinene is found in high amounts (more than 20%) in the headspace of lemon and citron oils and also in sweet lime. The negative effect of α-pinene on the yeast membrane function has been already studied. This monoterpene hydrocarbon was found to be particularly effective against *E. coli* O157:H7 (Takikawa et al., 2002).

Fisher and Phillips (2008) studied the mechanism of action of a citrus oil blend against *Enterococcus faecium* and *Enterococcus faecalis*. The results suggest that the antimicrobial action of a bergamot:orange (1:1 v/v) EO on *Enterococcus* spp. may be affected by the cell uptake of the oil. Vacuoles are observed within the cells, which were not present in the control cells. The lipophilic characteristic of the EOs will allow the preferential partitioning from the aqueous phase into the membrane structure of the cell and thus could result in the oil uptake into the cell to form vacuoles and thus having an antimicrobial activity from the inside. Furthermore, after exposure of *En. faecalis* and *En. faecium* to the citrus blend, increased cell membrane permeability was observed for the EO or vapor with an increase of two and 32–40 times, respectively. This might suggest a different mechanism for the vapor or a different (smaller) molecular size so that the vapor may be more effective at penetrating the membrane and thus causing damage to the cell. Although an increased permeability of the cell membrane is observed, the loss of adenosine triphosphate (ATP) that occurs within *Enterococcus* cells does not leak into the surrounding fluid (Fisher and Phillips, 2008). This finding suggests that the loss of intracellular ATP may be due to the lack of synthesis or increased hydrolysis of ATP. The possible uptake of the oil/vapor into the cell may be interfering with normal cellular function rather than increased permeability of the cell membrane to ATP.

BITTER OIL AND ANTIFUNGAL ACTIVITY FOR FOOD PRESERVATION

Hsouna et al. (2013) investigated also the antifungal activity of *Ca*EO by measuring the growth inhibition zone (GIZ) and minimum fungicidal concentrations (MFC) against various fungal species. They reported the capacity of this EO to control *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, and *Aspergillus fumigants* (GIZ = 15–22 mm; MFC = 0.078–1.25 mg/mL), *Fusarium graminearum*, *Fusarium oxysporum*, and *Fusarium culmorum* (GIZ = 19–22 mm; MFC = 0.078–0.156 mg/mL), and *Alternaria alternata* (GIZ = 15 ± 0.4 mm; MFC = 1.25 ± 0.8 mg/mL), all considered a challenge for food industries as they are responsible for spoilage of many foods. The antifungal activities of the EO have been attributed to limonene and (E)-nerolidol in particular, as literature reports strong antifungal activity of these molecules (Kim et al., 1995).

Caccioni et al. (1998) examined the effect of volatile components of *Citrus* fruit EOs on *P. digitatum* and *P. italicum* growth. Antifungal activity was studied by determining the dry weight of the pathogen mycelium after incubation in a liquid medium added to the oils, and the median effective doses (ED_{50}, ppm) were determined by log probit graphs. *C. aurantium* oil ED_{50} values were 1015.4 ppm for *P. digitatum* and 1490.6 ppm for *P. italicum* (Table 2).

BITTER OIL AND ANTIOXIDANT ACTIVITY FOR FOOD PRESERVATION

EOs and some of their components are highly lipophilic and have been shown to possess antioxidant properties. However, little is known about the antioxidant properties of EOs from edible plants and in particular of citrus EOs.

Hsouna et al. (2013), as above described, analyzed an EO of fresh *C. aurantium* L. flowers from Tunisia, and named *Ca*EO, also for its antioxidant activity. The 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay and β-carotene bleaching inhibition activity were used to test the antioxidant activity of this EO. The DPPH test showed a strong radical scavenging activity of *Ca*EO (median inhibitory concentration (IC_{50}) = 1.85 μg/mL) compared with the standard ascorbic acid. A good inhibitory effect on lipid peroxidation was also reported (IC_{50} = 15.3 μg/mL), suggesting that the addition of *Ca*EO could be useful to improve stability of food products.

Sarrou et al. (2013) studied the volatile constituents of the EOs of the peel, flowers, and leaves of *C. aurantium* L. growing in Greece. All the EOs were able to effective scavenge DPPH radicals. The oil of the old leaves showed the highest scavenging activity (94.36%) followed by flowers (53.98%), young leaves (22.79%), and peel oil (19.29%).

Aazza et al. (2011) studied the antioxidant activity of EO of *C. aurantium* L., along with other EOs isolated by steam distillation by a company in Morocco. Linalool (59%) and linalyl acetate (23%) constituted the major part of this EO. A good antioxidant activity was observed when measured as lipid peroxidation inhibition by evaluating the production of thiobarbituric acid reactive species (TBARS) (IC_{50} = 0.652 ± 0.125 mg/mL), and as scavenging of the stable radical DPPH (IC_{50} = 4.786 ± 0.476 mg/mL).

Jabri Karoui and Marzouk (2013) characterized bioactive compounds in peel and juice of *C. aurantium* L. from Tunisia and determined their antioxidant activity. Antioxidant activity was measured by: (a) the reduction of Mo(VI) to Mo(V)

TABLE 2 Antifungal Activity of Bitter Orange (*Citrus aurantium* L.) Oil

Antifungal Activity	References
Aspergillus niger: IZ = 17 ± 0.4 mm; MFC = 1.25 ± 0.3 mg/mL	Hsouna et al. (2013)
Aspergillus flavus: IZ = 22 ± 1.1 mm; MFC = 0.078 ± 0.2 mg/mL	
Aspergillus nidulans: IZ = 15 ± 0.3 mm; MFC = 0.625 ± 0.2 mg/mL	
Aspergillus fumigants: IZ = 18 ± 0.6 mm; MFC = 0.321 ± 0.2 mg/mL	
Fusarium graminearum: IZ = 22 ± 0.6 mm; MFC = 0.078 ± 0.4 mg/mL	
Fusarium oxysporum: IZ = 20 ± 0.9 mm; MFC = 0.156 ± 0.3 mg/mL	
Fusarium culmorum: IZ = 19 ± 0.4 mm; MFC = 0.156 ± 0.1 mg/mL	
Alternaria alternata: IZ = 15 ± 0.4 mm; MF = 1.25 ± 0.8 mg/mL	
Penicillium digitatum: ED_{50} = 1015.4 ppm	Caccioni et al. (1998)
Penicillium italicum: ED_{50} = 1490.6 ppm.	

The above values show minimum fungicidal concentration and ED_{50} of bitter orange (*Citrus aurantium* L.) oil against fungal strains.

TABLE 3 Antioxidant Activity of Bitter Orange (*Citrus aurantium* L.) Oil

Source	Antioxidant Activity	References
EO of fresh *Citrus aurantium* L. flowers from Tunisia	• DPPH assay: IC_{50} = 1.85 µg/mL • β-carotene bleaching test: IC_{50} = 15.3 µg/mL	Hsouna et al. (2013)
EO of peel, flowers, and leaves of *Citrus aurantium* L. growing in Greece	• DPPH assay (% inhibition) ◦ peel oil: 19.29% ◦ flower oil: 53.98% ◦ young leaves oil: 22.79% ◦ old leaves oil: 94.36%	Sarrou et al. (2013)
EO of *Citrus aurantium* L. isolated by steam distillation by a company in Morocco	• TBARS assay: IC_{50} = 0.652 ± 0.125 mg/mL • DPPH assay: IC_{50} = 4.786 ± 0.476 mg/mL	Aazza et al. (2011)
Peel and juice of *Citrus aurantium* L. from Tunisia	• Total antioxidant capacity (TAC) assay: ◦ peel extract = 5.23 ± 0.05 mg GAE/g of FW ◦ juice extract = 823.13 ± 17.18 mg GAE/L • DPPH assay: ◦ peel extract IC_{50} = 190 ± 0.01 µg/mL ◦ juice extract 97.05 ± 0.38 %I • β-carotene bleaching test: ◦ peel extract IC_{50} = 5.81 ± 0.03 ◦ juice extract %I = 15.92 ± 0.66	Jabri Karoui and Marzouk (2013)
Fruit juice of *Citrus aurantium* L. from north east Tunisia	• DPPH assay: %I = 96.1 • β-carotene bleaching test: %I = 18.27%	Tounsi et al. (2011)

The above values show antioxidant activity concentration of bitter orange (*Citrus aurantium* L.) oil.

and subsequent formation of a green phosphate/Mo(V) complex at acid pH to evaluate the total antioxidant activity, and expressed as mg gallic acid equivalents per gram of fresh weight in the case of peel extracts (5.23 ± 0.05 mg GAE/g of FW) and per liter in the case of the juice extract (823.13 ± 17.18 mg GAE/L); (b) DPPH assay: peel IC_{50} = 190 ± 0.01 µg/mL, and juice 97.05 ± 0.38% inhibition; (c) β-carotene bleaching test to estimate sample methanolic extract capacity to inhibit the β-carotene bleaching, and expressed as IC_{50} values for the peel extract (5.81 ± 0.03 mg/mL) and as % Inhibition for the juice extract (15.92% ± 0.66).

Tounsi et al. (2011) carried out a study on juices extracted by fruit press using ripe fruits of *C. aurantium*, along with other three varieties of citrus, all harvested in the north east of Tunisia. *C. aurantium* juice contained a total of phenolic acids of 86.4 ± 0.8 mg GAE/mL. Aromas were extracted from juices with a mixture of ether–pentane (1:1, v/v), and limonene (48.85%) was the most abundant component in bitter orange aroma followed by carvacrol (30.05%). Antioxidant capacity of juice was evaluated by: (a) DPPH assay: %I = 96.1; and (b) β-carotene bleaching method: %I = 18.27%.

Limonene is used as flavoring agent in a wide range of food. Because it is lipid soluble, limonene is often added to foods as oil-in-water emulsions, which are susceptible to both physical instability and oxidative degradation, leading to loss of aroma and formation of off-flavors. Whey protein isolate (WPI) could inhibit the oxidative deterioration of limonene in oil-in-water emulsions, through the formation of a cationic emulsion droplet interface and/or the ability of amino acids in WPI to scavenge free radical and chelate prooxidative metals. A sodium dodecyl sulfate–chitosan complex has appeared able to inhibit the oxidative deterioration of limonene due to the formation of a cationic and thick emulsion droplet interface that could repel prooxidative metals (Djordjevic et al., 2008). Taken together, these results show that *C. aurantium* EO antioxidant activity is high enough to be used as a potential source of natural antioxidants for the food industry (Table 3).

BITTER OIL AND FOOD SPOILAGE

Coksever and Saricoban (2010) studied the effect of adding bitter orange albedo (BOA) at 0, 1, 2.5, and 5% levels on the quality characteristics of naturally fermented Turkish sausage, called sucuk. Fermentation and ripening processes were followed during the 21 days; pH, value, and moisture content decreased and weight loss increased. BOA addition decreased weight loss and penetrometer values, indicating its positive effects. The TBARS values of all samples were lower than 1 mg of malonaldehyde/kg. The lowest TBARS value occurred in the sucuk with added BOA thus showing an

evident antioxidant effect, while the control sample had the highest TBARS value. The authors suggest that use of low concentrations (<5%) of BOA might be also a potential dietary fiber source to enhance the quality and characteristics of sucuk samples.

Giatropoulos et al. (2012) studied the bioefficacy against *Ae. albopictus* of three *Citrus* EOs and of their components. The tested citrus EOs were toxic against mosquito larvae, and of the *Citrus* EOs components tested, γ-terpinene was the most toxic (median lethal concentration (LC_{50}) = 20.21 mg/L followed by both enantiomeric forms of limonene (LC_{50} = 35.99 and 34.89 mg/L, for R-(+)-limonene and S-(−)-limonene, respectively).

Tripathi et al. (2003) investigated d-limonene fumigant toxicity, ovicidal effects, oviposition-deterrent, development inhibition, and feeding-deterrent activities against three stored-product beetles (Coleoptera): lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); and red flour beetle, *Tribolium castaneum* (Herbst). They found that contact and fumigant toxicity decreased as larvae aged. Contact toxicity was similar for adults of the three species tested, but *R. dominica* was most susceptible to fumigant activity. *T. castaneum* oviposition decreased as concentration of d-limonene increased and d-limonene reduced oviposition up to 92.3% at the concentration of 2.14 mg/cm^2. Hatching of d-limonene-treated eggs of *T. castaneum* was reduced by 94.5% with no subsequent larval and adult survival at 2.14 mg/cm^2 concentration. A flour disc bioassay indicated 87.7–96.8% feeding-deterrency by d-limonene toward all three insect species tested at the highest concentration of 60 mg/g food. Their findings suggest that d-limonene can be effectively used to suppress populations of stored-product beetles.

SUMMARY POINTS

- The most important oils obtained from the tree of bitter orange are cold pressed oil, petitgrain bigarade oil, and neroli oil.
- The most abundant component of bitter oil is the monoterpene limonene that represents 65–97% of the oil.
- Bitter oil is traditionally used for the treatment of disorders related to digestive system such as constipation, flatulence, slow digestion, gastric problems, etc.
- It has also strong relaxing properties and is used as mild sedative, antidepressant calming oil in state of anxiety, and its odor creates a sense of well-being.
- Bitter oil is also used in colds, dull skin, flu, gums and mouth, and chronic bronchitis. It has potent antimicrobial activity against a variety of microorganisms including various Gram-positive and Gram-negative bacteria.
- Bitter oil is also demonstrated as an antifungal, antioxidant, and flavoring agent.
- Bitter oil or its component limonene, along with other EOs, can produce synergistic antispoilage effects.
- Bitter oil or its components can be used for food and food products preservations.

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Chapter 30

Black Cumin (*Nigella sativa*) Oils

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List of Abbreviations

BHA Butylated hydroxyanisole
BHT Butylated hydroxytoluene
DPPH 2,2-diphenyl-1-picrylhydrazyl
GC Gas chromatographic
GC-MS Gas chromatography–mass spectrometric
RP-HPLC Reverse phase-high performance liquid chromatography

INTRODUCTION

Medicinal and aromatic plants have been utilized as spices and condiments to confer aroma and flavor to food and beverages. Due to their bioactive constituents, these plants can act as stabilizers, increasing the shelf-life of foods and beverages (Salgueiro et al., 2010). Scientific research has focused its interest on essential oils from medicinal plant as natural sources of antimicrobial and antioxidant compounds. Ancient Egyptians, Greeks, and Romans were aware of the therapeutic characteristics of *Nigella sativa* seeds and its essential oil. The seeds have a pungent bitter taste and smell and are used primarily in confectionery and liquors. *Nigella sativa* is also used in Armenian string cheese and in a braided string cheese called Majdouleh or Majdouli in the Middle East. Seeds can be ground and used with near abandon like black pepper.

Nigella sativa seeds contain protein (26%), carbohydrates (25%), crude fiber (8.4%), and ash (4.8%). Seeds contain good levels of carotene and minerals such as Cu, P, Zn, and Fe (Ahmad et al., 2013) and also contain 36–38% fixed oil, alkaloids, and saponins (Lautenbacher, 1997; Burits and Bucar, 2000; Singh et al., 2005). *Nigella sativa* seeds contain 0.4–2.5% essential oil and is used in folk medicine as a bread or cheese flavoring and as a spice in various kinds of meals (Wajs et al., 2008). To extract the volatile oil, crushed seeds are extracted with organic solvents. The solvent is removed and the brownish residue is steam distilled. Recovery of *N. sativa* essential oil by Soxhlet extraction and hydrodistillation gave a yield of 0.41–0.44% (Burits and Bucar, 2000). Through distillation with Clevinger apparatus, 0.18% of essential oil could be obtained (Singh et al., 2005).

BOTANICAL ASPECTS

Nigella sativa Linn. is an annual herbaceous flowering plant belongs to the family Ranunculaceae. *Nigella sativa* is native to south and south west Asia where the plant is cultivated and grows. *Nigella sativa* is widely cultivated in Mediterranean countries, middle Europe, and western Asia. It grows to 20–30 cm tall with finely divided, linear leaves (Figure 1). Flowers are usually pale blue and white in color with 5–10 petals. In its natural form, the flowers are bluish with a variable number of sepals and are characterized by the presence of nectaries. The gynoecium is composed of a variable number of multiovule carpels, developing into a follicle after pollination, with single fruits partially connected to form a capsule-like structure. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing seeds.

The seeds of *N. sativa* are small in size (1–5 mm), with corrugated integuments (Figure 2). Seeds are commonly known as black seed, black cumin (English), and habbatu sawda or habbatu el baraka (Arabic) (Benkaci–Ali et al., 2007).

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FIGURE 1 *Nigella sativa* plant has a stiff, erect, branching stem, bears deeply cut greyish-green (gray in print versions) leaves, and terminal greyish-blue flowers (white in print versions) followed by odd, toothed seed vessels, and filled with small compressed seeds.

FIGURE 2 *Nigella sativa* seeds are three-cornered, with two sides flat and one convex, black or brown externally, and white and oleaginous within. Seeds have a strong, agreeable aromatic odor, like that of nutmeg, and a spicy, pungent taste.

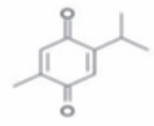
USAGE AND APPLICATIONS

Essential oils are utilized in food (as flavorings), perfumes, and pharmaceuticals (Burt, 2004). *Nigella sativa* had a rich historical and religious background. Seeds or their oils were claimed for medicinal applications dating back to the ancient Egyptians, Greeks, and Romans (Benkaci-Ali et al., 2007). In Islamic literature, *N. sativa* is considered as one of the greatest forms of healing medicine. It has been recommended for use on regular basis in *Al-Tibb-Al-Nabwi* (prophetic medicine). *Nigella sativa* seeds have been utilized for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, influenza, eczema, as a diuretic, lactagogue, and vermifuge (Lautenbacher, 1997; Eschborn, 1997; Burits and Bucar, 2000; Ramadan, 2007). In general, *N. sativa* seeds and its oil have a very low degree of toxicity (Ali and Blunden, 2003).

Gas chromatography and gas chromatography–mass spectrometry analysis of volatile oil resulted in the identification of bioactive compounds representing around 85% of the total amount. The main identified compounds were *p*-cymene (36.2%), thymoquinone (11.2%), and α-thujene (10.0%) as shown in Table 1 and Figure 3 (D'Antuono et al., 2002; Harzallah et al., 2011).

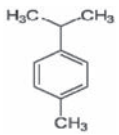
TABLE 1 Main Identified Compounds in *Nigella sativa* Essential Oil

Compound	Area (%)
α-Thujene	10.03
p-Cymene	36.20
Limonene	1.76
Terpinen-4-ol	2.37
Thymoquinone	11.27
Carvacrol	2.12
Longifolene	6.32



Thymoquinone

2-Isopropyl-5-methylbenzo-1,4-quinone

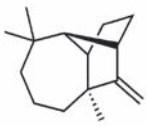


***p*-cymene**

1-Methyl-4-(1-methylethyl)benzene

α-thujene

1-isopropyl-4-methylbicyclo[3.1.0]hex-3-ene



Longifolene

(1*R*,2*S*,7*S*,9*S*)- 3,3,7-trimethyl-8-methylenetricyclo-[5.4.0.0^{2,9}]undecane

FIGURE 3 Structure of the identified compounds in *Nigella sativa* essential oil.

A lower level of *p*-cymene (14%) was detected by Nickavar et al. (2003) in the oil of Iranian *N. sativa*. Thymoquinone, dithymoquinone, thymohydroquinone, and thymol were the major phenolic compounds (Venkatachallam et al., 2010). Traces of the esters of saturated and unsaturated fatty acids were also found in the essential oil (Burits and Bucar, 2000). Two monoterpenoids: *cis*- and *trans*-4-methoxythujane were identified in the essential oil (Wajs et al., 2008). Four terpenoids: (1) *trans*-sabinene hydrate methyl ether, (2) *cis*-sabinene hydrate methyl ether, (3) 1,2-epoxy-menth-4-ene, and (4) 1,2-epoxy-menth-4(8)-ene were identified in *N. sativa* essential oil by nuclear magnetic resonance (Bourgou et al., 2012).

Nigella sativa seeds also contain proteins, alkaloids (nigellicines and nigelledine), and saponins (α-hederin) in substantial levels. Thymoquinone has antibacterial activity which could be potentiated by antibiotics especially in case of *Staphylococcus aureus* (Halawani, 2009; Harzallah et al., 2011; Ahmad et al., 2013). The oil and thymoquinone showed beneficial immunomodulatory characteristics, augmenting the T cell and natural killer cell-mediated immune responses (Venkatachallam et al., 2010). Terpenoids isolated from Tunisian *N. sativa* essential oil exhibited in vitro antioxidant potential and inhibited nitric oxide release by lipopolysaccharide-activated RAW 264.7 macrophages (Bourgou et al., 2012).

Nigella sativa essential oil exhibited strong ex vivo antioxidant activity, inhibiting dichlorofluorescin (DCFH) oxidation with a median inhibitory concentration (IC_{50}) of 1.0 μg/mL, high antiinflammatory activity, and inhibiting nitric oxide radical excretion. The oil was found to inhibit the growth of A-549 and DLD-1 cancer cell lines and to exert antibacterial activity against *S. aureus* and *Escherichia coli* with IC_{50} values of 12.0 and 62.0 μg/mL (Bourgou et al., 2010). Rats fed an aflatoxin-contaminated diet and treated with *N. sativa* oil were protected against aflatoxicosis (Abdel-Wahhab and Aly, 2005). Evidence is available supporting the utilization of *N. sativa* and its bioactive components in a daily diet to improve health (Butta and Sultana, 2010).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Preservation of food materials from degradation, mainly by oxidation processes or by microorganisms, during processing, storage, and marketing is an important issue in the food industry. To achieve this goal, the food industry has used synthetic additives which diminish microbial growth and delay the oxidation of easily oxidizable materials, such as lipids. However, owing to the economical impact of spoiled foods and consumers' growing concerns over the safety of foods containing synthetic antioxidants, much attention has been paid to natural bioactive compounds (Alzoreky and Nakahara, 2003; Viuda-Martos et al., 2011).

The seeds of *N. sativa* have been used for years as a spice and food preservative. Black cumin seeds have been added as a spice to a variety of Persian foods such as yogurt, pickles, sauces, and salads (Hajhashemi et al., 2004; Venkatachallam et al., 2010). The seeds are used extensively as a spice for flavoring purposes, especially bakery products and cheese. Seeds of *N. sativa* are used in the preparation of a traditional sweet dish and eaten with honey and syrup as well as for sprinkling on bread (Cheikh-Rouhou et al., 2007; Hamrouni-sellami et al., 2008). Seeds are of importance as a carminative; often they are used as a condiment in bread and other dishes (Lautenbacher, 1997; Eschborn, 1997; Burits and Bucar, 2000; Ramadan, 2007). Volatile and fixed *N. sativa* seeds oil is used worldwide for functional foods and nutraceuticals.

Essential oils are aromatic oily liquids recovered from plant parts (e.g., flowers, buds, seeds, leaves, bark, herbs, fruits, and roots). Essential oils are characterized by a strong odor and are formed by medicinal or aromatic plants as secondary metabolites (Bakkali et al., 2008). Recovery of essential oils could be obtained by expression, fermentation, or extraction. Essential oils are usually obtained by hydrodistillation, steam distillation, or dry distillation of the plant or of some parts of it. The main advantage of essential oils is that they can be used in any food and are generally recognized as safe (USFDA, 2006).

Antimicrobial Activity

Investigations using meat products, fish, milk, dairy products, vegetables, fruit, and cooked rice have shown that the concentration of essential oils needed to achieve a significant antibacterial impact is around 20 μL/g in foods and around 10 μL/mL in solutions for washing fruit and vegetables (Burt, 2004). *Nigella sativa* essential oil showed complete zone inhibition against *Bacillus cereus*, *Bacillus subtilis*, *S. aureus*, and *Pseudomonas aeruginosa* at 2000 and 3000 ppm. The antibacterial results obtained from the essential oil are given in Table 2. The oil was found to be a complete zone inhibitor against *B. subtilis* and *S. aureus* at 1000 ppm. The essential oil gave antibacterial activities comparable to some standard antibiotics such as ampicillin and cloxacillin (Singh et al., 2005).

The antifungal activities for *N. sativa* volatile oil obtained by the poison food and inverted petriplate techniques are given in Figure 4. The essential oil revealed good zones of inhibition against *Aspergillus flavus*, *Fusarium graminearum*, *Fusarium monoliforme*, and *Penicillium viridicatum* at different doses. In addition, the essential oil showed complete growth inhibition against *Penicillium citrinum* using 6 μL (Singh et al., 2005). *Nigella sativa* essential oil exhibited also strong antifungal activity against *Aspergillus* species and it was found to be effective for *F. graminearum*. For other fungi, the essential oil exerted moderate to good zones of inhibition (Singh et al., 2005). A higher volatility of the aromatic components of an essential oil will lead to a higher vapor concentration in the surrounding air space. This might be responsible for the increase in antimicrobial activity with an increase in dose concentration.

Most of the antimicrobial activity in essential oils derived from spices and culinary herbs is believed to derive from phenolic compounds. *Nigella sativa* essential oil contains good amounts of phenolic compounds (e.g., *p*-cymene, thymol, and carvacrol) which might be the reason of the antimicrobial potential of *N. sativa* essential oil. The strength of inhibition and the spectrum of antimicrobial activity of *N. sativa* essential oil suggest that interactions between individual components led to the overall activity (Singh et al., 2005).

Khosravi et al. (2011) evaluated the effects of *N. sativa* essential oil on growth and aflatoxins production by *A. parasiticus*. Determination of aflatoxins (AFB1, AFB2, AFG1, and AFG2) was performed by immunoaffinity column extraction using reverse phase–high-performance liquid chromatography. In the broth microdilution method, *N. sativa* oil exhibited strong activity (MIC90: 2.75; MFC: 6.25 mg/mL) against *A. parasiticus*. The study suggested that *N. sativa* oil might be used as natural inhibitors in foods at low levels to protect food from fungal and toxin contaminations by *A. parasiticus*.

Viuda-Martos et al. (2011) determined the effectiveness of the Egyptian *N. Sativa* essentials oil on the inhibition of the growth of some indicators of spoilage bacteria strains. They selected three bacterial spices (*Listeria*, *Pseudomonas*, and *Serratia*) commonly associated with refrigerated foods, eggs, meat, milk, poultry, seafood, and vegetables. *Nigella sativa* oil exhibited high percentage of inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical: 95.89% and high ferric reducing antioxidant capacity (FRAC) values of 3.33 mmol/L Trolox). Essential oil of *N. sativa* showed also inhibitory effects on *Listeria innocua*.

TABLE 2 Antibacterial Activity of *Nigella sativa* Essential Oil

		Inhibition Zone (mm)			
	Concentration (ppm)	*Bacillus subtilis*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aeruginosa*
Nigella sativa essential oil	1000	CI[a]	CI	70.4	60.7
	2000	CI	CI	CI	CI
Ampicillin	1000	–[b]	19.1	–	–
	2000	12.2	22.2	14.4	–
Cloxacillin	1000	–	22.8	12.3	–
	2000	–	26.4	13.3	–

[a]*Complete inhibition.*
[b]*No inhibition.*

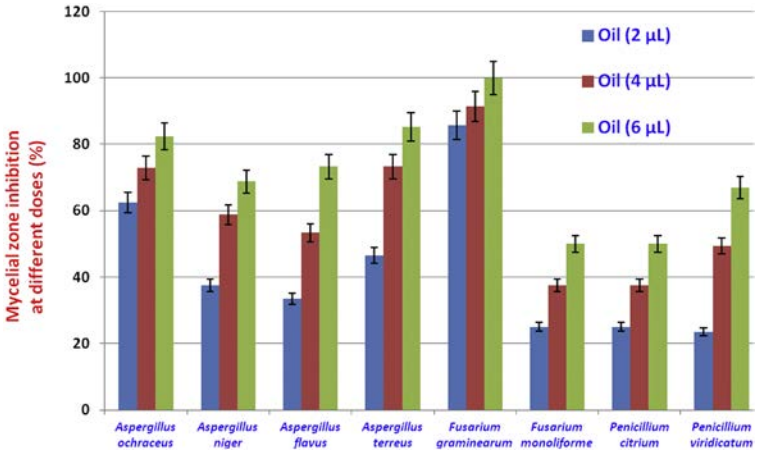


FIGURE 4 Antifungal impact of *Nigella sativa* essential oil against different food pathogenic fungi by the poison food medium method.

Mahgoub et al. (2013) studied the impact of adding *N. sativa* oil at levels of 0.1% and 0.2% (w/w) to Domiati cheese supplemented with probiotic cultures on the inhibition of food-borne pathogens (*S. aureus*, *E. coli*, *Listeria monocytogenes*, and *Salmonella enteritidis*) inoculated in cheese during storage. *Nigella sativa* oil showed antimicrobial impact where a concentration of 0.2% oil had the most effective antimicrobial potential on pathogens when compared with the control. Storage life of oil-supplemented cheeses was extended under refrigerated conditions with low microbial loads. In addition, oil-supplemented Domiati cheese had also improved physicochemical and sensory properties.

Antioxidant Activity

Lipid oxidation constitutes one of the major changes that can occur during the processing, marketing, and storage of foods. To control the rancidity, there is a growing demand for natural bioactive substances that can be introduced directly into the food product to delay or inhibit these processes.

The antioxidant activity of *N. sativa* essential oil was evaluated by measuring peroxide, thiobarbituric acid (TBA), and total carbonyl values of rapeseed oil enriched with *N. sativa* essential oil at time intervals. *Nigella sativa* essential oil showed strong antioxidant potential compared with butylated hydroxyanisole and butylated hydroxytoluene. In addition, their inhibitory action in the linoleic acid system was studied by monitoring the accumulation of peroxide concentration. Their radical scavenging capacity was carried out on DPPH radical and showed excellent scavenging potential compared with synthetic antioxidants. Their reducing power was also determined, demonstrating strong antioxidant capacity of *N. sativa* essential oil (Singh et al., 2005).

To evaluate the antioxidant potential of *N. sativa* essential oil, lipid inhibitory activity was compared with those of selected standard antioxidants using the ferric thiocyanate method. High absorbance was an indication of a high concentration of peroxides formed. The absorbance of linoleic acid emulsion without the addition of essential oil increased rapidly. *Nigella sativa* essential oil reduced the formation of peroxides and showed considerable antioxidative properties in the linoleic acid system (Singh et al., 2005). Edris (2011) showed that there was a correlation between the *N. sativa* volatile oil content and the oxidative stability of the corresponding crude *N. sativa* oil. High γ-terpinene content in the constitution of volatile oil can be another contributing factor for enhancing the oxidative stability of the crude oil.

Ramadan (2013) prepared healthy blends of high linoleic sunflower oil with *N. sativa* oil and future studied the functionality, stability, and antioxidative characteristics of blends. By increasing the amounts of *N. sativa* oil oils in sunflower oil, linoleic acid level decreased, while tocols increased. Inverse relationships were noted between peroxide value as well as anisidine value and oxidative stability at termination of storage under accelerated oxidative conditions. Levels of conjugated dienes and conjugated trienes in sunflower oil and blends increased with increase in time. *Nigella sativa* oil blends gave about 70% inhibition of DPPH· radicals.

SUMMARY POINTS

- *Nigella sativa* essential oil has been widely used in functional foods, nutraceuticals, and pharmaceutical products.
- *Nigella sativa* essential oil exhibited different biological characteristics including antifungal, antibacterial, and antioxidant potentials.
- The main bioactive compounds identified in *N. sativa* essential oil include *p*-cymene, thymoquinone, α-thujene, longifolene, β-pinene, α-pinene, and carvacrol.
- The diversity of food applications for *N. sativa* essential oil gives this essential oil great industrial importance.

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Chapter 31

Black Pepper (*Piper nigrum* L.) Oils

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List of Abbreviations

EO Essential Oil
SFE Supercritical Fluid Extraction

INTRODUCTION

The black pepper plant, also known as "black gold" or "king of spices" is the most commonly used spice in the world due to its commercial, economical, nutritional, and medicinal value. Its common names include peper (Portugal), poivre (France), marich (Nepal), kalimirch, and kalamari (India). The seeds are commonly used to enhance the taste and flavor of food, while the essential oil (EO) plays a significant role in food processing, health, and nutrition by preventing or delaying spoilage of foods due to its antioxidant and antimicrobial properties. It is also used in the production of perfumes and insecticides, as medicinal ingredients in pharmaceutical industries, and several eastern and western recipes (Meghwal and Goswami, 2012).

The antimicrobial activity of EOs of different spices against food-borne pathogens, as well as their antioxidant activity has been developed and proposed for use in packaged foods (Brewer, 2011), thus the growing interest in substituting synthetic preservation methods with natural ones, have heightened consciousness and increased demand for these EOs including that of black pepper (Meghwal and Goswami, 2012). Piperine, an alkaloid and major principle present in black pepper and responsible for its pungent and irritant action has been shown to possess antioxidant and antimicrobial properties. It is also used in alternative and complementary medicine to treat various disorders. The antioxidant constituents found in EOs of black pepper include *p*-cymene, α-terpinene-4-ol, and piperine (Table 1) (Brewer, 2011), while α-pinene, β-pinene, δ-3-carene, limonene, and α-terpinene-4-ol inhibit bacterial growth (Dorman and Dean, 2000). The composition of these constituents in the EO can be affected during production, especially the extraction method employed (Polovka and Suhaj, 2010).

BOTANICAL ASPECTS

The family Piperaceae is derived from the Latin word *Piper* meaning "pepper" and is composed of several species, one of which is *Piper nigrum* L., commonly called black pepper. Another species, *Piper guineense*, is also known as black pepper, but is specifically called the "African black pepper" or "West African black pepper" as it is mostly found in West African countries like Ghana and Nigeria (Ekanem et al., 2010). However, *P. nigrum* is the true pepper of commerce and the species is widely known as black pepper. It is indigenous to Southern India, but also cultivated in tropical regions such as Malaysia, China, Brazil, and Thailand (Meghwal and Goswami, 2012). It is a woody, climbing vine of about 9 m in length, with several rootlets growing from its stem nodes. The stem roots allow the vine to attach to living or nonliving standard supports, such as other plants or structures, for the vines to be established (Nelson and Edger, 2011).

The leaves are glossy, ovate, and about 13–25 cm long (Figure 1). They are dark green above and pale green beneath, with elongated, slender spikes or catkins (1.6–2 cm in length) bearing minute, white flowers. The flower spikes always appear on stems opposite the leaves, and bear from 50 to 60 single-seeded berries, hence, the yield of the berries depends on the number of leaves on the stem. The seeds in their dried forms are often referred to as "peppercorns" (Nelson and Edger, 2011).

Although black pepper is the most popular form of pepper spice, there are also the less pungent white and green peppers with marginal commercial value. For white pepper, the berries are harvested when about 75% of them are red or

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TABLE 1 Constituents of Black Pepper (*Piper nigrum* L.) EO

Components	Percentage in Sample
α-Thujene	1.4
α-Pinene	5.7
Camphene	0.1
β-Pinene	10.7
Sabinene	16.5
Myrcene	2.0
α-Phellandrene	0.7
α-Terpinene	0.2
δ-3-Carene	1.7
ϱ-Cymene	0.2
Limonene	18.8
β-Phellandrene	2.9
(E)-β-Ocimene	0.5
γ-Terpinene	0.4
trans-Sabinene hydrate	0.3
Terpinolene	0.4
Linalool	1.1
cis-Sabinene hydrate	0.1
Terpinen-4-ol	1.7
Δ-Terpinoel	0.5
Δ-Elemene	1.9
A-Copaene	1.2
B-Elemene	0.8
A-Cedrene	0.3
B-Caryophyllene	15.1
A-Gurjunene	0.2
A-Humulene	1.1
Δ-Muurolene	0.5
B-Selinene	0.7
Zingiberene	0.2
Furanodiene	2.1
A-Selinene	0.6
B-Bisabolene	1.1
B-Curcumene	0.1
Δ-Cadinene	0.7
B-Elemol	0.3
Germacrene B	1.4

TABLE 1 Constituents of Black Pepper (*Piper nigrum* L.) EO—cont'd

Components	Percentage in Sample
β-Caryophyllene oxide	0.9
(*E*)-Nerolidol	0.3
T-Cadinol	0.3
T-Muurolol	0.2
α-Eudesmol	1.2
Monoterpene hydrocarbons	62.2
Oxygenated monoterpenes	3.7
Sesquiterpene hydrocarbons	26.2
Oxygenated sesquiterpenes	3.2
Others	2.1
Total	**97.4**

Black pepper EO contains many components at different concentrations with antioxidant and antibacterial properties.
Reprinted from Martins et al. (1998) with permission.

FIGURE 1 Black pepper (*Piper nigrum* L.) leaves and green berries taken at Kumthiang plants market, Chiang Mai, Thailand. The leaves of black pepper (*P. nigrum* L.) are green, glossy, ovate, and about 13–25 cm long with spikes bearing the seeds on stems opposite the leaves. *This picture was taken by Miss Sopitsuda Maivun of the Faculty of Fine Arts and Architecture, Rajamangala University of Lanna, Chiang Mai, Thailand.*

reddish-orange in color. The skin of the berries is peeled (after soaking in water for about 8 days) and sun-dried to a white-beige color (Figure 2). As for green peppers (Figure 3), unripe (green) pepper berries are harvested and dried or preserved in vinegar or citric acid (Nelson and Edger, 2011).

Historical Cultivation

Historically, black pepper was cultivated by seed in small homestead gardens. This was aborted due to variations in seedling progenies which prevent seedlings raised from seeds to breed true to type. Also, seed production yields only few heterogenous progenies as they become dioecious and come to bearing very late due to their short viability and high sterility in post generation stages. Direct field cuttings, grafting, layering, and budding methods were also aborted as they result in poor establishment, due to unavailability of sufficient shoots to produce enough cuttings and establish big plantations (Sivaraman et al., 1999).

FIGURE 2 White pepper berries of *Piper nigrum* L. To obtain white peppercorns, the berries are harvested when about 75% of them are red or reddish-orange in color. They are soaked in water for about 8 days to peel the skin and sun-dried to a white-beige color. *The photograph was taken at Pratu Chiang Mai market, Chiang Mai, Thailand, by Miss Sopitsuda Maivun of the Faculty of Fine Arts and Architecture, Rajamangala University of Lanna, Chiang Mai, Thailand.*

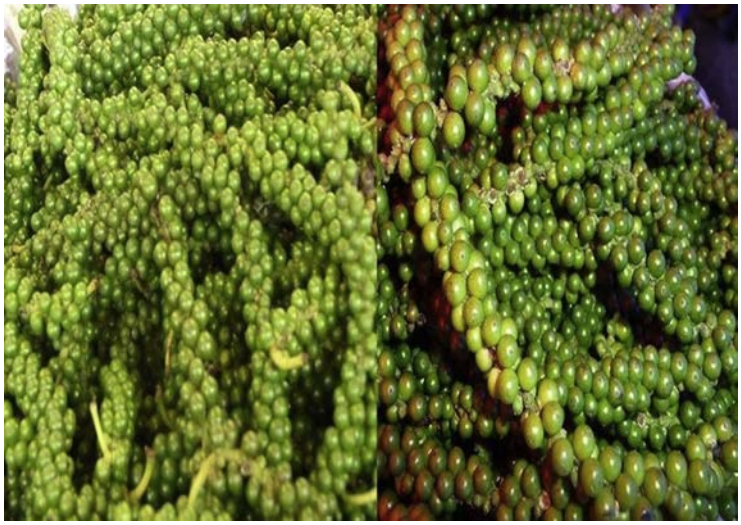

FIGURE 3 Freshly harvested peppercorns of black pepper (*Piper nigrum* L.). The seeds of black pepper are harvested at maturity when they are greenish yellow and the berries separate easily from the spike. *This photograph was taken at Pratu Chiang Mai market, Chiang Mai, Thailand, by Miss Sopitsuda Maivun of the Faculty of Fine Arts and Architecture, Rajamangala University of Lanna, Chiang Mai, Thailand.*

Present Day Cultivation

Black pepper is cultivated vegetatively by rooted cuttings, which is preferred for commercial cultivation. Very long runner shoots with several nodes are cut into smaller shoots with at least two or three nodes, raised in a nursery and transferred to the field after attaining sufficient growth. The bamboo method, a rapid multiplication technique is also used to generate quality planting materials (Sivaraman et al., 1999). In vitro propagation using tissue culture techniques is now generally used in place of traditional practices as it allows production of genetically stable and identical progeny (Abbasi et al., 2010).

Black pepper grows on heavy and light sandy clays with a rainfall requirement of about 2000 mm and average temperature of 28 °C (Sivaraman et al., 1999).

The seeds are harvested at maturity when their color is greenish yellow and the berries separate easily from the spikes, that is, usually from the third year after planting (Sivaraman et al., 1999). The greenish yellow pepper spikes

FIGURE 4 Black pepper berries of *Piper nigrum* L. The greenish yellow pepper spikes are cleaned by winnowing, dipped in boiling water for a few minutes to disinfect, and the fermentation process is started that turns the seed black. They are then sun-dried until the outer skin becomes black and wrinkled. *The photograph was taken at Pratu Chiang Mai market, Chiang Mai, Thailand by Miss Sopitsuda Maivun of the Faculty of Fine Arts and Architecture, Rajamangala University of Lanna, Chiang Mai, Thailand.*

are cleaned by winnowing, dipped in boiling water for a few minutes to disinfect, and then fermented. They are then sun-dried until the outer skin becomes black and wrinkled (Figure 4). The dried seeds are graded, cleaned, and stored in insecticide and fungicide-treated bags (Sivaraman et al., 1999; Nelson and Edger, 2011).

USAGE AND APPLICATIONS

Historical Usage and Applications

There is no doubt why India remains the largest producer of black pepper as Indians have known and used black pepper since 2000 BC, while it became known by the Chinese from the second century BC. Black pepper has been largely used as a flavoring agent in food. According to the fifth-century Syriac Book of Medicine, black pepper was used for the treatment of diarrhea and fever. It was also used to enhance the effect of other herbs, for example, it was used together with long pepper to treat gangrene, heart disease, indigestion, insect bites, diseases of the lungs and liver, toothaches, etc. (Srinivasam, 2009).

The ancient Ayurvedic Materia Medica (600–300 BC) described "Trikatu," meaning "three acrids," as the use of black pepper in combination with long pepper (*Piper longum*) and ginger (*Zingiber officinale*) to balance psychophysical components of the body in order to prevent disease (Srinivasam, 2009).

EOs of various spices including black pepper were developed in the middle ages by the Arabs, who were the first to recognize their therapeutic potential. The oils were used for the preservation of food, as antiseptic, and fragrance. They also served as analgesic, sedative, antiinflammatory, spasmolytic, and local anesthesic remedies. They were also applied during purification and embalming rituals (Bakkali et al., 2008).

Traditional Usage and Applications

To this day, the use of black pepper and its EOs has not changed much. However, more is now known about the proper applications of the oils in food, medicine, and pharmaceutical industries. Also, some of their mechanisms of action, particularly at the molecular level, have been understood (Karsha and Lakshmi, 2010; Zarringhalam et al., 2013).

The people of Kumaragiri Hills, Tamil Nadu, India, combine black pepper with other herbs to treat snake bite, scorpion sting, cholera, headaches, asthma, skin diseases, hemorrhoids, and ulcers (Alagesaboopathi, 2009).

The EO of black pepper is used as an energizing oil to treat respiratory infections, sooth muscular aches and pains, rheumatism exhaustion, physical and emotional coldness, and fever. It is also used to increase the flow of saliva, stimulate appetite, tone the colon muscles, and is a general digestive tonic (Kumoro et al., 2010; Aziz et al., 2012).

There are numerous reports on the physiological and drug-like actions of black pepper and its EO (Table 2). The bioactive molecule, piperine, has major pharmacological impacts on the nervous and neuromuscular systems and helps in digestion (Ahmad et al., 2012).

TABLE 2 Different Functions of Black Pepper (*Piper nigrum* L.)

Components	Effects Demonstrated	References
Peppercorns	Enhances secretion of saliva and activity of amylase	Glatzel (1968)
Piperine, EO	Inhibits lipid peroxidation; antioxidant	Vijayakumar et al. (2004) and Andrade and Ferreira (2013)
Piperine	Enhances bioavailability of drugs by inhibiting hepatic and intestinal aryl hydrocarbon hydroxylase	Atal et al. (1985)
Fruits, EO	Antimutagenic and anticancer	El Hamss et al. (2003)
Fruits, EO, Piperine	Inhibits growth of both Gram-positive and Gram-negative bacteria such as *Bacillus substilis*, *B. Sphaericus*, *Staphylococcus aureus*, *Klebsiella aerogenes*, and *Chromobacterium violaceum*	Jayaweera (1982), Ouattara et al. (1997) and Zarringhalam et al. (2013)
EO	Reduces risk of pneumonia in older people by activating the or bitofrontal cortex which improves reflexive swallowing movement	Ebihara et al. (2006)

The fruits and extracts EO of black pepper have been shown to possess different functions.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Essential Oil of Black Pepper as Preservative

The increasing demand for packaged food products and concern about the safety of synthetic preservatives and additives used to extend their shelf life has revived the use of traditional/natural food preservatives in the food industry (Brewer, 2011). The generation of reactive oxygen species during oxidative processes that occur in food is one of the causes of chemical spoilage, resulting in rancidity, deterioration of nutritional quality, development of undesirable off-flavors, and a decrease in safety and acceptability of foods (Polovka and Suhaj, 2010). Like humans, food tissues undergo oxidative stress but are equipped with antioxidant defense components that scavenge free radicals (Polovka and Suhaj, 2010). Herbs and spices are known to contain large amounts of antioxidants, such as flavonoids, phenols, carotenoids, and antimicrobial components, hence the growing interest in their use in food and pharmaceutical industries (Meghwal and Goswami, 2012).

Antibacterial Activities of Essential Oil of *Piper nigrum*

The significance of EOs as food preservatives cannot be overemphasized because, ordinarily, spices have to be used in sufficient quantities for their antimicrobial properties to be significant, whereas a very small quantity of the EO would maintain the organoleptic properties, retard microbial contamination, and ultimately reduce the onset of food spoilage (Dorman and Dean, 2000).

Studies by Dorman and Dean (2000) examined the antimicrobial activity of some EOs against 25 different genera of bacteria and demonstrated that the EO of black pepper was effective against both Gram-positive and Gram-negative bacteria. They attributed this to its ability to inhibit electron transport, protein translocation, phosphorylation, and enzymes within the bacterial cell membrane. Terpinene-4-ol was found to inhibit all bacteria except *Leuconostoc cremoris* because it may act as either a protein denaturing agent, solvent, or dehydrating agent. The terpenoids β-pinene and α-pinene exert their inhibitory activity due to their lipophilic properties, potency of their functional groups, and stability in aqueous environment (Dorman and Dean, 2000). Karsha and Lakshmi (2010) attributed the bactericidal action of black pepper oil to its ability to alter membrane permeability resulting in the leakage of intracellular materials and causing cell death.

Zarringhalam et al. (2013) attributed the antibacterial activity of the EO to the piperine and oleic constituents. Though the antibacterial activity of piperine has been reported, there are conflicting results on the inhibitory effects of fatty acids on some bacteria (Ouattara et al., 1997).

In another study, the EO of black pepper was reported to inhibit six meat spoilage organisms only when used at high concentrations (Ouattara et al., 1997). Sahadeo and Vilas (2011) also demonstrated that the EO had an inhibition rate of 43–64% when tested against Gram-positive and Gram-negative organisms, but did not inhibit *Escherichia coli* and *Salmonella typhi*. This is inconsistent with reports by Dorman and Dean (2000) who demonstrated that the EO of black pepper inhibited both organisms.

Antioxidant Activities of Essential Oil of *Piper nigrum*

Antioxidants are compounds that eliminate undesirable effects of reactive oxygen in foods and neutralize free radicals, thus, prevent food browning, rancidity, breakdown of nutrients, natural food flavors and colors. They improve the shelf life of food, profitability and palatability, and reduce waste. Antioxidants provide a natural solution to maximizing the shelf life of foods (Polovka and Suhaj, 2010).

The antioxidant activities of EO of black pepper have been reported by several researchers. In a study by Singh et al. (2005), crude mustard oil containing the EO of black pepper as an additive was shown to have significantly lower peroxide and thiobarbituric acid values which were comparable to butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Also, the oil exhibited moderate radical scavenging ability and reducing power. Masharina et al. (2009), while studying the antioxidant activity and stability of hexane solutions of some EOs, demonstrated that EO of black pepper had the least antioxidative activity compared with other oils. This is consistent with reports by Tipsrisukond et al. (1998) that black pepper oil is a less effective antioxidant compared with its extracts and oleoresins.

Nakatani et al. (1986) established the antioxidant activities of five of the six active components isolated from black pepper oil, namely *N*-feruloyl tyramine, *N-trans*-feruloyl piperidine, *N*-5-(4-hydroxy-3-methoxyphenyl)-2E,4E-pentadienoyl piperidine, and N-5-(4-hydroxy- 3-methoxyphenyl)-2E-pentenoyl piperidine, with *N*-5-(4-hydroxy-3-methoxyphenyl)-2E,4E-pentadienoyl piperidine being as active as BHA and BHT.

Essential Oil of Black Pepper for Preservation of Orange Juice

Oranges are a rich source of vitamins A and C, folate, calcium, iron, and thiamine. They also contain significant amounts of carotene and flavonoids. Thus, orange juice is no doubt nutritious and is one of the most common ways of preserving orange; however, without proper preservation, it decays, while its color, taste, and aroma diminishes (Kapoor et al., 2012). The juice can be preserved by thermal pasteurization, high hydrostatic pressure, pulsed electric, etc. Also, chemicals such as potassium sorbate and sodium benzoate are used. Natural preservatives such as bacteriocins, organic acids, phenolic acids, and EOs have shown promising results in their applications (Rupasinghe and Yu, 2012).

Kapoor et al. (2012) demonstrated that the EO of black pepper may be beneficial if applied in industries due to its ability to increase the shelf life of orange juice. From their results, orange juice containing EO of black pepper had significantly lower microbial load, pH, titratable acidity, nonenzymatic browning, and percent loss, while ascorbic acid content was high compared with fruit juices stored with oleoresins and ethanol.

Extraction of Essential Oil of Black Pepper

The extraction of EOs from plants has been shown to have effect on the composition and consequently the antioxidant and antimicrobial activity of the oil. The EO of black pepper has a spicy, pungent aroma, and appears from a colorless to a greenish liquid. It is levorotatory with specific gravity between 0.860 and 0.884, and refractive index between 1.478 and 1.488 at 20 °C (George et al., 1988). The most common method used in extracting EOs is by distillation (hydrodistillation, steam distillation, water and steam distillation), but the use of supercritical fluids or gaseous extraction methods is more common due to the problem of solvent residues in food (Rouathbi et al., 2007). In steam distillation, volatile EO separation is followed by extraction of oleoresins with liquid solvent, separation of solvent from oleoresin using distillation, and mixing oleoresin and EO in ratios occurring in the original spice, whereas in supercritical fluid extraction (SFE), the oil is extracted in one step without any organic solvent residues or change in aroma or flavor (Sovova et al., 1995).

Extraction by Superheated Steam

This method was described by Rouathbi et al. (2007) and involves the passage of superheated steam usually between 100 and 250 °C through powdered or whole fruits of black pepper in an experimental distillation device. The steam and extracted volatiles are collected in a condenser at different times and EO isolated from water by extraction with diethyl-ether-n-pentane (50/50, v/v) before drying on anhydrous sodium sulfate. A yield of 1.6% was obtained and the composition of oil was within literature values.

Extraction by Dense Carbon Dioxide

This can be done by either static or dynamic method using a dense gas. In the static method, the sample is sealed in a vessel with known amount of gas at constant temperature, until equilibrium is reached and the solvent containing the solutes are removed (Kerrola, 1995).

Supercritical Fluid Extraction (SFE)

SFE has gained attention over traditional techniques as it protects extracts from thermal and environmental degradation and solvent contamination. Supercritical fluids are fluids at their supercritical state, that is, the state reached when both temperature and pressure exceed the critical conditions of the fluid (Kumoro et al., 2010).

SFE of EO of black pepper can be done by the Lack's plug flow model or a model based on adsorption–desorption mechanism coupled with linear driving force (Sovova et al., 1995; Perakis et al., 2005). Generally, SFE involves placing ground black pepper in a high-pressure stainless steel extraction vessel using a dynamic extraction apparatus immersed in a water bath controlled by an electrical heater. Carbon dioxide is passed through the extraction vessel at a desired temperature until the system pressure is reached. A pressure regulator is then used to depressurize the carbon dioxide and oil extracts collected (Kumoro et al., 2010).

Effect of Radiation and Heat Treatment on Composition of EO of Black Pepper

The contamination of herbs, spices, and vegetables with microorganisms capable of causing spoilage can be controlled by radiation and heat treatment. However, it has been reported that such treatments can affect the composition or modify EO constituents, and ultimately affect their antioxidant properties (Suhaj et al., 2006). To avert such, the Directive 1999/3/EC gave the maximum average dose of ionizing radiation on herbs, spices, and vegetables as 10 kGy, while the limitation of the United States Food and Drug Administration is 30 kGy.

In a study by Suhaj et al. (2006), γ-irradiation of ground black pepper from 5 to 30 kGy was found to affect the radical scavenging and reducing power of pepper extracts within the first month of irradiation, which gradually disappeared after long-term storage. In another study, γ-irradiadion at 10 kGy was found to increase quinine radical and decrease total ascorbic acid and carotenoid content of black pepper. However, no significant change in the volatile oil content of black pepper was observed after γ-irradiation at doses of 10, 20, and 30 kGy, while in another report, γ-irradiation at 30 kGy decreased some volatiles, while lower doses had no effect (Polovka and Suhaj, 2010). Thus, the method of radiation processing, dose, and time of antioxidant measurement play a significant role on the antioxidant properties of spices and their EOs.

Very little has been reported on the effect of heat treatment on bioactive components of herbs and spices, but it is known that heat may inactivate some antioxidant compounds, and sometimes increase them by inhibiting some enzymes. Also, some antioxidants are formed during heat treatment due to the primary effect of Malliard browning reactions (Polovka and Suhaj, 2010).

SUMMARY POINTS

- Black pepper is known as the "king of spices" due to its commercial and economical value.
- The EO of black pepper is used to treat several ailments, and plays a significant role in food and pharmaceutical industries.
- Due to the antioxidant and antibacterial properties of the EO, methods have been developed and proposed for its potential use as food preservatives.
- SFE of the EO has gained attention over traditional methods as the oil is protected from thermal degradation and solvent contamination.
- Composition of black pepper EO can be affected by handling and production processes.

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Chapter 32

Caraway (*Carum carvi* L.) Essential Oils

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List of Abbreviations

DPPH 2,2-diphenylpicrylhydrazyl
GC-MS Gas chromatography–mass spectrometry
kGy KiloGray
MBC Minimal bactericidal concentrations
MDG Microwave dry-diffusion and gravity
MIC Minimal inhibitory concentration

INTRODUCTION

Aromatic plants, such as those from the Apiaceae family (Umbelliferae) produce large number of secondary metabolites, namely monoterpenes, sesquiterpenes, and phenylpropanoids, that have demonstrated several therapeutic properties, mainly antioxidant and antiinflammatory effects. So far more than 100 cultivated Apiaceae species have been identified with different applications, mainly as medicinal plants. Some of the Apiaceae species are used as vegetables (parsley, celery, parsnips, and carrots) and food spices (cumin, anise, coriander, and caraway). The fruit of caraway is a schizocarp, which at harvest splits into two halves called "seeds." The genus *Carum* is an important genus of the Apiaceae family and comprises about 20–30 species from Europe, North Africa, and Asia. The best-known species of this genus is *Carum carvi* L. (caraway) which is one of the oldest aromatic and medicinal plants known.

Caraway is best known as a good remedy for colds and congestion; it contains mild antihistamines and antimicrobial compounds that help to relax the muscles that cause coughing spasms. Caraway is often used alone or in combination with peppermint oil to relieve the symptoms of irritable bowel syndrome and indigestion. The antimicrobial action and other pharmacological properties of caraway are mainly assigned to the active components extracted from caraway essence or essential oils.

BOTANICAL ASPECTS

Caraway (*C. carvi*), also known as meridian fennel or Persian cumin, is a biennial plant in the family Apiaceae native to western Asia, Europe, and Northern Africa. The plant is similar in appearance to other members of the carrot family, having finely divided, feathery leaves with thread-like divisions, growing on 20–30 cm stems. The main flower stem is 40–60 cm tall, with small white or pink flowers in umbels. Caraway fruits (seeds) are crescent-shaped achenes, about 2 mm long, with five pale ridges (Figure 1). Caraway has been called by different names mainly derived from the Latin *cuminum* (cumin), the Greek *karon* (again, cumin) which was adapted into Latin as *carum*, and the Sanskrit *karavi*, sometimes translated as "caraway." English usage of the term caraway dates back to at least 1440, and is considered by Skeat to be of Arabic origin, though Katzer believes the Arabic *al-karawya* (cf. Spanish *alcaravea*) to be derived from the Latin *carum*. In Persian language caraway seeds are called *Zireh Siah*; *Zireh* means caraway and *Siah* means black.

The fruits have a pungent, anise-like flavor and aroma that comes from essential oils, mostly carvone and limonene. They are used as a spice in breads, especially rye bread, and other food products. Caraway is the best-known species of this genus and believed to be one of the oldest aromatic and medicinal plants known (Papini et al., 2007). There is a great variability of morphological traits within different caraway genotypes. Due to their high content of essential oils (>40%), production of essential oils from plants has been commercialized as food supplements.

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FIGURE 1 A view of caraway fruits (left) and a magnified view of the fruits (right). *Adopted from Wikipedia.*

Morphological traits such as the number of branches and lateral shoots, the number of umbel per plant or the seed size have a great influence on fruit crop. The examination of morphological traits and their interaction indicated stability of the analyzed traits with years (Seidler-Lozykowska and Bocianowski, 2012). The so-called black caraway refers to the caraway seed which is black in color, and the green caraway refers to Cumin (*Cuminum cyminum* L.) which also belongs to the Apiaceae family are green in color.

USAGE AND APPLICATIONS OF CARAWAY

Caraway is a spice that is added in small amounts to food and food products for its taste and flavor. The dried ripe fruits of caraway (*Carvi fructus*) are used in folk medicine as a carminative (Madisch et al., 2004).

In addition to the antipasmodic, carminative, emmenagogue, expectorant, galactagogue, stimulant, stomachic, and tonic properties of caraway, it is safely added to young children's dishes to help digestion. In vivo studies show that essential oil prepared from caraway exhibit cancer chemopreventive action against colon premalignant injuries induced by dimethylhydrazine, a well-known chemical carcinogen (Dadkhah et al., 2010).

Essential oils are usually volatile, odorous, and may contain up to 100 individual components that are composed mainly of monoterpenes, sequiterpenes, phenylpropanoids, and isothiocyanates. The oil possesses bactericidal, virucidal, fungicidal, analgesic, sedative, antiinflammatory, spasmolytic, and anesthetic activities. Caraway extract is also added to cosmetics, toothpaste, and some other pharmaceutical preparations as a source of carvone. Caraway essential oil (*Carvioleum*) obtained from fruit by hydrodistilling consists mainly of monoterpenes such as carvone and limonene, which usually make up 95% of all the oil (Kallio et al., 1994). According to El-Sawi and Mohamed (2002), caraway aldehyde is the main component of seed oil (53.6%) and herb oil (40.5%). There is considerable variation in the essential oil content and composition in commercially available caraway samples (Raal et al., 2003).

Aromatic hydrocarbon mixtures are obtained from aromatic plants, most often by means of water distillation or supercritical fluid extraction (Pavela et al., 2009). The essential oil yields ranged from 0.6% to 5.4%, and 18 compounds, comprising more than 97% of the composition of oil, were identified in the caraway essential oils. Carvone (44.5–95.9%) and limonene (1.5–51.3%) are probably the main components of the oils in caraway seeds. Analysis of the essential oils extracted from caraway cultivated in Iran showed that 23 known compounds are present in the oils (Fatemi et al., 2011). Comparison of the active components identified in essential oils extracted from caraway and cumin seeds revealed that the oils extracted from these seeds share common components. Regardless of the differences in the cultivation conditions, there are 28 components that are common to the two seeds (Jalali-Heravi et al., 2007). According to Laribi et al. (2013), the essential oil and fatty acid composition of three caraway seeds with different cultivation origin of Tunisia, Germany, and Egypt showed some differences in their proportions. This comparative study revealed that a total of 41 volatile compounds are detectable in the oil samples. Ketones (61.88–77.89%), representing carvone, *trans*-dihydrocarvone, and *cis*-dihydrocarvone, were the major compound of caraway seed essential oil. The essential oil composition (%) of Tunisian, German, and Egyptian caraway seed ecotype has been summarized in Table 1 (Laribi et al., 2013).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Caraway has been used traditionally as a condiment or a spice due to its pleasant flavor (Johri, 2011). Caraway seeds and crude extracts are used as a flavoring of bread (e.g., rye bread), cheese, sauerkraut, candies, meat products, pickled fruits, sauces, and chewing gums. Ground caraway seeds are used as a component of teas and herbal mixtures.

TABLE 1 Essential Oil Composition (%) of Tunisian, German, and Egyptian Caraway (*Carum carvi* L.) Seed Ecotypes

Compound	RI[a]	RI[b]	Identification[c]	TCE	GCE	ECE
Monoterpene Hydrocarbons						
α-Pinene	1032	934	RI, MS	0.09[b] ± 0.06	0.16[a] ± 0.01	0.16[a] ± 0.14
Camphene	1086	951	RI, Co-GC	0.03[c] ± 0.00	0.28[a] ± 0.08	0.12[b] ± 0.02
β-Pinene	1123	980	RI, Co-GC	0.03[c] ± 0.00	0.25[a] ± 0.08	0.14[b] ± 0.01
β-Myrcene	1166	991	MS	0.16[b] ± 0.03	0.11[b] ± 0.01	3.97[a] ± 3.76
Limonene	1206	1030	MS	19.52[b] ± 3.31	16.15[c] ± 0.95	29.11[a] ± 0.27
γ-Terpinene	1255	1062	RI, MS, Co-GC	0.04[b] ± 0.01	0.16[a] ± 0.05	0.07[b] ± 0.01
(E)-β-Ocimene	1266	1050	RI, MS, Co-GC	0.08[b] ± 0.06	0.18[a] ± 0.05	0.08[b] ± 0.02
p-Cymene	1280	1026	RI, MS, Co-GC	0.03[b] ± 0.00	0.07[a] ± 0.01	0.07[a] ± 0.01
Terpinolene	1290	1092	RI, MS, Co-GC	0.01[b] ± 0.00	0.12[a] ± 0.03	0.05[b] ± 0.01
Aldehydes						
Z-3-Hexenol	1370	855	RI, MS, Co-GC	0.01[b] ± 0.00	0.13[a] ± 0.03	0.19[a] ± 0.15
trans-Limonene oxide	1463	1136	RI, MS	0.01[c] ± 0.00	0.11[a] ± 0.01	0.05[b] ± 0.00
Cuminaldehyde	1785	1238	RI, MS, Co-GC	0.03[a] ± 0.00	0.01[b] ± 0.01	0.02[a,b] ± 0.00
Perilla–aldehyde	1789	1272	RI, MS, Co-GC	0.09[b] ± 0.00	0.19[a] ± 0.00	0.12[a,b] ± 0.01
Ketones						
trans-Dihydrocarvone	1627	1204	MS	0.15[b] ± 0.02	0.29[a] ± 0.07	0.15[b] ± 0.01
cis-Dihydrocarvone	1645	1197	MS	0.08[c] ± 0.02	0.25[a] ± 0.02	0.15[b] ± 0.00
Carvone	1740	1241	MS	76.37[a] ± 4.73	77.35[a] ± 0.17	61.58[b] ± 2.3
Oxygenated Monoterpenes						
Camphor	1532	1143	MS, Co-GC	0.01[b] ± 0.00	0.06[a] ± 0.01	0.02[b] ± 0.00
Linalool	1545	1100	MS, Co-GC	0.02[b] ± 0.00	0.07[a] ± 0.01	0.03[b] ± 0.00
Terpinene-4-ol	1611	1178	RI, MS, Co-GC	0.06[b] ± 0.01	0.10[a] ± 0.06	0.09[a] ± 0.03
α-Terpineol	1700	1189	RI, MS, Co-GC	0.06[a] ± 0.01	0.07[a] ± 0.04	0.03[b] ± 0.01
Dihydrocarveol	1720	1253	MS	0.14[a] ± 0.02	0.08[b] ± 0.01	0.11[a] ± 0.01
Citronellol	1766	1229	RI, MS, Co-GC	0.09[a] ± 0.06	0.08[a] ± 0.01	0.05[b] ± 0.00
Nerol	1797	1228	MS, Co-GC	0.02[b] ± 0.00	0.05[a] ± 0.01	0.03[b] ± 0.00
trans-Carveol	1841	1218	MS	0.05[c] ± 0.01	0.51[a] ± 0.08	0.27[b] ± 0.13
cis-Carveol	1869	1230	MS	0.05[c] ± 0.01	0.08[b] ± 0.01	0.10[a] ± 0.04
Perilla–alcohol	2001	1296	RI, MS, Co-GC	0.03[a] ± 0.01	tr	0.04[a] ± 0.03
Sesquiterpene Hydrocarbons						
β-Elemene	1600	1594	RI, MS, Co-GC	0.03[c] ± 0.00	0.27[a] ± 0.08	0.08[b] ± 0.01
β-Caryophyllene	1612	1419	RI, MS, Co-GC	0.06[b] ± 0.01	0.15[a] ± 0.05	0.06[b] ± 0.00
Allo-aromadendrene	1661	1474	RI, MS, Co-GC	0.04[c] ± 0.01	0.17[a] ± 0.02	0.09[b] ± 0.00
Germacrene-D	1719	1480	RI, MS, Co-GC	tr	0.01[a] ± 0.01	0.03[a] ± 0.01
β-Selinene	1742	1481	RI, MS, Co-GC	0.41[a] ± 0.41	0.10[b] ± 0.10	0.13[b] ± 0.13
α-Selinene	1745	1485	RI, MS, Co-GC	0.82[b] ± 0.82	0.14[a] ± 0.04	1.09[a] ± 0.86

Continued

TABLE 1 Essential Oil Composition (%) of Tunisian, German, and Egyptian Caraway (*Carum carvi* L.) Seed Ecotypes—cont'd

Compound	RI[a]	RI[b]	Identification[c]	TCE	GCE	ECE
α-Farnesene	1755	1508	RI, MS, Co-GC	0.69[a] ± 0.02	0.26[c] ± 0.03	0.44[b] ± 0.24
δ-Cadinene	1772	1517	RI, MS, Co-GC	0.37[c] ± 0.06	0.77[a] ± 0.00	0.56[b] ± 0.05
γ-Cadinene	1776	1511	RI, MS, Co-GC	0.16[c] ± 0.00	0.37[a] ± 0.02	0.24[b] ± 0.02
Oxygenated Sesquiterpenes						
Spathulenol	2125	1575	RI, MS, Co-GC	0.04[c] ± 0.01	0.65[a] ± 0.60	0.12[b] ± 0.01
Eugenol	2192	1356	RI, MS, Co-GC	0.02[b] ± 0.01	0.01[b] ± 0.01	0.04[a] ± 0.02
Thymol	2198	1290	RI, MS, Co-GC	0.04[b] ± 0.01	0.03[b] ± 0.03	0.12[a] ± 0.06
Carvacrol	2215	1296	RI, MS, Co-GC	0.02[c] ± 0.00	0.06[b] ± 0.02	0.10[a] ± 0.04
Esters						
Linalyl acetate	1556	1257	RI, MS, Co-GC	0.04[b] ± 0.01	0.08[a] ± 0.01	0.07[a] ± 0.01
Others						
Nonadecane	1900	1900	RI, MS, Co-GC	0.02[a] ± 0.00	0.02[a] ± 0.02	0.03[a] ± 0.01

Values are the mean of three replicates ±SD. Mean values in a row with different letters (a–c) are significantly different at $P < 0.05$ (Duncan's test).
TCE, Tunisian caraway seed ecotype; GCE, German caraway seed ecotype; ECE, Egyptian caraway seed ecotype; tr, trace.
[a]*Retention index on an apolar column (HP-5).*
[b]*Retention index on a polar column (HP-Innowax).*
[c]*RI, identification by retention index relative to C_7–C_{20}n-alkanes on HP-Innowax column; MS, mass spectrometry; Co-GC, coinjection with authentic compound.*
Information in Table 1 is reproduced with permission from the article by Laribi et al. (2013).

In other regions an aqueous extract of caraway is added to food as an aperitif, tranquilizer, carminative, diuretic, emenagogue, galactagogue, spasmolytic, gastric stimulant, and as an aphrodisiac (Bellakhdar et al., 1991). It has been shown that essential oils when used as mixture are more effective suggesting the additive effects of the oil components (Lopez et al., 2008). Cumin is also an ingredient in Ayurvedic medicine for curing stomach ailments, diarrhea, and dysentery (Oroojalian et al., 2010).

Furthermore, it has been demonstrated that caraway seed possess antioxidant properties as determined by in vitro assays such as 2,2-diphenylpicrylhydrazyl (DPPH) assay, β-carotene–linoleic acid test, and chelating and reducing power of the plant material. The antioxidant action of caraway is assigned to the active antioxidant components such as phenolic compounds. The antioxidant activity of essential oils extracted from caraway seed determined by DPPH assay revealed that the antioxidant power of the essential oils is significantly higher compared with standard antioxidant compounds such as trolox. The ability of the essential oils to suppress the formation of DPPH free radicals and peroxidation products further attests to the antioxidative action of the caraway oils (Fatemi et al., 2011). Carvone, a major component of caraway essential oil, is also used to inhibit sprouting in stored potatoes and onions (Sanli et al., 2010). Krkic et al. (2013) reported that a chitosan-caraway coating prepared by 0.20% caraway essential oils is useful for preservation of a dry fermented sausage. This natural coating could prevent the food from lipid oxidation and maintained the higher score of odor and taste after storage for five months. The impact of caraway seed meal on fish growth and performance has been evaluated by supplementation of the fish diet with different concentrations of caraway seed meal (5, 10, 15, and 20 g/kg). The data obtained during a 12-week period showed that the fish weight gain and feed utilization were significantly increased in fish fed diet containing caraway seed meal at 10 g/kg (Ahmad and Abdel-Tawwab, 2011). The use of caraway seed as a feed additive in fish diets affected growth performance, feed utilization, and whole-body composition of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings.

The information available on the antimicrobial effects indicates that among the phytochemicals studies, caraway has medium antibacterial activity in addition to its antioxidant properties. Caraway essential oils possess antibacterial (Shan et al., 2007), fungicidal (Soliman and Badeaa, 2002), insecticidal (Lopez et al., 2008), acaricidal (Damiani et al., 2009), larvicidal (Pitasawat et al., 2007), and molluscicidal (Kumar and Singh, 2006) activities. Therefore, it is suggested that the essential oil may be considered as an interesting source of antispoilage agent which can be used in food preservation.

The antibacterial action of the caraway essential oils tested in our laboratory using the disk diffusion technique based on minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations showed that *Bacillus subtilis* is

highly susceptible to the oil effects. Likewise, the caraway essential oils had potential antibacterial effects on *Escherichia coli* (Fatemi et al., 2011). Although the mechanism(s) of antimicrobial action of the caraway essential oils is not well understood, it seems that the major target of the cells is outer coating, mainly the lipid bilayer of the cell membrane (Burt, 2004). However, concerning the antibacterial action of the caraway preparations, there is a great variability among caraway populations and cultivars. The variability is estimated based on morphological and yielding traits, essential oil content and composition (Seidler-Lozykowska and Bocianowski, 2012) that have also been genetically determined (Toxopeus et al., 1995). The antibacterial action of the essential oils prepared from three Apiaceae species was assessed on several food-borne pathogens, namely *Staphylococcus aureus*, *Bacillus cereus*, *E. coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. According to this experiment, the ranges of MIC of the oils were 0.03–0.5, 0.18–3.0, and 0.37–3.0 mg/mL, respectively, for *Carum copticum*, *Bunium persicum*, and *C. cyminum*. Moreover, it was found that the combination of *B. persicum* and *C. cyminum* essential oils confirmed synergistic and additive activities against the pathogens (Oroojalian et al., 2010).

The antifungal properties of 18 essential oils have also been examined in vitro by adding the oils to the growth medium of different pathogens (*Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Alternaria citrii*, *Botrytis cinerea*, and *Penicillium digitatum*) isolated from mango, avocado, citrus, grapes, and cactus pear. The inhibitory properties of some of the major compounds of the oils, identified and quantified by gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionization detection (GC-FID), were also determined. Most of the oils were selected on the basis of commercial availability and for containing a predominant compound. The visual observation method of fungal growth is often used to estimate the lowest concentration of the extract capable of inhibiting fungal growth.

EXTRACTION AND PURIFICATION TECHNOLOGIES FOR THE EXTRACTION OF ESSENTIAL OILS

Different techniques have been developed for the extraction of essential oils from caraway in order to improve the yield of the essential oils. Supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, and hydrodistillation by direct induction heating assisted by magnetic field are major methods applied for the extraction of essential oils of caraway. In our laboratory (Fatemi et al., 2011), the essential oils prepared from caraway were extracted using a Clevenger-type apparatus. The extraction was carried out for 2 h and the extraction yields of the essential oils from the seeds were about 7% (w/w). GC analysis was performed using a GC-FID (9-A Shimadzu, Kyoto, Japan). The analysis was carried out using a DB-5 fused-silica column (30 m, film thickness 0.25 mm) with a temperature program of 40–250 °C at a rate of 4 °C/min, injector temperature of 250 °C, detector temperature of 265 °C, and helium as the carrier gas. The individual oil components were further characterized by GC-MS analysis. The oil constituents were identified with the help of the data in the computer library and by comparing with authentic standards. The identifications were confirmed by comparison of their retention indices either with selected authentic standards or with the literature data.

Another technique, called Green technique, has also been developed for extraction of essential oils from caraway seeds without any added solvent or water. In this technique, extraction is facilitated by microwave dry-diffusion and gravity (MDG). Essential oils isolated by MDG were quantitatively (yield) and qualitatively (aromatic profile) similar to those obtained by the conventional hydrodistillation method. Analysis of fatty acid content of caraway indicated that petroselinic acid is the major fatty acid (55.9%) followed by palmitic (23.82%) and linoleic (12.40%) acids. Generally, the environmental factors such as water deficit can affect the palmitic acid content and the double bound index of the fatty acid pool and thus the oil quality (Bettaieb Rebey et al., 2012).

SAFETY ISSUES OF DRYING AND STORAGE OF CARAWAY SEEDS

It has been reported that caraway seeds are susceptible to insect and disease attacks, contamination by pesticides, and aflatoxins through different ways such as environmental pollution during cultivation, harvest, and storage conditions (Abou-Arab et al., 1999). Therefore, the approach used to prevent contamination of the dried seed during storage and transport is important for maintaining the quality and the biological properties of the product. It appears that γ-irradiation is the method of choice in food preservation of dried fruits and medicinal plants that can be an effective way to prevent contamination of food products on storage. Irradiation treatment of food is becoming an increasingly accepted processing option for developing countries willing to meet growing sanitary and regulatory requirements in international trade. A review published by Chmielewski and Migdal dealt with the impact of γ-irradiation treatment at a dose of 10 kGy on the stability of main biologically active substances of spices (Chmielewski and Migdal, 2005). Meanwhile, there are concerns about the consumption of γ-irradiated products because of the generation of toxic metabolites and possibilities of a decrease in the

biological activity of the active components present in the food products. Black cumin (*Nigella sativa* L.) samples irradiated at doses ranging from 2.5 to 10 kGy indicated that increasing radiation dose can raise both free fatty acid and peroxide values, whereas oil contents, iodine numbers, refraction index, and Rancimat values were decreased. These changes were associated with changes in the composition of fatty acids (Arici et al., 2007). There are evidences which show that despite the irradiation-related changes in chemical composition of the oils, biological properties of essential oils remain unaffected.

SUMMARY POINTS

- Caraway is among the widely used spices which are used as crude or essence in various food products for its pleasant flavor and antispoilage properties.
- Crude and purified preparations of caraway seeds have antimicrobial and antioxidant properties and can be used as a natural food preservative.
- The essential oils derived from caraway are rich in nutraceutical compounds with pharmacological properties and can be used in alternative and complementary medicine.
- Like other dry fruits, caraway seeds are susceptible to contamination; hence, they need careful drying and storage.

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Chapter 33

Cardamom (*Elettaria cardamomum* Maton) Oils

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INTRODUCTION

The term cardamom refers to several plants of genera *Elettaria* and *Amomum* in the Zingiberaceae family. The plants of both of these genera are recognized by their spindle-shaped pods with small black seeds and characteristic aroma. Corresponding to the said two genera are two different types of cardamom, one is known as true cardamom, green cardamom, or small cardamom (*Elettaria cardamomum*) and the other is recognized as large cardamom or black cardamom (*Amomum subulatum*) (Bhide, 2010).

Green cardamom (*E. cardamomum*) is distributed in several Asian regions from India to Malaysia. The dried fruits (seed pods) of this perennial herb are marketed as one of the most expensive and valued spices. The small black seeds of cardamom, which are embedded in a thin papery outer shell or pod, have a pleasant aroma and a characteristic, slightly pungent taste (Padmakumari et al., 2010).

Green cardamom has been used all over the world for culinary and traditional pharmaceutical applications. This spice is not only employed as a flavoring and spicy ingredient in several food products, but is also used traditionally as a folk remedy to treat teeth and gum infections, lungs and pulmonary tuberculosis, and digestive and kidney disorders (Hamzaa and Osman, 2012; Saeed et al., 2014). The volatile oils, and other high-value gastroprotective and antioxidant bioactives present in cardamom seeds mainly contribute to its characteristic aroma and role as a functional food, as well as its pharmaceutical and nutraceutical value (Hamzaa and Osman, 2012). The current chapter is mainly designed to highlight the traditional pharmaceutical as well as the food science applications of cardamom and cardamom essential oil.

BOTANICAL ASPECTS

Green cardamom (*E. cardamomum* L. Maton), form the family Zingiberaceae, is an aromatic shrub that can grow up to a height of 2–5 m. Cardamom (*E. cardamomum*) is primarily native to southern India and Sri Lanka but is also widely naturalized in Tanzania and Guatemala. This spice is widely cultivated in several other regions of the world including Nepal, Thailand, Myanmar, Vietnam, Combodia, Morocco, Malaysia, and Central America (Ravindran and Madhusoodanan, 2002). *Elettaria cardamomum* is a herbaceous perennial plant with erect shoots bearing two rows of alternate green or dark green leaves. The leaves are long (around 40–60 cm), lanceolate and glabrous on both surfaces with acuminate apex.

Flowers and Fruit

The flowers of cardamom are white to lilac or pale violet, and are produced in the form of a loose spike 30–60 cm long. The fruit is a triocular, ovoid or oblong, yellow-green pod (1–2 cm long), containing about 15–20 black and brown seeds. The seeds, embedded inside the pod, have a warm, slightly pungent aromatic odor and taste (Telja et al., 2006). Cardamom plants are propagated by vegetative division of the rhizomes or by the seedlings. For good growth, the plant prefers loamy soils rich in organic matter, which are usually acidic in nature with a pH range of 5.0–6.5. It thrives best under moderate shade in evergreen forests. A typical photograph of cardamom plant is presented in Figure 1, while Figures 2 and 3 are representative of flowers and developing seeds and fruit (pods) of cardamom, respectively.

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FIGURE 1 A typical cardamom (*Elettaria cardamomum*) plant. *http://gardentia.net/2013/11/03/cardamom-velchi-ilaychi/. Courtesy of Nandan Kalbag.*

FIGURE 2 Cardamom (*Elettaria* cardamomum) flowers and developing seeds. *http://gardentia.net/2013/11/03/cardamom-velchi-ilaychi/. Courtesy of Nandan Kalbag.*

FIGURE 3 Pods of cardamom (*Elettaria cardamomum*).

USES AND APPLICATIONS

Cardamom is valued as one of the most expensive spice in the world after saffron and vanilla due to its unique flavor and odor. It has been one of the most important herbal spices traded between several civilizations through western Asia (Middle East) to Ancient Egypt, Greece, and Rome. The traditional pharmaceutical properties of this aromatic spice have been narrated in ancient Vedic literature dating back 3000 years. The aboriginal Egyptians used cardamom in perfumes and aromatic biotic materials which release fragrant smoke when burnt. The Greek physicians, botanists, and pharmacologists demonstrated its use as a folk remedy for the treatment of coughs, abdominal pain, spasms, and sciatica (Davis, 2005). In the Greco–Arab (Unani) system of traditional medicine, cardamom is considered as a powerful stimulant, carminative, stomachic, and diuretic; these properties are due to the presence of essential oil and specific chemical constituents (Said, 1983).

The traditional pharmaceutical uses of this spice are mainly attributed to the presence of volatile oils and several bioactives such as phenolics, 1,8-cineole, limonene, linalool, terpinolene, and myrcene (Marongiu et al., 2004; Kurt, 2011). In modern era, cardamom is used in eastern and western practices for curing different ailments like influenza, infection, asthma, bronchitis, cardiac disorder, diarrhea, nausea, and cataracts (Gilani et al., 2008; Khan et al., 2011). Cardamom seeds possess multiple biological roles including antioxidant, antihypertensive, antidiabetic, gastroprotective, laxative, antispasmodic, antibacterial, antiplatelet aggregation, and anticancer activities (Marongiu et al., 2004; Padmakumari et al., 2010). Like ginger and turmeric, cardamom can reduce pain and swelling, especially in mucus membranes, the mouth, and throat. The seeds being carminative, stomachic, and desiccant, are ingested for the treatment of gastrointestinal disorders (Jamal et al., 2006). Likewise, phytonutrients, vitamins, and essential oils in cardamom act as antioxidants, and help to clean up free radicals and resist cellular aging (Saeed et al., 2014).

Moreover, cardamom is a well-known remedy to treat impotence and improve low sexual response (Jamal et al., 2006; Lwasa and Bwowe, 2007). Knowing the highest per person consumption in Saudi Arabia (Ravindran and Madhusoodanan, 2002), the desire or culture of multiple marriages among men may be linked to the wide scale consumption of a popular hot drink (*Gahwa*) with essential ingredient, cardamom. In aromatherapy as massage oil, cardamom essential oil is useful to comfort the digestive system and throat problems and has refreshing effects. Cardamom essential oil, with antimicrobial, antispasmodic, analgesic, and antiinflammatory activities, is reported to inhibit the growth of viruses, bacteria, fungi, and molds (Al-zuhair et al., 1996). The cardamom essential oil is known to provide relief from headache by acting as a gentle tonic for the nervous system. Moreover, cardamom essential oil blended with fennel, ginger, German chamomile oils can relieve symptoms of colic in infants. The essential oil, due to its high 1,8-cineole and terpinyl acetate contents, has profound effects on the respiratory system and thus promotes clear breathing and respiratory health (Ravindran and Madhusoodanan, 2002; Davis, 2005).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Cardamom has well-established gastronomic values and is widely used as an ingredient in a broad range of curries, sweets, and confectionery products. In south Asia, cardamom is commonly used as a flavoring and spice agents in the cuisine of Pakistan, India, Sri Lanka, and Iran. It is often used in traditional Indian sweets and in *masala chai* (spiced tea). In Scandinavia as well as in Germany and Russia it is used to improve the flavor of cakes, pastries, and sausages. In particular, in Finland and Sweden, it is used in traditional treats such as the Finnish sweet bread *pulla* and in the Scandinavian Christmas bread *Julekake* (Mahmud, 2008).

Guatemala is the largest producer of cardamom in the world followed by India. Despite having numerous applications in the cooking and folk medicine systems of the South Asia, about 60% of the world production of cardamom is exported to Arab regions such as south west Asia (western Asia/Middle East) and north African countries where it is mainly used for coffee preparation. Worldwide, India and Saudi Arabia are the two major consumers of cardamom. To meet their domestic needs, these two countries together require more than 50% of the world's cardamom production. Saudi Arabia has the maximum per capita consumption of cardamom where this organic spice is mostly used to prepare a traditional drink *Gahwa*, which is a mixture of cardamom seed powder and coffee with ratio varying between 30:70 to 50:50 (w/w), and is also famous in some other Arab countries such as Kuwait, Muscat, and Qatar (Ravindran and Madhusoodanan, 2002).

In the Middle East cardamom powder is also used as a spice as well as a traditional flavoring agent in coffee and tea. The ground seeds of cardamom, the most important part of the plant, are not only useful in spice mixtures, they are also employed in curries, beverages such as tea and coffee, baked foods, confectionaries, meat products, custards, wines, and liqueurs. The ancient Egyptians chewed green cardamom as a tooth cleaner and to aid digestion (Lwasa and Bwowe, 2007).

From the perspective of modern food science applications, cardamom fruit/pod, its extract, and essential oils can be used as a natural antioxidant and food preservative due to the presence of an appreciable content of phenolics and antimicrobial

agents (Martins et al., 2001; Nair et al., 1998). It has been shown that cardamom considerably inhibited the formation of toxic histamine in mast cells and thus can be explored as a natural antidote to minimize histamine food poisoning (Shakila et al., 1996). In view of the developing microbial resistance of food-borne pathogens against synthetic antimicrobial drugs, there is currently a greater need to explore plant sources of antimicrobials (Saeed et al., 2014).

The essential oil from cardamom has shown strong antibacterial activity against microorganisms (Shahnaz and Ali, 2014). The essential oil inhibited the growth of *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, *Aspergillus niger*, *Geotrichum candidum*, and *Rhodotorula* (Elgayyar et al., 2001; Shahnaz and Ali, 2014). The volatile oil from this spice is reported to inhibit the growth of food-borne viruses and fungus. Moreover, the oil, due to insecticidal activity, has the potential to act as a wheat grain protectant by attacking on *Tribolium castaneum* and *Sitophilus zeamais*, via contact and fumigant action (Huang et al., 2000), suggesting its potential uses as preservative in the food industry.

There is strong evidence that natural antioxidants are safer and possess several potential health benefits due to their effective free radical scavenging properties (Saeed et al., 2014). Cardamom fruit and its essential oils have considerable amounts of antioxidant phenolics and flavonoids (quercetin, kaempferol, luteolins, and pelargonidin), and can be used as a natural antioxidant additive in the food industry (Nair et al., 1998). The essential oil is effective in increasing levels of glutathione (a natural antioxidant in the body) in a dose-dependent manner from 100 to 5000 ppm (Badei et al., 1991).

The essential oil can be blended with vegetable oils such as coconut oil to develop essential lotions and ointments for healthy skin cell functioning. Due to its powerful cleansing, purifying, and insect repellent activities, cardamom essential oil can be mixed with lemon essential oil to develop natural wood preservative and polish. The essential oil, which is mostly employed for flavoring purposes in processed foods, can also be used in some liquid products including beverages and fruit drinks, alcoholic preparations, and liquors. Currently, the perfume and cosmetic industry uses essential oils as well as fruit extracts from several herbs and plants including cardamom to blend with other volatile and vegetable oils and develop naturally flavored cosmetic products and perfumes. Cardamom essential oil and oleoresins are also used as a soluble spice in the processed food industry for flavoring purposes (Ravindran and Madhusoodanan, 2002).

In line with the new development of natural and novel foods, a range of marketable and cardamom-derived products have been launched. For example, a special aromatic tea is prepared by adding cardamom along with tea and lime having a refreshing taste and odor. Cardamom-flavored hot water along with a variety of other products such as cardamom-flavored biscuits, drinks, milk, cheese, and chocolate, etc., are marketed in India and other regions. In order to diversifying and enhancing the commercial uses of cardamom for development of innovative products, cardamom flavor has been encapsulated using modern approaches. Encapsulated spices have unique features such as uniform flavor strength, instant flavor release and dispersion, free flowing ability, and ease of applications. In this regard, cardamom cola, cardamom coffee, and cardamom tea with added cardamom instant flavor are some of the novel processed products developed by the researchers. There is an extensive need to initiate innovative cardamom products development programs to further diversify the potential uses of this valuable herb leading to preparation of novel cardamom flavour-based foods and drinks (Ravindran and Madhusoodanan, 2002).

ESSENTIAL OIL YIELD AND CHEMICAL COMPOSITION

Essential oils are volatile products derived from different parts of plants such as flowers, leaves, stem bark, and roots. The extraction of essential oil from plant material can be performed by different methods such as supercritical fluid extraction, solvent extraction, hydrodistillation, steam distillation, cold or hot pressing, effleurage, and phytonic process (Samer et al., 2008). The percentage yield of the essential oil obtained from cardamom may vary from 0.2% to 5.6% depending upon the part of the plant used and technique of the extraction (presented in Table 1). The composition of cardamom essential oil, as studied by different researchers, reveals that 1,8 cineole (20–60%) and α-terpinyl acetate (20–55%) are the major chemical components (Padmakumari et al., 2010). The characteristic cardamom aroma is actually developed by a combination of the major components, 1,8- cineole and α-terpinyl acetate (Olivero-Verbel et al., 2010). The major chemical constituents that impart the sweet flavor to the oil are α-terpinyl acetate, geranyl acetate, nerol, and α-terpineol; while 1,8- cineole imparts a harsh odor. The essential oil of *E. cardamomum* harvested from Tehran (Iran) showed the presence of two compounds, 1,8-cineole and α-terpineol, as major compounds along with terpinene and fenchyl alcohol (Marongiu et al., 2004). It is well known that the chemical composition of cardamom essential oil varies considerably in relation to genetic makeup of the variety, region, and age of the plant as well as depending upon the isolation method used (Shahnaz and Ali, 2014).

Cardamom essential oil samples from Italy contained α-terpinyl acetate and 1,8-cineole as the major constituents followed by linalyl acetate, limonene, and linalool. The seeds volatile oil of *E. cardamomum* from south India mainly contained α-terpinyl acetate (56.87%) 1,8-cineole (15.13%), α-terpineol (4.67%), and limonene (4.05%)

(Savan and Kucukbay, 2013). Similarly, Sharma et al. (2011) reported that cardamom essential oils from different regions of India contained α-terpinyl acetate (61.65–68.10%), 1,8-cineole (7.23–11.76%), and α-terpineol (2.91–5.51%) as the principal components among others. In another study, the major components of cardamom essential oil from Turkey were found to be 1,8-cineole (25.6%), linalool (6.3%), and α-terpinyl acetate (40.7%) (Gochev et al., 2012). The major components of cardamom essential oil from Gorakhpur were noted to be α-terpinyl acetate (44.3%), 1,8-cineole (10.7%), α-terpineol (9.8%), and linalool (8.6%). Moreover, the extract from the seeds of cardamom obtained from Guatemala showed the presence of terpinyl acetate (36.8%), 1,8-cineole (29.2%), linalyl acetate (5.2%), sabinene (3.9%), and linalool (3.1%) (Singh et al., 2008). The chemical composition of cardamom essential oil from various regions is presented in Table 2.

The structure of some important chemical compounds of cardamom essential oils are shown in Figure 4.

TABLE 1 Essential Oil Yield from Different Parts of Cardamom (*Elettaria cardamomum*)

Parts	Technique	Yield (%)	Reference
Seeds	Steam distillation and oil is extracted with n-hexane	Nr	Huang et al. (2000)
Leaves	Hydrodistillation	Nr	Mahmud (2008)
Seeds	Steam distillation	0.21–5.60	Subaddarage et al. (1985)
Fruits	Hydrodistillation	3.25	Shahnaz and Ali (2014)
Fruits	Hydrodistillation	1.00	Ebru and Zehra (2013)
Leaves	Super critical fluid extraction	Nr	Samer et al. (2008)
Seeds	Super critical fluid extraction	0.40–0.80	Narayanan and Cadavallur (1991)

Nr, not reported.

TABLE 2 Chemical Composition of Cardamom (*Elettaria cardamomum*) Essential Oil

Origin/Parts	Major Components	Yield (%)	Reference
South India/seeds	Limonene	4.05%	Savan and Kucukbay (2013)
	1,8-cineole	15.13%	
	α-terpineol	4.67%	
	α-terpinyl acetate	56.87%	
Turkey/seeds	1,8-cineole	25.6%	Gochev et al. (2012)
	linalool	6.3%	
	α-terpinyl acetate	40.7%	
Guatemala/seeds	terpinyl acetate	36.8%	Singh et al. (2008)
	1,8-cineole	29.2%	
	linalyl acetate	5.2%	
	Sabinene	3.9%	
	Linalool	3.1%	
New Delhi/fruits	1,8-Cineole	85.2%	Shahnaz and Ali (2014)
	cis-ocimene	3.7%	
	α-terpinene	2.2%	
	Formic acid	2.1%	

Continued

TABLE 2 Chemical Composition of Cardamom (*Elettaria cardamomum*) Essential Oil—cont'd

Origin/Parts	Major Components	Yield (%)	Reference
Sri Lanka/fruits	α-Terpinyl acetate	74.6%	Subaddarage et al. (1985)
	1,8 Cineole	4.4%	
	Linalol	12.7%	
	β-Terpineol	1.8%	
Pakistan/leaves	4-Terpineol	30.26%	Mahmud (2008)
	1,8-Cineol	25.74%	
	α-terpiene	9.807%	
	Linalool	2.67%	

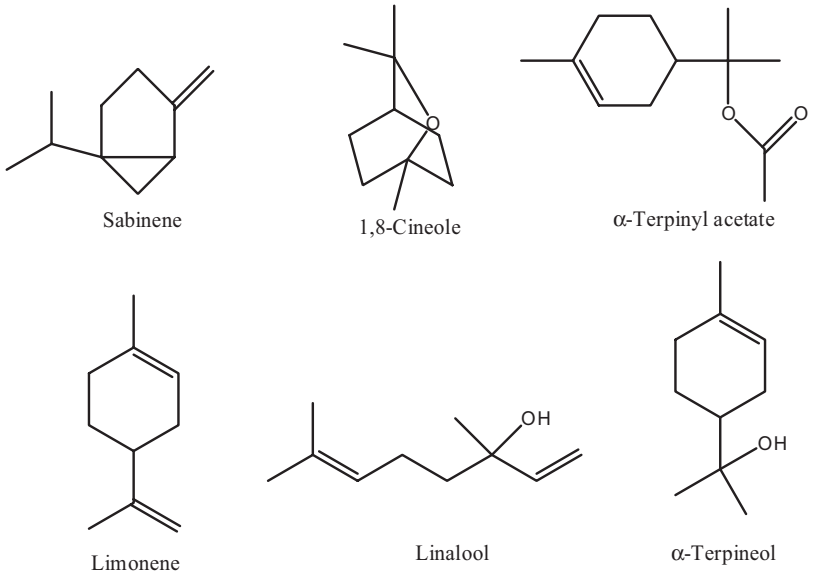

FIGURE 4 Structure of some important chemical components of cardamom (*Elettaria cardamomum*) essential oil.

SUMMARY POINTS

- Cardamom (*E. cardamomum*) is an aromatic herb from the family Zingiberaceae with a pleasant aroma and a characteristic, pungent taste.
- In South Asia, cardamom is mainly used as a spice and flavoring ingredient in cooking.
- In the Gulf region, cardamom is commonly employed as flavoring ingredient for coffee and tea, while in Saudi Arabia, the largest consumer in the world, it is mainly used for preparation of *Gahwa*.
- Cardamom possesses multiple traditional pharmaceutical uses.
- Cardamom contains considerable amounts of valuable natural antioxidants and volatile constituents with multiple bioactivities.
- The characteristic aroma of cardamom and its essential oils is due to the presence of the major components, 1,8- cineole and α-terpinyl acetate.
- The percentage yield of the cardamom essential oil ranges from 0.2 to 5.6% depending on the part of the plant used and the origin of harvest.
- A range of cardamom-flavored traditional and novel processed products have been developed.

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Chapter 34

Carrot (*Daucus carota*) Oils

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INTRODUCTION

Carrots produce a diversity of phytochemicals including α-tocopherol, carotenoids, phenolics, and polyacetylenes, many of which have antioxidant and other health promoting effects (Metzger and Barnes, 2009). They are highly nutritious and rich in provitamin A, vitamins C, D, E, K, B1, B6, and biotin, minerals such as magnesium, calcium, potassium, phosphorus, organic sodium, and many other trace minerals. According to traditional Chinese medicine, carrots are beneficial to a variety of organs including the eyes, ears, lungs, stomach, liver, pancreas, intestine, and skin. They have been referred to as tiny ginseng. Ginseng is a very expensive medicinal material, but carrots are inexpensive and very popular, and are consumed in the form of carrot juice, carrot soup, salad, and carrot cakes.

BOTANICAL ASPECTS

Carrot (*Daucus carota*) is a biannual plant that can attain a height of 1 m. The flowering season spans from June to August. The umbels appear white and round in full blossom. The storage root accumulates a large quantity of carotenoids. Prior to domestication the carrot root appeared white. Carotenoid-rich orange carrots are the result of intensive breeding. Purple carrots and carrots of other colors are available. Roots exposed to light do not enlarge to the same extent as roots maintained in darkness, and they possess lutein-rich chloroplasts in lieu of β-carotene-rich chromoplasts found in underground roots (Rodriguez-Concepcion and Stange, 2013). Tomato yield can be augmented by intercropping with carrot. Wasps that predate on garden pests are attracted by carrot flowers. Irrigation water containing the heavy metals chromium and nickel can cross-contaminate potatoes and onions but not carrots grown in soil previously never contaminated with these heavy metals (Stasinos and Zabetakis, 2013).

Organic carrots have been reported to have a higher content of carotenoids, phenolic acids, vitamin C, and dry matter than conventional carrots. In contrast, Søltoft et al. (2011) did not find a higher content of carotenoids in organic food products. The method of cultivation did not exert an unequivocal effect on the nutritional quality of carrots or health-related biomarkers in a rat model.

Kjellenberg et al. (2010) studied the levels of three principal polyacetylenes including falcarindiol, falcarindiol-3-acetate, and falcarinol in carrots harvested several times per season and at different places in Sweden. Falcarindiol and falcarindiol-3-acetate first declined at high levels and then rose later in the harvest season while falcarinol did not display major changes during the harvest season. During storage, the amount of polyacetylenes rose in samples with initially low levels and declined in samples with initially high levels. The amounts of the polyacetylenes changed depending on ambient conditions and varied between fresh and stored samples. It has yet to be investigated whether it is possible to achieve in carrots a polyacetylene composition at harvest with a minimal risk of bitter off-taste and maximal health effects.

USAGE AND APPLICATIONS

The consumption of carrot and its products is on the rise owing to the rich content of antioxidants. Carrots can be eaten in different ways: fried, steamed, boiled, or cooked in stews and soups. In India, carrots are used in salads and in preparation of curries or added to spicy rice. Baby carrots or mini carrots are peeled carrots cut into cylindrical forms sold as snack food. Pureed carrots are fed to babies. Carrot juice, chips, flakes, powder, bread, biscuits, cakes, puddings, jams, and preserves are available. Carrot greens are harvested before the root has developed to a sizable extent, and eaten stir-fried, or in salads.

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Aliphatic C(17)-polyacetylenes of the falcarinol type, which occur in common food plants of the Apiaceae family such as carrot, celeriac, parsnip, and parsley exhibit antibacterial, antimycobacterial, and antifungal activities (Christensen, 2011). Carrot oil extract demonstrates antioxidant activity. Retinoic acid, the biologically active form of vitamin A present in abundance in carrots, acts on dendritic cells, B cells, T cells, invariant natural killer T (iNKT) cells, and macrophages in the immune system. Retinoic acid mitigates concanavalin A-induced hepatitis by downregulating interleukin-4 and interferon-γ production in disease-causing NKT cells in the liver (Nagy, 2012).

The LC_{50} (50% lethal concentration, i.e., concentration causing half of the treated organisms to die) values of acetone, ethanol, hexane, and methanol extracts for *D. carota* against fourth instars of *Culex annulirostris* using were 236, 37, 77, and 242 mg/L, respectively. The corresponding LC_{50} values of *Khaya senegalensis* extracts were 20, 5, 5, and 7.6 mg/L, respectively, indicating a higher potency (Shaalan et al., 2006).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Composition

Geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate) is a constituent of carrot prepared by fractional distillation of essential oil as well as a constituent of many other essential oils. It is used as perfumes for soaps and creams and also as a flavoring ingredient. Carrot root oil obtained by supercritical fluid carbon dioxide extraction was rich in carotenes, monoterpene volatiles, phenolics, phytosterols, sesquiterpene, and waxes. Its most prominent components were α-humulene, α-pinene, β-caryophyllene, β-sitosterol, campesterol, cycloartenol, 1-hexacosanol, linolenic acid, 24-methylencycloartanol, sabinene, stigmasterol, trilinolein, and C38 waxes (Ranalli et al., 2004).

The composition of the oils of *D. carota* subsp. *halophilus* from Portugal demonstrated variations in ontogenesis, especially in the levels of elemicin that rose in the mature umbels. In the essential oils from roots and flowers of *D. carota* L. ssp. *Maritimus*, there were six and 32 identified compounds, respectively. Root oil was composed of myristicin (29.7%), dillapiole (46.6%), and phenolic derivatives (76.3%). The flower essential oil had an abundance of monoterpene hydrocarbons (68.4%) and belonged to the sabinene (4; 51.6%) type. The oxygenated monoterpenes represented the second major fraction of the same oil among which terpinen-4-ol (11.0%) was the predominant compound. The main components in the essential oil prepared from flowering and mature umbels with seeds of Sardinian wild *D. carota* L. subsp. *carota* were11-α-(H)-himachal-4-en-1-β-ol (9.0–21.6%) and β-bisabolene (17.6–51.0%). On the other hand, oils from samples of Portuguese counterparts were mainly made up of α-pinene (3.5–37.9%) and geranyl acetate (5.2–65.0%). Supercritical extracts had more sesquiterpene hydrocarbons and fewer monoterpenes (Maxia et al., 2009).

The main components of the essential oil of the flowering and mature umbels with seeds of wild *D. carota* L. subsp. *carota* from Sejnane in Tunisia were α-selinene (7.4–8.6), 11-α-(H)-himachal-4-en-1-β-ol (12.7–17.4%), carotol (3.5–5.2%), eudesm-7(11)-en-4-ol (8.2–8.5%), and sabinene (12.0–14.5%), whereas the oils from Tunis in Tunisia were mainly made up of carotol (48.0–55.7%) and elemicin (31.5–35.3%) (Marzouki et al., 2010). The flavones luteolin, luteolin 3′-O-β-D-glucopyranoside, and luteolin 4′-O-β-D-glucopyranoside were pharmacologically active constituents of carrot seed extract (Kumarasamy et al., 2005). Carrot psyllid (*Trioza apicalis*) feeding ensued in a rise in the concentrations of β-pinene, limonene, and sabinene in the foliage of both carrot cultivars examined, Parano and Splendid (Table 1). Emission of (Z)-β-ocimene was the only significant volatile induction from Parano leaves (Table 2). Limonene treatment elevated the (Z)-β-ocimene and total phenolic concentrations in the foliage. Exposure to methyl jasmonate and limonene induced a defense response from the carrot, but limonene spray did not effectively deter egg laying by carrot psyllid (Nissinen et al., 2005).

Carrots produced with the application of a lower level of nitrogen fertilizers displayed a less bitter, less earthy, but a better, more fruity, intensive, and sweet taste. They possessed more total sugar and dry matter. There was a positive correlation between the sweet taste and the total sugar content, and the intensive taste and the quantity of essential oils (Schaller et al., 1998). Sesquiterpene hydrocarbons were identified as principal constituents in essential oils of carrots collected from 10 wild populations distributed in northern Tunisia. The main volatile compounds comprised β-bisabolene, elemicin, geranyl acetate, and sabinene. There were variations in the composition of volatile compounds among the populations (Rokbeni et al., 2013).

Carrot seed oil has a yellowish brown color and a soft, sweet earthy grounding aroma. Schaller et al. (1998) studied, in carrots, the association between the sensory quality and the contents and composition of essential oils and total sugars as affected by nitrogen fertilization. The carrot variety *Nanthya* F1 was cultivated at three different levels of inorganic nitrogen fertilization. Flavor components with medium and higher boiling points were extracted as essential oils and resolved by gas chromatography. Flavor components with lower boiling points were collected from the headspace and subjected to chemosensorial analysis. The results of sensory assessments employing an untrained panel of 300 people were compared with

TABLE 1 Concentrations (Mean ± Standard Error, µg/g of Fresh Weight) of Some Monoterpenes and Sesquiterpenes, and Propenyl Benzenes in the Leaves of Undamaged and Carrot Psyllid (*Trioza apicalis*)-Damaged Leaves of Two Carrot Cultivars, Parano and Splendid (n = 6)[a]

		Parano		Splendid		Main Effects	
Compound	KI[b]	Undamaged	Damaged	Undamaged	Damaged	Cultivar	Treatment
α-Pinene	939	4.5 ± 1.0	20.3 ± 6.0	9.7 ± 4.9	16.4 ± 6.2	ns	ns
Sabinene	976	66.9 ± 14.9	102.2 ± 10.1	0.9 ± 0.1	1.5 ± 0.2	0.000	0.027
β-Pinene	978	nd	4.9 ± 2.4	0.6 ± 0.2	2.7 ± 1.3	ns	0.008
Myrcene	984	61.6 ± 8.1	92.2 ± 13.0	47.9 ± 10.7	53.9 ± 10.0	0.045	ns
Limonene	1025	9.7 ± 0.8	14.3 ± 1.2	17.7 ± 4.2	28.7 ± 5.4	0.004	0.054
(Z)-β-Ocimene	1025	2.1 ± 0.4	5.4 ± 1.5	5.7 ± 1.8	2.3 ± 0.9	ns	ns
Terpinolene	1081	0.6 ± 0.4	4.8 ± 3.0	0.3 ± 0.2	0.8 ± 0.4	ns	ns
Bornyl acetate	1278	0.5 ± 0.2	1.4 ± 0.5	0.9 ± 0.2	3.0 ± 0.8	0.012	ns
β-Caryophyllene	1418	9.3 ± 1.7	15.6 ± 5.6	19.8 ± 6.6	26.6 ± 5.9	ns	ns
Methylisoeugenol		27.5 ± 18.2	42.3 ± 10.2	88.9 ± 18.7	99.4 ± 12.4	0.007	ns
α-Asarone	1587	0.4 ± 0.2	1.4 ± 0.6	2.3 ± 1.1	1.1 ± 0.4	ns	ns

[a]*Carrot psyllids were allowed to feed for 7 days on the carrots before they were removed. Terpenoids were extracted five or six days after the removal of the carrot psyllids. Main effects were tested using the generalized linear model (GLM) procedure using lg (x + 1) transformed data. ns indicates nonsignificant main effect, nd indicates not detected compound, and tr indicates a compound with concentration under 0.1 µg/g of formula weight (fw).*
[b]*Kovats indices, obtained from Davies (1990), Deng et al. (2004).*
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TABLE 2 Concentrations (Mean ± Standard Error, ng/plant/hour) of Some Monoterpenes and Sesquiterpenes, and Propenyl Benzenes in the Headspace Emission of Undamaged and Carrot Psyllid (*Trioza apicalis*)-Damaged Leaves of Two Carrot Cultivars, Parano (Control, n = 4; Damaged, n = 6) and Splendid (n = 6)[a]

		Parano		Splendid		Main Effects	
Compound	KI[b]	Undamaged	Damaged	Undamaged	Damaged	Cultivar	Treatment
α-Pinene	939	1.3 ± 0.6	26.2 ± 21.4	7.6 ± 2.8	17.2 ± 14.2	ns	ns
Sabinene	976	12.6 ± 4.0	100.6 ± 73.1	1.8 ± 0.7	2.2 ± 2.0	0.003	ns
β-Pinene + myrcene	978	21.5 ± 9.7	82.1 ± 31.5	52.0 ± 25.2	98.5 ± 88.6	ns	ns
(Z)-β-Ocimene	1025	0.4 ± 0.4	1.5 ± 0.5	nd	nd	0.000	0.013
Limonene	1025	2.5 ± 0.4	9.5 ± 4.6	105.4 ± 62.6	63.8 ± 47.6	0.008	ns
Terpinolene	1081	nd	10.0 ± 8.3	4.0 ± 4.0	1.7 ± 1.3	ns	ns
Nonanal	1104	nd	1.4 ± 1.4	nd	1.6 ± 1.6	ns	ns
Methyl salicylate	1234	0.4 ± 0.2	0.3 ± 0.2	1.7 ± 0.6	1.3 ± 0.6	0.038	ns
Bornyl acetate	1278	nd	2.0 ± 1.3	0.5 ± 0.2	0.6 ± 0.2	0.007	ns
β-Caryophyllene	1418	0.1 ± 0.1	19.2 ± 15.9	6.3 ± 2.6	19.0 ± 15.4	0.008	ns
Methylisoeugenol		nd	8.1 ± 6.7	17.8 ± 12.7	10.4 ± 5.0	0.051	ns

[a]*Carrot psyllids were allowed to feed for 7 days on the carrots before they were removed. Terpenes were collected within 32 h after the removal of the carrot psyllids. Main effects were tested with GLM procedure using lg (x + 1) transformed data. ns indicates nonsignificant main effect, and nd indicates not detected compound.*
[b]*Kovats indices, obtained from Davies (1990), Deng et al. (2004).*
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data from chemical analyses. Carrots grown with lower nitrogen fertilization were characterized by a less earthy and bitter, but sweeter and more fruity taste, and a higher content of dry matter and sugar. The essential oil composition and quantity depend on the level of nitrogen fertilization. The intensity and sweetness of the taste show a positive correlation with the amounts of essential oils and total sugars, respectively. Carrots can be distinguished from one other by using chemosensorial headspace analyses based on the nitrogen fertilization levels.

Antimicrobial Activity

Helicobacter pylori infection may cause gastritis, peptic ulcers, and gastric cancer. Although oral administration of carrot seed oil failed to reduce bacterial loads in the treated animals, *H. pylori* infection was cleared in one-fifth to nearly one-third of the animals. Thus carrot seed oil may be employed as food additives to complement treatment (Bergonzelli et al., 2003). The antibacterial effect of essential oils from flowers and roots resulted in the inhibition of a series of common human pathogenic bacteria, and of some clinically and environmentally isolated strains. Carrot seed oil was highly active against *Campylobacter jejuni*. Elemicin and (E)-methylisoeugenol in the essential oil of wild *D. carota* L. obtained from aerial parts at the end of the flowering stage exerted an inhibitory action on *Escherichia coli*, *C. jejuni* (Table 3), and *Campylobacter lari* (Rossi et al., 2007). The antibacterial effect of essential oils from flowers and roots resulted in the inhibition of a series of common human pathogenic bacteria, and some clinically and environmentally isolated strains (Jabrane et al., 2009). Sardinian carrot oils manifested potent antifungal activity toward dermatophytes and *Cryptococcus neoformans*. Doses of carrot oil exhibiting antifungal activity toward *Aspergillus* species, dermatophytes and yeasts exerted minimal cytotoxicity toward mammalian cells up to 24 h (Tavares et al., 2008). The minimum inhibitory concentration (MIC values) for antimicrobial activity of toward *E. coli* and *Staphylococcus aureus*, and clinical strains of *Candida albicans* and *Candida tropicalis*, as determined with the broth dilution assay, exceeded 2.5%(v/v) (Marzouki et al., 2010). The isolated carrot essential oils exhibited activity against Gram-negative *E. coli* and *Salmonella typhimurium*, Gram-positive *S. aureus*, and the yeast *C. albicans* in vitro (Rokbeni et al., 2013). Thus the essential oil has the potential to be used as for the control of food-borne pathogenic and food spoilage microbes.

Metabolic Effects

Consumption of 16 fl oz of daily freshly squeezed carrot juice for three months was devoid of any effect on plasma insulin, leptin, cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, apolipoprotein A, apolipoprotein B, body fat percentage, interleukin-1α, or C-reactive protein. Systolic pressure but not diastolic pressure was slightly reduced. Plasma total antioxidant capacity was significantly elevated and plasma malondialdehyde production declined (Potter et al., 2011).

Adverse Effects of Excessive Intake of Carrots

Carotenoids like β-carotene are beneficial to health when ingested at levels found in food, but adverse effects may appear when consumed in excessive dosages by smokers or after exposure to asbestos (Paiva and Russell, 1999). A middle-aged man who consumed six to seven pounds of carrots weekly experienced abdominal discomfort and exhibited hypercarotinemia, yellow/orange skin coloration, and elevated liver enzymes (Sansone and Sansone, 2012).

TABLE 3 Antibacterial Activities of Some Constituents of Carrot Essential Oil Against *Campylobacter jejuni*

	Minimum Inhibitory Concentration (μg/mL)
Isoeugenol	125
(E)-Methylisoeugenol	125
Eugenol	250
Methyleugenol	250
Elemicin	250
3,4-Dimethoxystyrene	250

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It can be seen from the foregoing account that carrot consumed in the right amount is good for health because of the antioxidant, antibacterial, and antifungal activities. Nevertheless, hepatotoxicity arises in cases of overdosage. With the dedicated effort of many investigators, a wider application of carrot essential oil can be anticipated.

SUMMARY POINTS

- Geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate) is a constituent of carrot essential oil.
- Carrot root oil is abundant in carotenes, monoterpene volatiles, phenolics, phytosterols, sesquiterpene, and waxes.
- Carrot flower essential oil is rich in monoterpene hydrocarbons.
- The flavones luteolin, luteolin 3′-O-β-D-glucopyranoside, and luteolin 4′-O-β-D-glucopyranoside are bioactive constituents of carrot seed extract.
- Carrot essential oil exhibited antibacterial and antifungal activities.
- Lycopene is a carotenoid present in carrots. It has antioxidant activity and may be of some therapeutic value in some diseases.

DISCLAIMER

This article was written in a personal capacity (E.F.F.) and does not represent the opinions of the United States Food and Drug Administration, the United States Department of Health and Human Services, or the United States Federal Government.

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Chapter 35

Carvone (*Mentha spicata* L.) Oils

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List of Abbreviation

EO Essential oil

INTRODUCTION

Carvone is utilized as a component of caraway and dill, two of the oldest cultivated spices, and the worldwide-used aromatic plant spearmint. It is a monoterpene, belonging to the terpenoids, a large and structurally diverse family of natural products derived from C5 isoprene units, generally joined in a head-to-tail fashion, first identified in turpentine (*Terpentin* in the German language). Typical structures contain carbon skeletons represented by $(C5)_n$. Terpenoids are classified as hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes, (C20), sesterterpenes (C25), triterpenes (C30), and tetraterpenes (C40). Most monoterpenes are optically active: there are many examples known where enantiomeric forms of the same compound can be isolated from different sources. Carvone is one of such example: S-(+)-carvone (or D-carvone) is present in relevant amount in caraway and dill, whereas R-(−)-carvone (or L-carvone) can be found in spearmint. The individual enantiomers produce different biological responses, especially toward olfactory receptors in the nose that, thanks to the presence of chiral groups, respond to the two mirror image forms differently. Thus, the characteristic odor of caraway is due to D-carvone, whereas L-carvone smells of mint. D-carvone has a mentholated, spicy aroma with rye notes and medium strength, whereas L-carvone has a minty and sweetish medium-strength odor. Moreover, the aroma of the stereoisomers may be perceived at very different concentrations: D-carvone can be perceived at 600 ppb, whereas L-carvone has an aroma threshold of just 43 ppb.

On the contrary, the two stereoisomers have the same safety use threshold, quantified as a maximum daily intake of 2390 μg/capita/day.

D-carvone is the main component (50–76%) of the essential oil extracted from caraway seeds (*Carum carvi* Linneus 1753), but it occurs even in relevant quantities in the oils extracted from dill (*Anethum graveolens* Linneus 1753) ranging from 30% to 60% and from Indian dill (*Anethum sowa*) at a concentration of 20–60%. *Mentha spicata*, the major source of L-carvone, is a very profitable cultivation for farmers. Carvone high-content genotypes are now commercially available; however, there is still room for further improvement, as demonstrated by the finding of accessions with exceptionally high carvone content, exceeding 70% of the whole oil (Chauhan et al., 2009).

BOTANICAL ASPECTS

Carum carvi belongs to the genus *Carum*, Apiaceae family. Papini et al. (2007) used internal transcribed spacer DNA sequence analysis and revealed that this genus is polyphyletic. In particular, *C. carvi* L. clustered within the tribe *Careae* Baill. (formerly *Aegopodium* clade). Caraway can be found in several European and Mediterranean natural environments and its habitats are moist meadows, arable land, and waste places from lowland to mountain elevations. It is andromonoecious, has perfect florets, and is protandrous and strongly dichogamous, the stigmata not being receptive until several days after pollen is shed. Caraway is pollinated by bees and the fruits are schizocarps. There are both annual and biennal caraway varieties, the latter yielding more and is therefore preferentially cultivated. Moreover, breeding activities have produced biennal cultivars whose seeds are enriched with essential oil (around 7%). The current area of cultivation is extended from northern temperate to tropical climates, resulting in a seed oil world production of an estimated 10 t/year (de Carvalho and da Fonseca, 2006).

Anethum graveolens (genus *Anethum,* Apiaceae family) is a diploid (2n = 22), out-crossing plant that has its primary center of origin in central Asia, but wild and weedy types are widespread in the Mediterranean basin and in west Asia.

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FIGURE 1 ***Mentha spicata*, a source of L-carvone.**

Depending on the growth environment, it can be an annual or a perennial herb. Dill is cultivated in Europe, India, and the United States. Successful cultivation requires warm-to-hot summers with high sunshine levels and rich, well-drained soil. Dill varieties have been developed, characterized by different yielding and aroma. In the work of Sundan et al. (2013) random amplified polymorphic DNA analysis was used to study genetic similarity among 135 dill accessions collected in different continents. Although several groups were identified, all the genotypes collected in two main clusters showed little-to-no association with the geographic origin of the material. However, such information can be useful for the management of dill germplasm and to plan new breeding programs.

Ferrie et al. (2011) used doubled haploid techniques in both caraway and dill to generate lines with the potential for commercial production as well as lines to be incorporated into breeding program for superior agronomic or quality characteristics.

Mentha spicata belongs to the genus *Mentha*, Lamiaceae family (Figure 1). The genus has a wide distribution across Europe, Africa, Asia, Australia, and America as perennial herbs. Based on cpDNA data, *Mentha* is strongly supported as being monophyletic (Bunsawat et al., 2004).

Spearmint oil production worldwide is estimated around 1500 t/year. About 80% of the total mint production comes from India. Yield, content, and composition of spearmint is a function of cultivation environment and harvesting time (Zheljazkov et al., 2010). Spearmint requires long days to reach flowering and accumulate essential oil with desirable composition, therefore is generally cultivated in cold areas, but great interest is in developing new varieties adapted to production areas in more temperate environments in which water availability and longer growing seasons may increase harvest per season.

USAGE AND APPLICATIONS

Several historical sources report that caraway, dill, and spearmint are among the oldest herbs known and used (Plinius, 77 AD). Their use is not limited to food, but involves even herbalist and pharmaceutical preparations. In this regard, dill has been reported to be a galactogogue and to have antihyperlipidemic, antihypercholesterolemic, and antioxidant activities (Sundan et al., 2013). Caraway seeds can have antispasmodic, carminative, emmenagogue, expectorant, galagtogogue, stimulant, stomathic, and tonic effects (de Carvalho and da Fonseca, 2006). The seeds can be chewed after a meal in order to relieve heartburn. New medicinal uses such as antihyperglycemic potential have also been reported (Eddouks et al., 2004). Spearmint has stimulant, carminative, and antispasmodic properties. Moreover, carvone is a potential chemoprotective agent, because of its ability to increase detoxifying activities in murine cells (Zheng et al., 1992).

The essential oils extracted from the three species are extensively used in perfumery, as perfuming agents and in cosmetics. In particular, there is an increasing demand and use of spearmint essential oil for toothpaste and other oral care products. This oil is now even used in aromatherapy.

Carvone can even work as environmentally friendly parasiticides and insecticides (Franzios et al., 1997). This monoterpene, together with other essential oil components, is effective for the control of termites, ants, and carpenter ants (Bedoukian et al., 2012).

The insecticidal, fumigant, and repellent activities of *Mentha* essential oil has been reviewed by Kumar et al. (2011). *Mentha spicata* oil is particularly effective in the control of *Tribolium castaneum, Musca domestica*, and *Spodoptera littoralis*.

Carvone can even have a role in aquaculture, working as anesthetic by contact (Patent Pub. No.: US 2011/0212196 A1).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Carvone has been used for millennia in food as a flavoring. Caraway seeds, raw or cooked, thanks to their spicy flavor—due to D-carvone content—are used as a flavoring anywhere a caraway or rye note is required, like in confectionery, in bread preparations, in cheese, in sauerkraut, in sauces, and in cordials. They are in fact an essential ingredient of the traditional German liqueur Kümmel. The crushed seeds can be used to prepare a tea. The extracted essential oil is a flavoring in ice creams, candy, and soft drinks. The young leaves, characterized by lower essential oil content and therefore much less spicy than the seeds, can be consumed in salads, whereas older leaves can be cooked like spinach. Dill leaves and seeds are used as seasoning in eggs, meats, salads, sauces, and soups. Dill seeds flavor cakes, pastries, vinegars, soups, salads, potatoes, meats, sauerkraut, and pickles.

Spearmint leaves are used as flavor for beverages (like tea) and for a wide range of traditional food preparations. The essential oil is extensively used in confectionery and chewing gum. Moreover, L-carvone can also be blended to fruit flavors used in bubble gum and in fruit gum, to enhance flavor, sweetness, and prolong flavor duration, as patented by Wrigley in 1992 (US Patent Number 5158790). The producers of spearmint gum can use the spearmint oil or the natural L-carvone, extracted from spearmint oil, or the synthetic L-carvone, derived from chemical conversion of D-limonene from citrus fruits.

Carvone now plays a new role in the firsts steps of the agro-food chain. In particular this compound has been found to inhibit sprouting in potatoes (Teper-Bamnolker et al., 2010). This is a serious problem for potato fresh market: during storage the break of dormancy leads to tuber sprouting, associated with weight loss, turgidity, and softening. The same problem can affect seed tubers. Dormancy is a state of growth suspension used by the plant to face environmental stresses, such as overwintering or drought. Tubers in particular activate, through endogenous signals, endodormancy and this growth suppression can be terminated by time. Untreated potatoes sprout after about three months of storage at 8 °C. The length of time can be further shortened by environmental signals, like very low temperatures. On the contrary, temperatures of 2–4 °C can delay this time but results in the sweetening of the stored tubers. Potato storage therefore needs both sprout inhibitors and proper storage management. However, the use of some chemicals, like chlorpropham, as an antisprouting agent pose several environmental and health risks. An environmental friendly alternative has been found in spearmint essential oil: fogging with this essential oil inhibits completely potato sprouting, causing black necrotic symptoms in the bud meristems, with no visible damage to the tuber skin. The inhibition can be reversed by washing potato with water. The active molecules present in spearmint essential oil has been found to be L-carvone, probably affecting the cell membrane and in particular the meristem tips. Interestingly, Teper-Bamnolker et al. (2010) found that mint essential oil at very low concentrations induces early sprouting of potatoes, suggesting that two different physiological mechanisms are activated at the two different L-carvone concentrations. This dose-dependent phenomenon is known as hormesis and this mechanism in potato tuber treated with monoterpenes has been elucidated by Rentzsch et al. (2012). These authors found that carvone interact with gibberellin-induced bud dormancy in a hormesis-type manner and that low dose of the monoterpene induces the accumulation of α and β-amylase in a tissue-specific manner. On the contrary, high monoterpene doses activate in the plant's general stress-type response pathways and decrease the gibberellin-mediated responses. Carvone is commercialized in the Netherlands as an antisprouting agent under the name "Talent."

Carvone is an antimicrobial agent, with both antibacterial and antifungal activities. *In vitro* evaluation has shown that both optical isomers have antimicrobial properties. Table 1 shows works demonstrating the inhibitory effects on a wide range of bacterial and fungal pathogens.

Interestingly, in food systems, the efficacy of carvone antibacterial activity seems to be strongly related to food characteristics. Tassou et al. (1995) demonstrated that mint oil antibacterial activity is more effective in tzatziki—the greek traditional cucumber and yogurt salad—compared with high-fat products like patè and fish roe salads.

Burt (2004) reviewed the antibacterial properties of essential oils in the frame of food science and gave the following essential oils general ranking in order of decreasing antibacterial activity:

oregano/clove/coriander/cinnamon > thyme > mint > rosemary > mustard > cilantro/sage

Generally speaking, carvone can be therefore defined as good antimicrobial agent. Its mechanism of action is not completely understood. Oosterhaven et al. (2012) demonstrated that it acts by disturbing the metabolic energy status of the cell, whereas Helander et al. (1998) found that it did not affect the membrane system or the adenosine triphosphate pool.

Another interesting point is the synergism/antagonism that can result between components of an essential oil and between them and preservation methods or other food preservatives. Delaquis et al. (2002) showed that dill, mixed in

TABLE 1 Carvone Antimicrobial Activity

Source/Compound	Target Microorganism	References
Caraway	*Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Fusarium moniliforme*	Soliman and Badeaa (2002)
Carvone	*Salmonella typhimurium*	Ceylan and Fung (2004)
D-Carvone	*Listeria monocytogenes*	Friedman et al. (2002)
D-Carvone	*Escherichia coli, Salmonella typhimurium, Photobacterium leiognathi*	Helander et al. (1998)
D-Carvone	*Fusarium subglutinans, Fusarium cerealis, Fusarium verticillioides, Fusarium proliferatum, Fusarium oxysporum, Fusarium sporotrichioides, Aspergillus tubingensis, Aspergillus carbonarius, Alternaria alternata and Penicillium* sp.	Morcia et al. (2012)
Dill	*Aspergillus niger, Saccharomyces cerevisiae, Candida albicans*	Jirovetz et al. (2003)
L-Carvone	*Campylobacter jejuni*	Friedman et al. (2002)
L-Carvone	*Botrytis cinerea, Geotrichum candidum, Monilinia laxa, Mucor piriformis, Nepenthes alba, Penicillium expansum, Penicillium italicum, Penicillium digitatum*	Caccioni and Guizzardi (1994); Plotto et al. (2003); Neri et al. (2006, 2007, 2009)
L-Carvone	*Enterococcus faecium, Escherichia coli, Aspergillus niger*	Naigre et al. (1996)
Spearmint	*Listeria monocytogenes, Salmonella enteriditis*	Tassou et al. (1995)
Spearmint	*Penicillium digitatum*	du Plooy et al. (2009)
Spearmint	*Fusarium oxysporum*	Nosrati et al. (2011)
Spearmint	*Aspergillus parasiticus*	Alinezhad et al. (2011)

Table 1 shows some results obtained after evaluation of antimicrobial properties of carvone, caraway, dill, or spearmint.

various combinations with other aromatic plants like cilantro and coriander can give very different effects, including additive, synergistic, and antagonistic. In taramosalata (Tassou et al., 1995), sodium chloride and mint oil are synergic.

The antimicrobial properties of carvone can play a role in agriculture. There is great interest in the finding natural compounds for crop protection, in the frame of "greener" approaches. Morcia et al. (2012) evaluated the antimicrobial activity of carvone against some phytopathogens that can have a strong negative impact on cereal production, both in term of yield and safety, because of the mycotoxin production. EC90, as determined *in vitro* using a set of *Fusaria* strains (Morcia et al., 2012), can be reached in the presence of 0.09% of carvone. The fungistatic or fungicide effect has been found dose-dependent. Of the five natural molecules tested by Morcia et al. (2012), different levels of potency were observed according to the following scale: thymol > eugenol > carvone > terpinen-4-ol > 1,8-cineole. This is in agreement with Kurita and Koike (1983) who found that the antifungal activity of essential oils depends on their major components and follows the rule: phenols > alcohols > aldehyde > ketones > ethers > hydrocarbons. Figures 2 and 3 report the *in vitro* evaluations of antifungal activity of different D-carvone concentrations against two species of mycotoxigenic *Fusaria* and *Pyrenophora graminea*, agents of seed-borne disease in cereals.

Carvone can be phytotoxic. To evaluate this, experiments on seed germination were performed to test its potential phytotoxic effect. Germination rate was calculated on 100 seeds (two replicates) each of seven hulless and seven hulled barley genotypes per treatment. The oil component percentages that were used to treat the seeds before germination tests were fixed at equal or higher that the mean EC_{90} values found in the fungal growth experiments (Morcia et al., 2012). Figure 4 shows the results obtained. The conclusion is that D-carvone, at the antifungal concentrations, did not affect seed germination. This means that this is a possible approach to protect seeds and plants without affecting their viability.

SUMMARY POINTS

- Carvone is the major component of essential oils from caraway, dill, and spearmint.
- Two different optical isomers are present, characterized by different aromas.
- Caraway and dill are used as flavorings for several traditional foods.

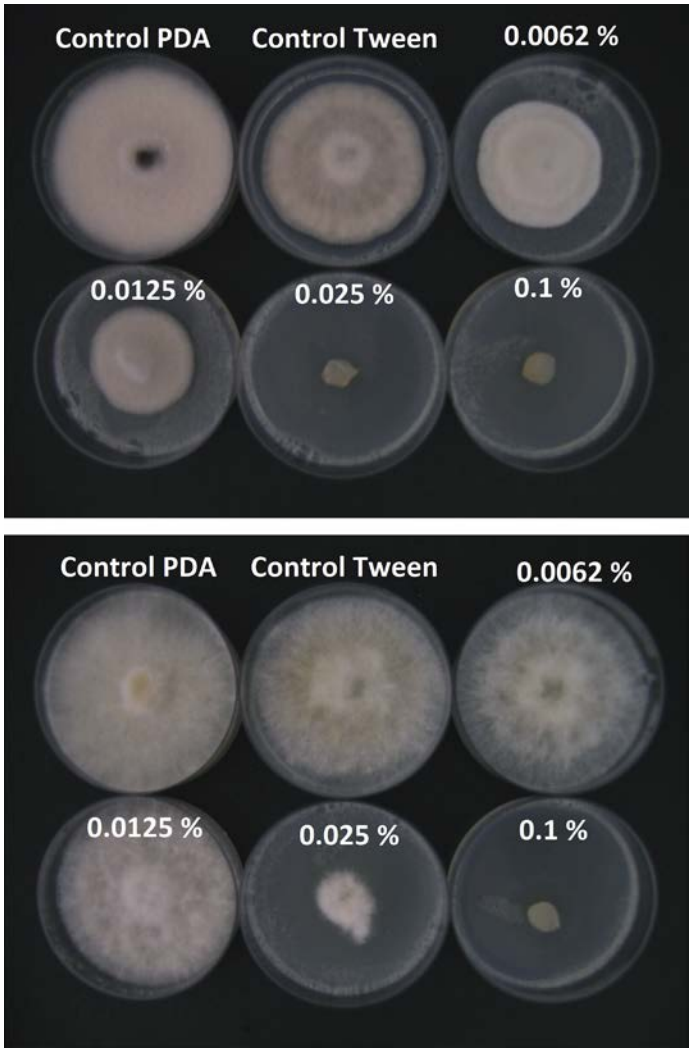

FIGURE 2 In vitro evaluation of carvone antifungal activity on *Fusarium graminearum* and *Pyrenophora graminea*. The effects of different concentrations of D-carvone on mycelium growth of *F. graminearum* (upper) and *P. graminea* (lower). The mycelia were inoculated on petri dishes containing D-carvone at concentrations ranging from 0% to 0.1%. At 0.1% D-carvone complete growth inhibition was observed for both fungi.

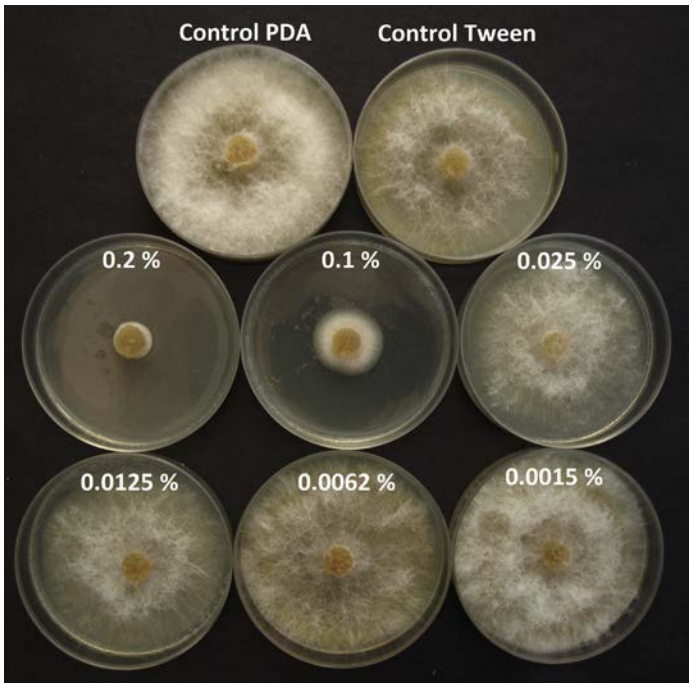

FIGURE 3 In vitro evaluation of carvone antifungal activity on *Fusarium sporothrichioides*. The effects of different concentrations of D-carvone on mycelium growth of *F. sporothrichioides*. The mycelia were inoculated on petri dishes containing D-carvone at concentrations ranging from 0% to 0.2%. At 0.1% D-carvone the mycelium growth was strongly inhibited, whereas at 0.2% D-carvone no growth was observed.

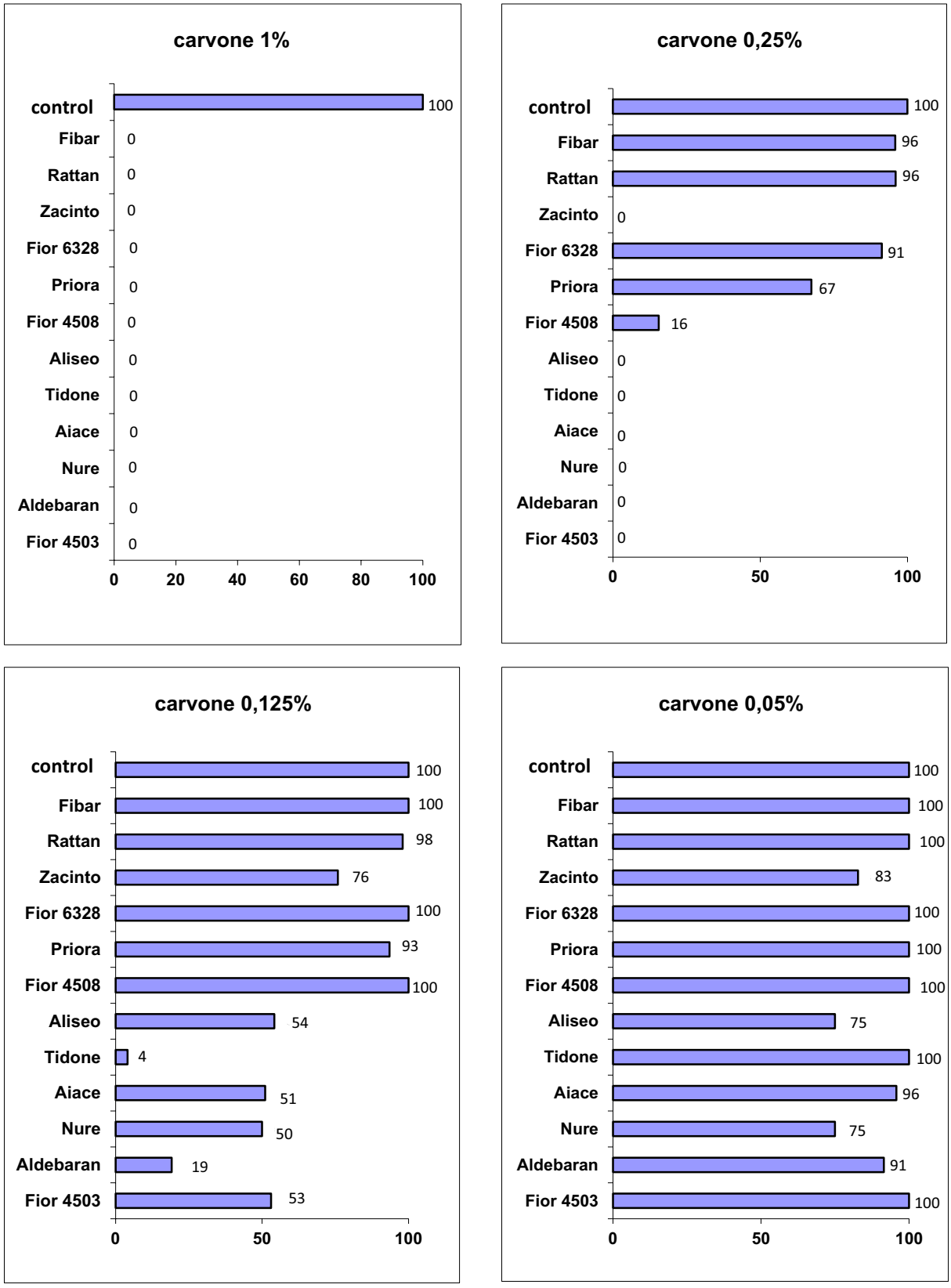

FIGURE 4 Carvone phytotoxicity evaluated by barley seed germination. Seeds of 12 barley varieties, including covered and naked, were treated with different percentages of D-carvone and seed germination tests performed. Some carvone phytotoxicity effects were observed at 0.125% treatment, that is, a carvone concentration higher than those required to have antifungal effects.

- Spearmint is an important ingredient of chewing gum.
- Carvone has good antimicrobial properties that can be useful against pathogen and spoilage microorganisms.
- Carvone-based products can have important applications in crop protection and in agro-food storage.

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Chapter 36

Cedar (*Cryptomeria japonica*) Oils

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List of Abbreviations

EO Essential oil
GC–MS Gas chromatography–mass spectrometry
IC_{50} 50% Inhibitory concentration
USB Used sake barrel

INTRODUCTION

It has been reported that the essential oil (EO) from the Japanese cedar *Cryptomeria japonica* possesses a variety of different medicinal properties, including antimicrobial activities (Matusnaga et al., 2000; Cha et al., 2007; Cheng et al., 2005, 2009). *Cryptomeria japonica* is one of the most commercially important conifers in Asia. "Sake" is a rice-based alcoholic beverage of Japanese origin, known as *Nihonshu* in Japan, meaning "Japanese alcohol". This drink is also referred to in English as rice wine. A type of sake known as "taru-sake" was traditionally distributed throughout Japan in Japanese cedar barrels, although nowadays the same beverage is generally sold in bottles. Furthermore, bottled taru-sake is growing in popularity because it provides a convenient way to enjoy the woody aroma. This type of sake makes up a significant portion of the overall sake market.

The main focus of the current study was to find a useful way to recycle used sake barrels (USBs), making use of the wood chips derived from USBs. We have demonstrated that EO can be extracted by steam distillation from the waste wood chips derived from USBs made of Japanese cedar wood (*C. japonica*). To determine whether the EO extracted from the USBs (EO–USB) possessed any antimicrobial activity, we investigated the effectiveness of EO–USB in terms of its ability to inhibit the growth of microorganisms related to food decay, and through a comparison of this material with other EOs derived from the xylem tissue of several other Japanese woods.

We have established an in vitro assay method to identify direct inhibitors of DNA metabolic enzymes, such as DNA polymerase (Mizushina et al., 1996, 1997, 1999), DNA gyrase (topoisomerase) (Mizushina et al., 2000), inosine-5′-monophosphate (IMP) dehydrogenase (Mizushina et al., 2007), polynucleotide kinase (Mizushina et al., 1996), and deoxyribonuclease (Mizushina et al., 1996). Furthermore, since the mid-1990s, we have discovered more than 100 inhibitors of these enzymes from natural materials (Mizushina, 2009, 2011; Sakaguchi et al., 2002). Using these assays, we investigated whether the components of the EO–USB could be used to inhibit the activities of these types of enzymes from *Escherichia coli* cells. In addition, we have provided a discussion of the antimicrobial mechanism used by EO–USB and its potential use for food preservation.

BOTANICAL ASPECTS

Japanese cedar (cryptomeria) is a monotypic genus of conifer belonging to the cypress family Cupressaceae (formerly belonging to the family Taxodiaceae). The genus itself only includes one species, *C. japonica* (syn. *Cupressus japonica* L.f.). *Cryptomeria japonica* is a very large evergreen tree that can reach up to 70 m (230 ft) in height with a trunk diameter of up to 4 m (13 ft). In terms of its appearance, this species has a red-brown bark that peels *japonica* in vertical strips. The leaves are arranged spirally and are long and needle-like in structure 0.5–1 cm (0.20–0.39 in). The seed cones are globular and about 1–2 cm (0.39–0.79 in) in diameter with about 20–40 scales. The growth rate is slow to medium (20 ft in 20 years). The tree is long-lived (there are specimens in Japan that are about 650 years old) and typically grows at its fastest rate when

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it is planted in forests in deep, well-drained soil beds under warm, moist conditions (Nagakura et al., 2004). It is intolerant of poor soils and cold, drier climates. *Cryptomeria japonica* is extensively used in forestry plantations in Japan, China, and the Azores, and is widely cultivated as an ornamental tree in other temperate areas, including the United Kingdom, Europe, and North America, as well as some regions of the eastern Himalayas, including Nepal and India. The wood is scented, reddish-pink in color, lightweight but strong, waterproof, and resistant to decay. It is favored in Japan as a building material for all types of construction work as well as being used for interior paneling. In the Darjeeling district and Sikkim in India, where it is one of the most widely grown trees, *C. japonica* is called *Dhuppi*, and is favored for its light wood, used extensively in house building. The pollen from Japanese cedar is a major cause of hay fever in Japan.

USAGE AND APPLICATIONS

Cryptomeria japonica is a widely distributed conifer, known as "sugi" in Japanese. *C. japonica* is well-known in Taiwan as one of the most important plantation tree species because of its beautiful yellowish red to red-colored heartwood (Chang and Cheng, 2001). Among plantation trees, it has one of the highest values as a building material for Japanese-style houses and is also commonly used as a material for ceiling boards, wall paneling, and posts (Hon and Minemura, 1991). In light of its industrial importance, the chemical components of *C. japonica*, including its terpenoids, have been investigated, and these compounds have been reported to show a variety of different bioactivities, including antifungal, termiticidal, antimite, antipathogenic, and antifeeding activities against snail species and the pill-bug, and have also developed resistance against the *Cryptomeria* bark borer (Cheng et al., 2005).

EO from *C. japonica* is predominantly derived from the foliage, although EO can sometimes be derived from the wood and roots of different types of conifers in the pine or cypress families. The EO of *C. japonica* has many uses in medicine and art, as well as perfumery, and although the characteristics of the oils derived from different species may vary, they all possess some degree of bactericidal and pesticidal activity. The EO of *C. japonica* is currently used for its aromatic properties, especially in aromatherapy, and can also be used to renew the smell of natural cedar furniture. This EO can also be used as an insect repellent, where it can be applied directly to the skin or used as an additive in sprays, candles, and other products.

The EO of *C. japonica* is a mixture of organic compounds that are generally considered to be safe for use as food preservatives by the United States Food and Drug Administration, although studies have shown that prolonged exposure to high levels of this EO can cause liver and pulmonary toxicity. The United States Environmental Protection Agency (EPA) does not expect these effects to occur in people using the currently registered products because their use and public exposure levels are much lower than those described in the case studies. The EPA believes there is negligible human environmental risk posed by exposure to registered Japanese cedar pesticide or food preservative products when they are used in the properly prescribed manner.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Preparation of EOs from USBs and the Xylem Tissue of Japanese Woods

EO–USB was prepared to make good use of the waste woods produced by rice wine (sake) companies. The waste wood chips were derived from USBs that had been constructed from *C. japonica* grown in the Nara Prefecture of Japan. The wood chips were subjected to steam distillation, producing a yield of 0.1% (v/w) of EO–USB [1] when the distillate was twice the weight of the wood chips, as described previously (Takao et al., 2012). The EOs derived from the xylem tissues of several other major Japanese woods, including Japanese cedar (*C. japonica*) [2], Japanese cypress (*Chamaecyparis obtusa*) [3], and false arborvitae (*Thujopsis dolabrata*) [4], purchased from the Tree of Life Co., Ltd (Tokyo, Japan).

Suppressive Effects of EOs from Japanese Woods on the Food Decay-Related Microbial Growth

Representative food decay-related microorganisms, including a Gram-positive bacterium (*Staphylococcus aureus* subsp. *aureus* NBRC12732), Gram-negative bacterium (*E. coli* NBRC3301), a yeast (*Candida albicans* NBRC1594), and a fungus (*Penicillium citrinum* NBRC6352), were obtained from the National Institute of Technology and Evaluation (Chiba, Japan). A second yeast strain (*Saccharomyces cerevisiae* IFO1950) was obtained from the Institute for Fermentation in Osaka (Osaka, Japan). These microbes were precultured using liquid media before being transferred to Mueller–Hinton agar plates, and used in an antimicrobial assay according to the Kirby–Bauer disc diffusion method that is well standardized and has been widely evaluated (Bauer et al., 1966; Figure 1). In this assay, the EOs that had been obtained from the different Japanese woods by steam distillation were diluted with ethanol (10 times).

TABLE 1 Antimicrobial Activities of EOs from USBs and Japanese Woods against Five Representative Microbes Related to Food Decay

Microbe	Strain	Inhibition Zone (mm)			
		[1]	[2]	[3]	[4]
Bacterium (Gram-positive)	*Staphylococcus aureus* Subsp. *aureus* (NBRC12732)	2.9 ± 0.6	2.5 ± 0.4	1.0 ± 0.2	6.0 ± 1.0
Bacterium (Gram-negative)	*Escherichia coli* (NBRC3301)	3.1 ± 0.5	2.6 ± 0.5	1.3 ± 0.2	4.6 ± 0.9
Yeast	*Candida albicans* (NBRC1594)	0.5 ± 0.1	0.5 ± 0.1	0	2.2 ± 0.4
Yeast	*Saccharomyces cerevisiae* (IFO1950)	2.1 ± 0.4	1.8 ± 0.3	1.6 ± 0.3	2.7 ± 0.5
Fungus	*Penicillium citrinum* (NBRC6352)	1.8 ± 0.4	1.7 ± 0.3	0	7.0 ± 1.2

[1], EO–USB (Japanese cedar); [2], EO of *Cryptomeria japonica* (Japanese cedar); [3], EO of *Chamaecyparis obtuse* (Japanese cypress); and [4], EO of *Thujopsis dolabrata* (False arborvitae). This assay was used as the disc diffusion method (Kirby–Bauer method). These EOs were obtained by steam distillation and used at 10 times dilution with ethanol. Data are presented as the mean ± SD (n = 5).

The inhibitory effects of these four EOs on microbial growth, based on the corruption of food using the disc diffusion method, are shown in Table 1. In particular, photographs of the growth suppression effects of the EOs toward *E. coli* are shown in Figure 1. The longer length of the inhibition zone indicates higher levels of antimicrobial activity. The suppressive effects of EO–USB were slightly greater than those of the commercial EO derived from the xylem tissue of *C. japonica*, even though these two EOs were derived from the same origin (i.e., Japanese cedar). Of the four EOs prepared in the current study, the greatest inhibitory effects were shown by *T. dolabrata*, followed by EO–USB, *C. japonica*, and then *C. obtuse*. The EOs from Japanese cedar effectively suppressed the growth of the five major microorganisms related to food decay, and were the strongest and weakest antimicrobial agents against the Gram-negative bacterium *E. coli* and the yeast *C. albicans*, respectively.

Effects of EOs from Japanese Wood on in vitro DNA Polymerase Activity from *Escherichia coli*

The bacterial cells of the Gram-negative bacterium *E. coli* were cultured and collected, and cell extracts containing the cellular enzymes were prepared (Takao et al., 2012). This extract was used to conduct DNA metabolic enzyme assays to allow for in vitro biochemical action of Japanese wood EOs to be investigated. The in vitro activity of DNA polymerase was assayed at 37 °C as described previously (Mizushina et al., 1996, 1997, 1999).

As shown in Figure 2, concentrations of 100 and 1000 μg/mL of EO–USB inhibited the activity of DNA polymerase in the cell extract taken from *E. coli*, and the 50% inhibitory concentration (IC_{50}) value of this material was determined to be 65 μg/mL. The inhibitory activity of EO–USB was approximately 1.5-fold greater than that of the commercially sourced EO of *C. japonica*. Of the four EOs tested, the EO of *T. dolabrata* provided the highest inhibition, with the inhibitory effects of the EOs occurring in the order of *T. dolabrata* (false arborvitae) > USB > *C. japonica* (Japanese cedar) > *C. obtuse* (Japanese cypress). The in vitro inhibitory activities of the EOs toward *E. coli* DNA polymerase were ranked in the same order as those determined for *E. coli* bacterial growth suppression using the disc diffusion method (Table 1 and Figure 1); therefore, inhibition of DNA polymerase activity might directly affect proliferation in *E. coli* cells. These results suggest that the components of EOs are able to penetrate the cells and reach the nucleus, inhibit DNA polymerase activity, and suppress of *E. coli* growth, possibly by inhibition of DNA polymerase activity.

In the interest of developing a way of reusing the waste wood chips derived for USBs, we focused on the use of EO–USB in the latter part of this study.

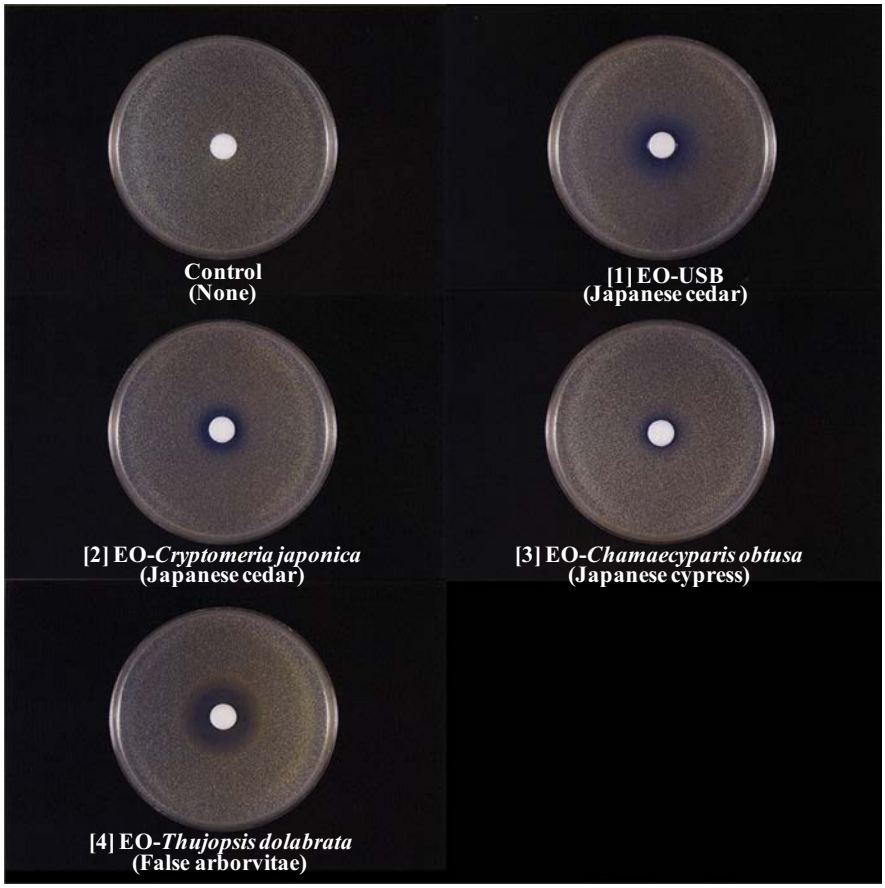

FIGURE 1 The results of the antimicrobial assay using the disc diffusion method (Kirby–Bauer method) of the EOs from USBs and several other Japanese woods against *E. coli* (NBRC3301). These EOs were obtained by steam distillation and used at 10 times dilution with ethanol. A 10% ethanol solution was used as the control.

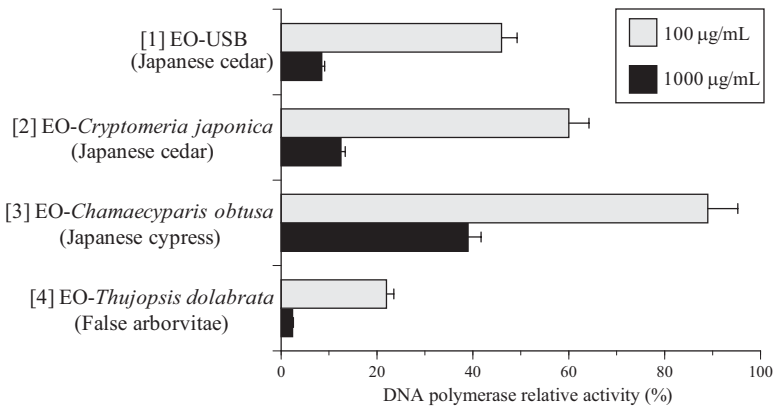

FIGURE 2 Inhibitory effects of the EOs from USBs and several other Japanese woods on the in vitro DNA polymerase activity of *E. coli* cell extracts. DNA polymerase activity (0.05 units) in the absence of EO was taken as 100%, and the relative activities are shown. Data are presented as the mean ± SD (n = 3).

Chemical Composition of EOs from Japanese Cedar

The components of the two Japanese cedar EOs, namely EO–USB and the commercial EO from the xylem tissue of *C. japonica*, were analyzed by gas chromatography–mass spectrometry (GC–MS), resulting in the identification of 14 different sesquiterpenes. Taken together, these sesquiterpenes accounted for 77% of the total compounds in these EOs. The compounds identified as a consequence of this GC–MS experiment are listed in Table 2 according to their elution order

TABLE 2 Composition of the Sesquiterpenes in the EOs Extracted from Japanese Cedar

	[1] EO–USB		[2] EO of *Cryptomeria japonica*	
Compound	**(μg/mL)**	**(%)**	**(μg/mL)**	**(%)**
α-Cubebene	24.05	3.03	17.16	2.05
α-Muurolene	73.85	9.29	71.14	8.51
δ-Cadinene	206.13	25.94	190.98	22.84
Gleenol	17.15	2.16	11.34	1.36
Cubenol	50.07	6.3	38.68	4.63
Epicubenol	91.81	11.55	65.74	7.86
Elemol	22.99	2.89	56.35	6.74
T-Cadinol	3.58	0.45	14.87	1.78
γ-Eudesmol	12.98	1.63	18	2.15
Torreyol	30.23	3.8	29.95	3.58
α-Eudesmol	19.53	2.46	32.54	3.89
β-Eudesmol	36.77	4.63	48.36	5.78
Kongol	5.35	0.67	6.38	0.76
Cryptomerione	19.69	2.48	39.7	4.75
Others	180.5	22.72	195.11	23.32
Total	794.68	100	836.28	100

The composition of EOs was analyzed by a gas chromatograph–mass spectrometry (GC–MS) QP2010 plus (Shimadzu, Kyoto, Japan). The instrument was equipped with a fused silica column ULBON HR-SS-10 (50 m × 0.25 mm, film thickness 0.25 μm) (Shinwa Chemical Industries Ltd, Kyoto, Japan) for sesquiterpenes. Helium was used as the carrier gas at a flow rate of 2 mL/min. Prior to GC–MS analysis, EOs were diluted 1000 times with *n*-hexane. *R*-(−)-Carvone dissolved in ethanol was used as an internal standard. For analysis of sesquiterpenes, the oven temperature was maintained at 60 °C for 1 min, increased from 60 to 220 °C at a rate of 5 °C/min, and then held at 220 °C for 7 min.

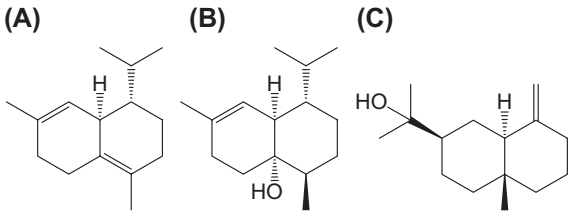

FIGURE 3 Structures of the sesquiterpenes present in EO–USB. (A) δ-Cadinene, (B) Epicubenol, and (C) β-Eudesmol.

from the capillary column. Although the total amount of sesquiterpenes in EO–USB was 0.95-fold lower than that found in the commercial EO of *C. japonica*, the two EOs had a similar composition, with δ-cadinene and epicubenol being identified as the two most abundant sesquiterpenes in both EOs. These two compounds were present in slightly higher amounts in the EO–USB than the EO of *C. japonica*, suggesting that the antimicrobial activity of EO–USB is greater than that of the commercial EO, based on the inhibition of DNA polymerase data.

Suppressive Effects of Sesquiterpenes from EO–USB on *Escherichia coli* Growth

The three different sesquiterpenes, δ-cadinene, epicubenol, and β-eudesmol, were identified as the first, second, and fifth most abundant components of the EO–USB, respectively (Table 2), and were prepared for analysis. The chemical structures of these compounds are shown in Figure 3. δ-Cadinene and β-eudesmol were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and Wako Pure Chemical Industries, Ltd (Osaka, Japan), respectively. Epicubenol was separated and purified from EO–USB using silica gel column chromatography as described previously (Takao et al., 2012).

TABLE 3 Antimicrobial Activity of the Sesquiterpenes from EO–USB (Japanese Cedar, *Cryptomeria japonica*) against *Escherichia coli* (NBRC3301)

Compound	Inhibition Zone (mm)
δ-Cadinene	2.1 ± 0.7
Epicubenol	3.1 ± 0.5
β-Eudesmol	0.4 ± 0.1

This assay was conducted in accordance with the disc diffusion method (Kirby–Bauer method). These compounds were used at 10 times dilution with ethanol. Data are presented as the mean ± SD (n = 5).

TABLE 4 IC_{50} Values of the Sesquiterpenes from EO–USB (Japanese Cedar, *Cryptomeria japonica*) against the activities of DNA Metabolic Enzymes from *Escherichia coli* (NBRC3301)

	Compound (μg/mL)		
Enzyme	δ-Cadinene	Epicubenol	β-Eudesmol
DNA polymerase	66 ± 3.6	45 ± 2.3	175 ± 8.5
DNA gyrase	>1000	>1000	>1000
IMP dehydrogenase	>1000	>1000	>1000
Polynucleotide kinase	>1000	>1000	>1000
Deoxyribonuclease	>1000	>1000	>1000

Data are presented as the mean ± SD (n = 3).

The antimicrobial inhibition zones of these sesquiterpenes are listed in Table 3. Epicubenol, which accounted for 11.55% of the total sesquiterpenes in EO–USB, elicited the strongest cell growth suppression of *E. coli*. Other constituents, including the most abundant compound δ-cadinene (25.94%), showed smaller inhibitory effects than epicubenol. β-Eudesmol was the found to be the weakest anti *E. coli* proliferative inhibitor of the three compounds tested. The combination of these different components in EO–USB must result in a synergistic antimicrobial effect, because EO–USB was as effective as the strongest inhibitor, epicubenol.

Inhibitory Effects of Sesquiterpenes from EO–USB on the Activities of the DNA Metabolic Enzymes in *Escherichia coli*

The in vitro activities of the DNA polymerase (Mizushina et al., 1996, 1997, 1999), DNA gyrase (topoisomerase) (Mizushina et al., 2000), IMP dehydrogenase (Mizushina et al., 2007), polynucleotide kinase (Mizushina et al., 1996), and deoxyribonuclease (Mizushina et al., 1996) of an *E. coli* cell extract were assayed at 37 °C as described previously. The three major sesquiterpenes in EO–USB inhibited the in vitro activity of *E. coli* DNA polymerase, and the IC_{50} values of these compounds were ranked as follows: epicubenol > δ-cadinene > β-eudesmol (Table 4). These results suggest that the inhibition of DNA polymerase by these sesquiterpenes is positively correlated with their bacterial growth suppression (Table 3). The IC_{50} values of epicubenol and EO–USB against *E. coli* DNA polymerase were 45 and 65 μg/mL, respectively (Figure 1). These data therefore suggest that this compound might be one of the DNA polymerase inhibitors in EO–USB. In contrast, these compounds had no effects on the activities of other DNA metabolic enzymes, such as DNA gyrase, IMP dehydrogenase, polynucleotide kinase, or deoxyribonuclease in the extract from *E. coli* bacterial cells.

In this study, we have used an in vitro assay method to determine the DNA polymerase activity of several EOs and their individual components in microbial cell extract. This assay can be completed within 3 h and did not require the culture of the target microbes, and could therefore be used as a convenient screening tool for antimicrobial agents.

In conclusion, the EOs from *C. japonica* (Japanese cedar) heartwood contain sesquiterpenes, such as epicubenol and δ-cadinene, in greater quantities than those present in sapwood, making them very effective for inhibiting the survival of

microorganisms associated with food decay. Given that USBs are made from *C. japonica* heartwood, the EO extracted from waste wood chips made from USBs could be used for food preservation.

SUMMARY POINTS

- Bad microorganisms are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by having an adverse impact on their nutritional value and sometimes producing toxins.
- EO–USB from the waste woods (Japanese cedar) produced from rice wine (sake) companies, as well as EOs derived from the xylem tissue of several major Japanese woods, such as Japanese cedar (*C. japonica*), Japanese cypress (*C. obtusa*), and false arborvitae (*T. dolabrata*), were prepared by steam distillation.
- The antimicrobial activities of the EOs derived from the Japanese woods against the microorganisms associated with food decay were ranked as follows using disc diffusion method: *T. dolabrata* > USB > *C. japonica* > *C. obtusa*.
- These four EOs also inhibited the in vitro activity of DNA polymerase in an extract from *E. coli* bacterial cells with the same ranking.
- The main sesquiterpenes in EO–USB were determined by GC–MS analysis to δ-cadinene (25.94%) and epicubenol (11.55%), and the amounts of these two compounds in the EO–USB were slightly higher than those in the EO of *C. japonica*.
- Pure samples of δ-cadinene, epicubenol, and β-eudesmol were prepared and evaluated in terms of their inhibition of the bacterial growth and DNA polymerase activities of *E. coli*, with epicubenol showing the strongest inhibition of the three compounds tested.
- These results suggested that the microbial growth suppression properties by EO–USB, which contains δ-cadinene and epicubenol, could be related to the inhibition of DNA polymerase.
- As well as their potential application in different medicines for human health, EO–USB could be exploited as herbal fumigants for the protection of stored foodstuffs from microorganisms associated with food decay.

ACKNOWLEDGMENTS

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Chapter 37

Celery (*Apium graveolens* var. *dulce* (Mill.) Pers.) Oils

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List of Abbreviations

DPPH 2,2′-Diphenyl-1-picrylhydrazyl
GRAS Generally recognized as safe

INTRODUCTION

Celery (*Apium graveolens*) is a popular vegetable belonging to the Apiaceae family. It is commercially cultivated worldwide (Sowbhagya et al., 2010) and occasionally found in its original wild form (Marongiu et al., 2013). The plant is a "typical example of man's improvement of a wild species", as it underwent extensive selection during the last few hundred years (de Vilmorin, 1950). Celery is valued not only for its nutritional properties (Roslon et al., 2010) but also for the distinctive aroma which it owes to the presence of phthalide-rich essential oil (Kurobayashi et al., 2006; Sellami et al., 2012). Thus, *Apium graveolens* is widely used as a spice and flavoring ingredient in food products (Sowbhagya et al., 2007, 2010). The current trend toward the use of natural preservatives triggered research on antibacterial, antifungal, and antioxidant properties of volatile oils, including celery (Alves-Silva et al., 2013). The present review covers the aspects of production and chemical composition of celery essential oil, its antioxidant properties, as well as its antibacterial and antifungal activity against food-borne pathogens.

BOTANICAL ASPECTS

Wild celery (*Apium graveolens* L.) is a scapose hemicryptophyte characterized by thin and hollow stems and slim roots (Marongiu et al., 2013). It is an erect plant, 60–90 cm tall, recognizable by pinnate or trifoliate leaves with three-lobed, ovate to suborbicular leaflets, around 4 cm long. Flowers, arranged in compound umbels, are small, white to greenish-white, with five ovate, acute petals. Schizocarp fruit is 1–2 mm wide and consists of two brown, pale-ridged, suborbicular to ellipsoid mericarps (Asif et al., 2011; Fazal and Singla, 2012). Beside wild celery, three main crop varieties exist, namely *A. graveolens* var. *dulce* (Mill.) Pers. (commonly referred to as stem, stalk, sweet, or cultivated celery, or simply celery), *A. graveolens* var. *rapaceum* (Mill.) DC. (better known as celeriac, root celery or turnip-rooted celery), and *A. graveolens* var. *secalinum* Alef. (i.e., leaf celery) (Roslon et al., 2010). Contrary to wild-type celery, these are annual or biennial herbs (Marongiu et al., 2013). As indicated by their common names, the above plants differ with respect to morphology. The sweet variety is recognized by thick, solid, and succulent leaf petioles (stalks), which are usually blanched during cultivation (de Vilmorin, 1950; Khalid and Hussein, 2012; Moradalizadeh et al., 2013). On the other hand, celeriac is distinguished by thickened hypocotyl, often incorrectly referred to as the "root" (MacLeod and Ames, 1989). The variety *secalinum* is the most similar to wild celery.

Among *A. graveolens* varieties, stalk celery is popular in India, the United States, and Europe, both as seed and vegetable crops (de Vilmorin, 1950; Rao et al., 2000; Tirillini et al., 2004). Celeriac is commonly grown in Europe and represents major celery variety in some countries, such as Poland (Roslon et al., 2010). In comparison with sweet and root celery, the leaf variety is less commonly cultivated. Wild plants are widespread, but particularly abundant in the Mediterranean region, where celery is considered to originate from (de Vilmorin, 1950; Marongiu et al., 2013).

With annual production exceeding 4000 t, India is currently the major supplier of celery seed (around two-thirds of the world production). Specialized seed crops are cultivated in Punjab (90% of Indian production), Amritsar, Haryana, and the

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north-western Himalayas on the area of 5000 ha. Over 3000 t of plant material is exported (Rao et al., 2000; Thappa et al., 2003; Sowbhagya et al., 2007, 2010; Fazal and Singla, 2012).

USAGE AND APPLICATIONS

Besides being a popular vegetable, *A. graveolens* has long been used as a medicinal plant. In its wild form, it has been known to humans since ancient times and is mentioned in Egyptian, Greek, Roman, Chinese, and Indian sources (de Vilmorin, 1950; Asif et al., 2011; Marongiu et al., 2013). At present, celery continues to play an important role in traditional medicinal systems. The plant is reported to possess antispasmodic, carminative, diuretic, emmenagogue, galactogogue, laxative, sedative, hepatoprotective, antihelminthic, nematicidal, and mosquitocidal properties. Celery seed is used to treat asthma, bronchitis, liver and spleen diseases, and also as diuretic and antirheumatic medicine (Mišić et al., 2008; Asif et al., 2011; Fazal and Singla, 2012; Khalid and Hussein, 2012; Sellami et al., 2012; Moradalizadeh et al., 2013). Essential oil of celery seed exhibits antiinflammatory activity in gout and rheumatism (Marongiu et al., 2013). These effects are attributed to the presence of phthalides, such as sedanolide and 3-*n*-butylphthalide, which have also been shown to possess anticarcinogenic and neuroprotective properties (Kurobayashi et al., 2006; Mišić et al., 2008; Peng et al., 2008). 3-*n*-butylphthalide inhibits platelet aggregation, reduces neurone apoptosis, improves mitochondrial function, and decreases oxidative damage. In China, it has been officially approved for the treatment of stroke patients (Peng et al., 2008).

Apart from medical applications, celery seed essential oil is used as an ingredient and fixative in perfumes, to which it imparts warm, floral, and clinging notes (Rao et al., 2000; Sowbhagya et al., 2007, 2010). It can also be added to cosmetics, such as lotions, soaps, and creams (Khalid and Hussein, 2012).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Two celery varieties are commonly cultivated as food crops: *A. graveolens* var. *dulce* (stalk celery) and *A. graveolens* var. *rapaceum* (celeriac). The first is especially valued for its thickened petioles which can be consumed in raw, boiled, and fried form. They are often added to salads, dressings, marinades, soups, sauces, and stews. The thickened "root" (hypocotyl) of celeriac and, to a lesser extent, leaves of different varieties (including *A. graveolens* var. *secalinum* also known as "leaf celery") can be used in a similar manner (MacLeod and Ames, 1989; Rao et al., 2000; Kurobayashi et al., 2006; Roslon et al., 2010; Sellami et al., 2012). Due to their high nitrate content (around 3–4%), dried and comminuted aerial parts of celery (also known as celery powder) are used as a curing agent in the meat industry (Sebranek et al., 2012). Although the consumption of celery is generally considered safe, the plant is a common cause of food allergies (for instance, 30–40% of food allergic patients in Switzerland and France are sensitized to celery) and consequently, its presence in food has to be declared by producers (Fuchs et al., 2012). Celery allergens belong to proteins (Api g 1, Api g 4) or glycoproteins (Api g 5) (Fuchs et al., 2012) and as such are not likely to be found in an appropriately steam-distilled essential oil (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2004). In some cases, handling of celery can cause phytophotodermatitis due to the presence of furanocoumarins (Lombaert et al., 2001), but the literature data indicate that these compounds are not found in *A. graveolens* essential oil obtained by hydrodistillation (Table 1).

Besides being a popular vegetable, *A. graveolens* var. *dulce* is grown for its aromatic fruits, commonly referred to as "seeds". The dry ripe seeds are used as a spice for flavoring salads, soups, sauces, pickles, tomato juice, beverages, desserts, bakery products, eggs, fish, and meat dishes. The mixture of ground celery fruits with table salt or pepper is commonly known as celery salt or celery pepper, and are also used as a condiment (Rao et al., 2000; Thappa et al., 2003; Sowbhagya et al., 2007, 2010; Mišić et al., 2008).

Celery owes its characteristic flavor to the presence of essential oil, composed of monoterpenes (represented by limonene, α- and β-pinene, myrcene), sesquiterpenes (mainly β-selinene) and phthlides, including 3-*n*-butylphthalide, sedanolide, and sedanenolide. As shown in Table 1, the composition of volatile oil varies depending on the part of the plant used, celery variety, and country of origin. Substantial differences were observed with respect to the concentrations of major constituents, such as limonene (1.8–78.9%), β-selinene (none to 37.4%), and sedanenolide (none to 34.2%), but samples with exceptionally high content of otherwise absent or minor compounds (e.g., β-caryophyllene, kessane, phellandrene, pulegone) were also reported (Table 1). The variations in phthalide concentrations may, to a certain degree, be explained by their relatively low volatility (Ludwiczuk et al., 2001; Tirillini et al., 2004), which makes essential oil isolation highly dependent on the efficiency of the applied distillation method.

TABLE 1 Composition of Hydro and Steam Distilled *Apium graveolens* L. Essential Oils Depending on the Type of Plant Material, Variety, and Country of Origin

Compound[a]	Fresh Aerial Parts, var. *dulce*, UK (MacLeod and Ames, 1989)[b]	Seeds, var. *dulce*, Poland (Ludwiczuk et al., 2001)[c]	Flowering Aerial Parts, Portugal (Marongiu et al., 2013)[d,e]	Flowering Aerial Parts, Portugal (Alves-Silva et al., 2013)[f]	Flowering Aerial Parts, Italy (Marongiu et al., 2013)[d,e]	Seeds, var. *rapaceum*, Serbia (Mišić et al., 2008)[d]	Seeds, var. *dulce*, India (Rao et al., 2000)	Seeds, var. *dulce*, India (Philippe et al., 2002)[g,h]	Seeds, India (Thappa et al., 2003)[i]	Seeds, Iraq (Kiralan et al., 2012)[f,j]	Fresh Aerial Parts, Iran (Moradalizadeh et al., 2013)[d,f]	Air-Dried Leaves, Cuba (Pino et al., 1997)[f,k]	Fresh Leaves, var. *dulce*, Tunisia (Sellami et al., 2012)
Borneol	—	—	—	—	—	—	—	—	—	—	—	—	2.1
3-*n*-Butyl-4,5-dihydrophthalide (sedanenolide)	28.1	—	8.2	—	1.0	4.8	—	0.2	2.2	—	—	32.1	34.2
3-*n*-Butyl-hexahydrophthalide	0.1	—	1.2	—	0.4	—	—	—	—	—	—	—	—
3-Butylidene-4,5-dihydrophthalide (ligustilide)	3.6	—	0.7	—	0.9	—	—	—	—	—	—	—	—
3-*n*-Butylidenephthalide	1.1	—	0.3	—	—	—	—	—	—	—	—	—	38.4
3-*n*-Butylphthalide	2.5	0.30	7.3	—	2.7	0.6	6.9	1.8	2.9	—	—	4.9	3.9
3-*n*-Butyl-3a,4,5,6-tetrahydrophthalide (sedanolide)	6.4	tr	45.2	—	7.6	2.5	0.7	0.3	—	—	—	—	—
Camphene	0.1	—	0.1	—	—	—	—	—	—	—	1.4	—	—
Carvacrol	—	—	—	—	3.4	—	—	—	—	—	6.3	—	—
Carveol	tr	tr	—	—	—	—	0.9	—	—	0.2	—	—	—
Carvone	—	—	—	—	—	—	1.9	—	—	0.1	—	—	—
β-Caryophyllene	1.2	0.1	1.7	3.7	8.1	0.2	tr	0.7	—	0.6	1.9	13.5	0.6
Caryophyllene oxide	—	tr	0.2	—	1.7	—	2.3	0.2	—	—	0.3	tr	—
p-Cymene	0.1	—	—	—	—	—	tr	tr	—	tr	—	—	—

Continued

TABLE 1 Composition of Hydro and Steam Distilled *Apium graveolens* L. Essential Oils Depending on the Type of Plant Material, Variety, and Country of Origin—cont'd

Compound[a]	Fresh Aerial Parts, var. *dulce*, UK (MacLeod and Ames, 1989)[b]	Seeds, var. *dulce*, Poland (Ludwiczuk et al., 2001)[c]	Flowering Aerial Parts, Portugal (Marongiu et al., 2013)[d,e]	Flowering Aerial Parts, Portugal (Alves-Silva et al., 2013)[f]	Flowering Aerial Parts, Italy (Marongiu et al., 2013)[d,e]	Seeds, var. *rapaceum*, Serbia (Mišić et al., 2008)[d]	Seeds, var. *dulce*, India (Rao et al., 2000)	Seeds, var. *dulce*, India (Philippe et al., 2002)[g,h]	Seeds, India (Thappa et al., 2003)[i]	Seeds, Iraq (Kiralan et al., 2012)[f,j]	Fresh Aerial Parts, Iran (Moradalizadeh et al., 2013)[d,f]	Air-Dried Leaves, Cuba (Pino et al., 1997)[f,k]	Fresh Leaves, var. *dulce*, Tunisia (Sellami et al., 2012)
β-Damascenone	—	—	—	—	—	—	—	—	—	—	—	0.2	—
Dihydrocarvone	—	tr	—	—	—	—	0.3	—	—	0.1	—	—	—
β-Elemene	—	tr	—	—	—	—	tr	—	—	—	—	0.1	—
Epiglobulol	—	—	—	7.7	—	—	—	—	—	—	—	—	—
β-Eudesmol	—	—	—	—	—	—	0.7	0.4	0.4	—	—	—	—
Geraniol	—	—	—	—	—	—	—	—	—	—	—	—	0.3
3-Hexenol	—	—	—	—	—	—	—	—	—	—	—	tr	—
α-Humulene	0.2	tr	0.2	—	0.7	—	—	—	—	—	0.4	1.7	—
Kessane	—	tr	1.7	—	—	0.1	—	7.6	—	—	—	—	—
Limonene	35.5	74.9	24.0	16.0	1.8	26.4	50.9	50.1	78.9	76.6	46.2	18.3	3.6
Limonene oxide	—	tr	—	—	—	—	2.7	tr	—	tr	—	—	—
Linalool	tr	tr	—	—	—	—	0.2	0.1	—	0.1	—	tr	—
Myrcene	0.9	0.9	3.4	—	0.2	1.6	1.3	1.4	0.4	1.2	1.2	—	—
Neophytadiene	—	—	0.5	—	34.6	—	—	—	—	—	—	—	—
β-Ocimene	0.1	tr	0.3	—	—	0.2	—	—	—	—	17.6	0.1	—
Phellandral	—	—	—	17.2	—	—	—	1.9	—	—	—	—	—
α-Pinene	0.5	0.3	0.5	—	—	0.1	tr	0.3	0.8	0.2	0.5	0.2	tr
β-Pinene	1.8	6.8	tr	2.5	—	1.8	1.2	2.1	—	1.0	1.1	9.5	—
Pulegone	—	—	—	—	—	—	—	—	—	—	8.3	—	—
Sabinene	0.2	—	0.1	—	—	tr	—	0.2	—	—	0.2	0.3	—
α-Selinene	0.2	1.7	—	4.3	—	0.3	1.6	—	—	1.9	0.4	—	—

β-Selinene	1.0	14.0	1.8	37.4	—	1.8	19.5	22.2	6.2	11.1	2.6	8.3	0.4
γ-Selinene	—	—	—	—	—	—	—	0.4	—	—	—	—	—
Terpineol	0.1	—	—	—	—	—	0.1	—	—	tr	—	tr	—
Terpinene	6.7	tr	—	—	—	—	—	—	—	tr	5.7	2.4	3.1
α-Thujene	0.1	—	—	—	—	—	—	—	tr	—	—	—	9.9
Thymol	—	—	—	—	6.1	—	—	—	—	—	0.6	—	0.7

Table 1 compiles the results of studies on chemical composition of celery oil obtained from plants from different varieties/cultivars grown in various parts of the world. Values (rounded to one decimal place) are expressed as % of essential oil (gas chromatography–mass spectrometry data). References are given to original research articles. tr, trace amounts (<0.1%); —, compound not detected.
[a]*Isomer is not specified The presented data refer to the sum of isomers; compounds listed alphabetically.*
[b]*Data for "Celebrity" cultivar.*
[c]*Data for "Orient" cultivar.*
[d]*Data for conventionally hydrodistilled sample.*
[e]*Plants collected from natural habitats.*
[f]*Variety of celery not specified.*
[g]*Data for kessane-rich oil sample.*
[h]*Philippe, J., Suvarnalatha, G., Sankar, R., Suresh, S., 2002. Kessane in the Indian celery seed oils. J. Essent. Oil Res. 14, 276–277.*
[i]*Data for unmodified "check" strain.*
[j]*Kiralan, M., Bayrak, A., Abdulaziz, O.F., Özbucak, T., 2012. Essential oil composition and antiradical activity of the oil of Iraq plants. Nat. Prod. Res. 26, 132–139.*
[k]*Pino, J.A., Rosado, A., Fuentes, V., 1997. Leaf oil of celery (*Apium graveolens *L.) from Cuba. J. Essent. Oil Res. 9, 719–720.*
Table by Adam Kokotkiewicz, previously unpublished.

Although limonene is the predominant constituent of celery volatile oil, it is the phthalide fraction which gives *A. graveolens* the characteristic spicy taste and aroma (MacLeod and Ames, 1989; Thappa et al., 2003; Kurobayashi et al., 2006). The cooking process affects celery flavor by reducing its "green spicy" note. However, the differences in aroma between raw and boiled celery do not result from the changes in phthalide concentrations, but the formation of thermally generated compounds, including β-damascenone, sotolon, and β-ionone (Kurobayashi et al., 2006).

The commercial production of *A. graveolens* essential oil is based on specialized seed crops of stalk celery. The seeds contain 1.5–2.5% essential oil, which is obtained by hydro or steam distillation. A study by Rao et al. (2000) included detailed research on the chemical composition and physical properties of volatile oil from Indian celery, as well as aspects of large-scale distillation, essential oil deterpenation, and the storage stability of oleoresin (Table 2). Research also investigated how to improve yield and quality of celery essential oil. Table 2 summarizes the results of these studies, which included the evaluation of different comminution methods (Sowbhagya et al., 2007), pretreatment of seeds with cellulolytic enzymes (Sowbhagya et al., 2010), and the use of fertilizers on celery crops (Khalid and Hussein, 2012). Efforts to improve the quality of celery seed essential oil by introducing genetically modified plants were also made. Due to the high limonene content (up to 80%), volatile oil in celery it is often not suitable for direct use in the food industry and thus needs to be deterpenated. However, selected genetically modified strains of *A. graveolens* are characterized by lowered limonene (45–50%) and increased sedanolide content (up to 34%) in the volatile fraction (Thappa et al., 2003). Some other work on technological aspects of celery was conducted with the use of green parts: studies included the use of microwave-assisted hydrodistillation or supercritical carbon dioxide extraction for the isolation of volatile fraction, the effects of raw material stabilization on essential oil yield, and the evaluation of *A. graveolens* leaves and waste material as an additional source of volatile compounds (Table 2).

The present trend toward the use of natural food preservatives has boosted research on antibacterial, antifungal, and antioxidant activities of essential oils. Volatile oils have a long history of use by humans as they are a common constituent of plant foods and spices. Currently, most of them have generally recognized as safe (GRAS) status, and as such they are positively perceived by consumers (Mišić et al., 2008; Patil and Kamble, 2011; Alves-Silva et al., 2013). Consequently, essential oils have been tested for their activity against a range of food-borne pathogens, including Gram-positive and Gram-negative bacteria, yeasts, and fungi. The results of investigations into the antimicrobial properties of celery volatile oil are summarized in Table 3. These studies provided mixed results, with activity against specific pathogens varying from none to moderate. The data are also difficult to interpret due to the use of different antimicrobial assays (e.g., disc diffusion or broth macrodilution), which were previously shown to provide results that were hardly comparable (Friedman et al., 2002). Moreover, studies conducted with a large number of essential oils often lack the data on their chemical composition (Table 3). This makes explanation of the results even more problematic, especially given the large variation in the concentrations of celery volatile oil constituents (Table 1). However, despite the aforementioned difficulties some general conclusions on antimicrobial activity of celery essential oil can be drawn. The volatile fraction of *A. graveolens* seems to be more active against Gram-positive than Gram-negative bacteria. For instance, the inhibitory activity of celery essential oil was recorded against *Staphylococcus aureus* and different *Listeria* strains whereas *Escherichia coli* and different *Salmonella* serovars often remained unaffected (Table 3). Several fungal or mold strains were also affected, although with varying efficiency. These results are to some extent supported by the reports on antimicrobial effects of limonene, which is one of the major constituents of celery volatile oil (Table 1). The above compound inhibited the growth of *Listeria monocytogenes* and some of the fungal strains included in the presented surveys (*Aspergillus flavus*, *Aspergillus niger*, *Alternaria alternata*) (Lis-Balchin et al., 1996; Singh et al., 2010). However, other than some preliminary antifungal tests (Momin and Nair, 2001) little is known about the activity of celery phthalides against food-borne pathogens.

Only a few studies on the antioxidant properties of celery essential oil have been conducted, the results of which are presented in Table 4. The screening analyses made by Wei and Shibamoto (2007) included several essential oils, which were tested for their antioxidant activity using different assays. Generally, celery oil showed moderate antioxidant potential as it was surpassed by rose, parsley, ylang–ylang, and jasmine essential oils in most of the tests applied. Alves-Silva et al. (2013) evaluated the antioxidant activity of celery volatile oil in several assays that demonstrated it to be less potent than bush-basil oil, but comparable to coriander. The results presented in the above mentioned reports are difficult to compare due to the use of different assays and essential oils. There are also some differences with respect to the antioxidant activity of celery essential oil, measured by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, included in these studies. In both cases, the volatile oil consisted mainly of limonene (around 75%) and β-selinene (11%). On the other hand, the essential oil with high β-selinene (37.4%) and low limonene (16%) content showed relatively weak antiradical activity (lower compared with oils of fennel, aniseed, and parsley) at a high concentration of 29 mg/L (Table 4).

TABLE 2 Studies on Selected Aspects of the Production of *Apium graveolens* L. Essential Oil

Studied Aspects of Essential Oil Production	Results	References
Comparison of physical properties and chemical composition of essential oils (steam and Clevenger distilled) obtained from raw and roasted celery (var. *dulce*) seeds from different regions of India (Mumbai, Amritsar, Nagpur); large scale extractions; essential oil deterpenation; preparation and storage stability of oleoresin.	More volatile oil obtained by hydrodistillation (mean of four seed series 2.3%) compared with steam distillation (2.1%); volatile oil content of roasted seeds lower (mean of two distillation methods 1.61%, Mumbai seeds) comparison with raw material (1.80%); no detrimental effect of seed roasting to oil quality; high limonene content (89.8%, Mumbai seeds) in essential oil obtained by large-scale (10 kg batch) steam distillation, as compared to small-scale process (66.9%); low limonene content (2.3%) and high phthalides concentration (>50%) in deterpenated oil (13% yield); oleoresin (ca. 24% yield) showed to be stable for 2 months when stored at 8–10 °C).	Rao et al. (2000)
Evaluation of genetically modified celery strains for the production of essential oil with increased phthalide content; evaluation of leaf material (by-product during seed production) as an additional source of essential oil.	Two modified celery strains showed to provide seed oil with high phthalide content (ca. 34% and 14% sedanolide, respectively) and lowered limonene concentration (ca. 51% and 46%, respectively), compared with control strain (ca. 5% phthalides and 79% limonene); fresh and dry leaf material showed to contain oil with composition similar to seed oil (up to 65–73% limonene and 5.7–8% phthalides).	Thappa et al. (2003)
The influence of size reduction on the yield and chemical composition of celery seed essential oil; evaluation of distillation time on the chemical composition of seed oil.	For small batches (200 g), the highest oil yield obtained for flaked seeds precooled to 5–8 °C (2.2% dry wt), followed by seeds flaked at room temp. (2.0%) and ground at ambient temp. (1.9%); higher oil yield from large batches (10 kg) obtained for flaked (1.76%) than hammer mill-powdered seeds (1.4%); essential oil collected at different distillation times characterized by different composition and flavor properties (30 min: >80% limonene, 10% β-selinene, phthalides in trace amounts; 120 min: 25% limonene, 45% β-selinene, 15% sedanolide, 10% 3-*n*-butylphthalide); clogging and choking associated with seed pulverization eliminated by replacing conventional milling with seed flaking.	Sowbhagya et al. (2007)
The effect of enzyme pretreatment on the yield and chemical composition of celery seed essential oil.	Higher oil yield (2.2–2.3%) from seeds pretreated with enzymes (cellulose, pectinase, protease or viscozyme) as compared to control (1.8%); higher limonene concentration (82.2%) and lower β-selinene (9.0%) and phthalide content (1.1% 3-*n*-butylphthalide, 1.1% sedanenolide) in essential oil from cellulase-treated seeds, compared with control group (63.9% limonene, 16.4% β-selinene, 5.3% and 5.7% 3-*n*-butylphthalide and sedanenolide, respectively); chemical composition of oil from viscozyme-treated seeds similar to untreated material (67.7% limonene, 17.6% β-selinene, 3.9% 3-*n*-butylphthalide, 2.1% sedanenolide).	Sowbhagya et al. (2010)
Evaluation of vegetable celery (var. *dulce*) by-products (pooled waste material resulting from trimming: leaf laminas, sheaths, top parts of petioles, undersized plants) as a source of essential oil.	Maximum essential oil yield (6.8 kg/ha) obtained at 180 kg nitrogen (N)/ha fertilization rate; productivity comparable to celery crops at the vegetative phase but lower in comparison to specialized seed crops (>30 kg oil/ha); decreased oil yield (3.7 kg/ha) at higher dose of fertilizer (300 kg N/ha); higher phthalides content (71%) and lower limonene concentration (4%) in essential oil achieved at 300 kg N/ha nitrogen, compared with 180 kg N/ha fertilization rate (51% limonene, 28% phthalides).	D'Antuono et al. (2002)[a]

Continued

TABLE 2 Studies on Selected Aspects of the Production of *Apium graveolens* L. Essential Oil—cont'd

Studied Aspects of Essential Oil Production	Results	References
The influence of organic fertilizers on the yield and chemical composition of celery fruit essential oil.	Positive effect of combined cattle manure + liquid manure treatment on essential oil yield (2.0–2.1%) compared with plants fertilized with cattle manure only (1.5%); increased limonene (36.9%), β-selinene (22.0%), 3-*n*-butylphthalide (11.0%), and sedanenolide (10.5%) concentrations in essential oil obtained from plants receiving combined cattle manure + liquid manure fertilizer, comparison with plants treated with cattle manure only (30.4, 20.4, 9.33, and 9.5%, respectively, data for 10 m^3 cattle manure dose).	Khalid and Hussein (2012)
The influence of stabilization of raw material on the yield and chemical composition of essential oil in celery leaves.	Leaves frozen for 6 months at −20 °C shown to contain comparable amount of essential oil (ca. 0.3–0.6%) as the fresh plant material; the amount of essential oil in dried (35 °C) leaves 2 to 4-fold higher than in fresh leaves; decrease in α-phellandrene and increase in limonene content in frozen and dried leaves, respectively; decomposition of some of minor essential oil constituents in frozen (camphene, sabinene, caryophyllene oxide) and dried (terpinene, humulene, caryophyllene oxide) leaf samples.	Roslon et al. (2010)
Evaluation of supercritical carbon dioxide extraction for the isolation of essential oil from aerial parts of wild-grown celery.	Lower limonene content (5.6%) and higher phthalides concentration (81%) in essential oil obtained by supercritical fluid extraction compared with hydrodistilled oil (24 and 63%, respectively; data for Portuguese celery)	Marongiu et al. (2013)
Evaluation of microwave-assisted hydrodistillation for the isolation of essential oil from aerial parts of celery.	Higher oil yield attained for microwave-assisted (1.4%) than conventional hydrodistillation (1.1%); similar composition of essential oils obtained by both distillation methods (74–77% monoterpene hydrocarbons, 15.5–16.4% oxygenated monoterpenes, 5.1–5.3% sesquiterpene hydrocarbons); substantial time saving in the case of microwave-assisted hydrodistillation (25 min, compared with 180 min for conventional hydrodistillation).	Moradalizadeh et al. (2013)

The table compiles the results of studies on technological aspects of celery oil production (e.g., the influence of fertilizers, enzyme pretreatment, different size reduction methods, and isolation procedures on the yield and quality of celery seed oil). References are given to original research articles.
[a]*D'Antuono, L.F., Neri, R., Moretti, A., 2002. By-products of vegetable celery (*Apium graveolens *L. var.* dulce*) as potential source of flavours. Acta Hortic. 576, 327–331.*
Table by Adam Kokotkiewicz, previously unpublished.

TABLE 3 Studies on Antimicrobial Properties of *Apium graveolens* L. Essential Oil

References	Microbial Strains Used[a]	Antimicrobial Tests Applied (Measured Parameters Given in Parentheses)[b]	Results[a,b]	Comments[b]
Lis-Balchin and Deans (1997)	20 strains of *L. monocytogenes*$^{G+}$	Disc-diffusion (IZ)	Inhibitory activity of celery essential oil against 18 of 20 examined *L. monocytogenes* strains; oil of celery shown to be more potent compared with fennel, aniseed (none of the strains affected), and dill (11 strains affected) essential oils.	*L. monocytogenes* strains isolated from different food products including meats, eggs, and vegetables; essential oils obtained from commercial sources (composition of celery oil not given).
Elgayyar et al. (2001)[c]	*A. niger**	Disc-diffusion (IZ) Tube dilution method (MLC)	Strong inhibitory activity of celery essential oil against *Y. enterocolitica*, *G. candidum*, and *Rhodotorula*; moderate inhibitory activity against *L. monocytogenes* and *S. aureus*; weak activity against *A. niger*, *E. coli*, *S.* Typhimurium and *P. aeruginosa*; inhibitory activity of celery, angelica, carrot, and parsley essential oils against most of the tested strains shown not to be statistically significant; MLC values of celery oil against the tested strains: >10% (*E. coli*, *L. plantarum*, *P. aeruginosa*, *S.* Typhimurium), 5% (*L. monocytogenes*, *Y. enterocolitica*), 2.5% (*S. aureus*); inhibitory activity of celery oil weaker compared with oregano, coriander, and basil oil	Research conducted with the use of commercially available essential oils (foodgrade, 100% pure essential oils, chemical composition not given).
	E. coli^{G-}			
	*G. candidum**			
	L. plantarum^{G+}			
	L. monocytogenes^{G+}			
	P. aeruginosa^{G-}			
	Rhodotorula†			
	S. Typhimurium^{G-}			
	S. aureus^{G+}			
	Y. enterocolitica^{G-}			
Friedman et al. (2002)	*C. jejuni*$^{G-}$ (four strains)	Modified microtiter plate bactericidal assay (BA_{50})	Celery seed oil inactive (BA_{50}>0.67%) against *E. coli* and *S. enterica*; weakly active against *L. monocytogenes* (BA_{50} 0.13–0.29%), high activity against *C. jejuni* (BA_{50} 0.0085%, among 11 most active oils tested); limonene (usually major or one of major compounds of celery oil) inactive against *E. coli*, *S. enterica*, and one strain of *L. monocytogenes* (BA_{50}>0.67%), weakly active against *C. jejuni* (BA_{50} 0.35%) and one *L. monocytogenes* strain (BA_{50} 0.25%).	Study conducted with the use of commercially available, 100% pure essential oils (chemical composition of oils not specified).
	E. coli^{G-} (six strains)			
	L. monocytogenes^{G+} (2 strains)			
	S. enterica^{G-} (four strains)			
Sipailiene et al. (2005)[d]	*E. coli*$^{G-}$	Disc-diffusion (IZ)	Antibacterial activity shown for both leaf and root supercritical carbon dioxide extracts; highest inhibitory activity against *B. cereus*; *C. freundii* and *P. vulgaris* resistant to both types of extracts at the concentrations used (1–10%); dose-dependent antibacterial activity against most strains; stronger inhibitory activity demonstrated for leaf extracts.	Major components of celery essential oil obtained by hydrodistillation of SFE from (1) leaves: limonene (32.2%), 3-*n*-butylphthalide (12.6%), myrcene (9.1%), δ-selinene (6.0%), α-humulene (4.3%), and (2) roots: limonene (21.9%), carvone (17.7%), 3-*n*-butylphthalide (11.6%), sedanolide (7.0%), sedanenolide (4.8%).
	B. cereus^{G+}			
	C. freundii^{G-}			
	E. aerogenes^{G-}			
	E. faecalis^{G+}			
	H. alvei^{G-}			
	L. monocytogenes^{G+}			
	P. vulgaris^{G-}			
	S. aureus^{G+}			

Continued

TABLE 3 Studies on Antimicrobial Properties of *Apium graveolens* L. Essential Oil—cont'd

References	Microbial Strains Used[a]	Antimicrobial Tests Applied (Measured Parameters Given in Parentheses)[b]	Results[a,b]	Comments[b]
Wannissorn et al. (2005)[e]	*C. jejuni*$^{G-}$	Disc-diffusion (IZ)	No antimicrobial activity against any of the bacterial strains tested shown for celery oil; substantial activity shown for *Cinnamomum bejoghota*, *Cymbopogon citratus*, *Mentha arvensis* var. *piperacens*, *Ocimum basilicum*, and *Zingiber cassumuna* essential oils.	Essential oils obtained by hydrodistillation (details of the procedure and chemical composition of oil not given).
	C. perfringens^{G+}			
	S. Enteritidis^{G-}			
	S. Typhimurium^{G-}			
Kamble and Patil (2008)[f]	Several fungal and mold strains, including *S. cerevisiae*[†]	Disc-diffusion (IZ)	Weak to moderate activity of celery oil against most of the strains used; weak inhibitory activity against *S. cerevisiae* (over three times weaker compared with 10 µg ketoconazole/disc; weaker than most of the essential oils tested, including allspice, anise, asafetida, basil, cassia, clove, coriander, cumin, fennel, and thyme).	Study conducted with the use of commercially available essential oils (production details and chemical composition not given).
Mišić et al. (2008)	*L. ivanovii*$^{G+}$ (three strains)	Broth macrodilution method (MIC).	Stronger inhibitory activity of SFE extract compared with hydrodistilled oil shown for *S. aureus*, (MIC 450 µg/mL, average for 8 strains tested, compared with >1100 µg/mL for hydrodistilled oil), *L. monocytogenes* (MIC 512 µg/mL, average for 8 strains tested, compared with >1100 µg/mL for hydrodistilled oil), and *L. ivanovii* (MIC 640 µg/ml, compared with >1280 for hydrodistilled oil).	Celery seed essential oil obtained by hydrodistillation or SFE with CO_2; major components of hydrodistilled oil: limonene (81.6%), sedanenolide (4.8%), sedanolide (2.5%), dictyotene (2.4%), β-selinene (1.8%) and β-pinene (1.8%); major components of SFE: sedanenolide (49.5%), limonene (26.4%), sedanolide (11.1%), β-selinene (3.6%), 3-*n*-butylphthalide (1.8%).
	L. monocytogenes^{G+} (five strains)			
	S. aureus^{G+} (eight strains)			
Patil and Kamble (2011)	*B. cereus*$^{G+}$	Disc-diffusion (IZ)	Inhibitory activity of celery oil against 50% of the tested bacterial strains (less than for allspice, cassia, clove, and nutmeg oils, more than for ginger, fennel, cardamom, black pepper, and turmeric oils); Gram-negative bacteria: no inhibitory effect of celery oil against *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris*, and *S.* Paratyphi A; mildly to moderately inhibitory activity against *E. aerogenes*, *P. aeruginosa*, and *S.* Typhi; Gram-positive bacteria: mildly to moderately inhibitory activity against *B. cereus*, *B. subtilis*, *S. aureus*, *S. pyogenes*.	Study conducted with the use of commercially available essential oils (production details and chemical composition not given); oils checked for sterility prior to antimicrobial tests.
	B. subtilis^{G+}			
	E. aerogenes^{G-}			
	E. coli (3 strains)$^{G-}$			
	K. pneumoniae^{G-}			
	P. vulgaris^{G-}			
	P. mirabilis^{G-}			
	P. aeruginosa^{G-}			
	S. Typhi^{G-}			
	S. Paratyphi A^{G-}			
	S. aureus^{G+}			
	S. pyogenes^{G+}			

Alves-Silva et al. (2013)	*A. denitrificans*[G–]	Colorimetric broth microdilution method (MIC) for yeasts and bacterial strains; disc-diffusion (IZ) for molds.	MIC values of celery essential oil against bacterial strains in the range 2.5–10.0 µL/mL, comparable to coriander and bush-basil oils; strongest inhibitory activity (2.5 µL/mL) against *L. innocua*, *S. marcescens*, and *S. putrefaciens*; MIC values against yeasts in the range 0.04–5.0 µL/mL, lowest for *D. hansenii* (0.04 µL/mL), *C. zeylanoides* (0.31 µL/mL) and *S. cerevisiae* (0.62 µL/mL); inhibitory activity against most yeasts tested stronger than for coriander and weaker than for bush-basil oils; celery oil inactive against *A. alternata*, activity against other molds weaker compared with bush-basil (*M. racemosus*, *P. chrysogenum*) and coriander (*P. chrysogenum*).	Essential oil obtained by microwave-assisted hydrodistillation from aerial parts of celery; major components: β-selinene (37.4%), phellandral (17.2%), limonene (16.0%), epiglobulol (7.7%), α-selinene (4.3%).
	A. faecalis[G–]			
	*A. alternata**			
	A. hydrophila[G–]			
	C. zeylanoides[†]			
	D. hansenii[†]			
	E. amnigenus[G–]			
	E. gergoviae[G–]			
	L. innocua[G+]			
	*M. racemosus**			
	*P. chrysogenum**			
	P. carsonii[†]			
	P. fluorescens[G–]			
	P. fragi[G–]			
	S. cerevisiae[†]			
	S. marcenscens[G–]			
	S. putrefaciens[G–]			
	Y. lipolytica[†]			
Marongiu et al. (2013)	Several fungal strains, including *A. flavus** and *A. niger**.	Broth macrodilution method (MIC, MLC).	Higher activity against *A. niger* and *A. flavus* shown for essential oil from wild-grown Italian celery (MIC 0.32 and 0.64 µL/mL, respectively; MLC > 1.25 µL/mL for both *Aspergillus* species); higher MLC values for Portuguese celery (>20 µL/mL); both essential oils more active against *Aspergillus* strains than amphotericin B.	Essential oils obtained by hydrodistillation from wild-grown plants; major components of oil from Italian celery: neophytadiene (34.6%), γ-himachalene (10.3%), caryophyllene (8.1%), sedanolide (7.6%); main constituents of Portuguese celery oil: sedanolide (45.2%), limonene (24.0%), sedanenolide (8.2%), 3-*n*-butyl phthalide (7.3%).

The table comprises the results of studies of antimicrobial activity of celery oil against different food-borne pathogens. Data are given on the antimicrobial assays used, as well as the composition of essential oil (if available). In the case of studies involving many oils, antimicrobial activity of celery oil is compared to volatile frations of other plants. Symbols: G+, Gram-positive bacteria; G–, Gram-negative bacteria; *, molds/fungi; †, yeasts.

[a]*Full Latin names of the microbial strains used (in alphabetical order): A. denitrificans, Achromobacter denitrificans; A. hydrophila, Aeromonas hydrophila; A. faecalis, Alcaligenes faecalis; A. alternata, Alternaria alternata; A. flavus, Aspergillus flavus; A. niger, Aspergillus niger; B. cereus, Bacillus cereus; B. subtilis, Bacillus subtilis; C. jejuni, Campylobacter jejuni; C. zeylanoides, Candida zeylanoides; C. freundii, Citrobacter freundii, C. perfringens, Clostridium perfringens; D. hansenii, Debaryomyces hansenii; E. aerogenes, Enterobacter aerogenes; E. amnigenus, Enterobacter amnigenus; E. gergoviae, Enterobacter gergoviae; E. faecalis, Enterococcus faecalis; E. coli, Escherichia coli; G. candidum, Geotrichum candidum; H. alvei, Hafnia alvei; K. pneumoniae, Klebsiella pneumoniae; L. plantarum, Lactobacillus plantarum; L. innocua, Listeria innocua; L. ivanovii, Listeria ivanovii; L. monocytogenes, Listeria monocytogenes; M. racemosus, Mucor racemosus; P. chrysogenum, P. chrysogenum; P. carsonii, Pichia carsonii; P. mirabilis, Proteus mirabilis; P. vulgaris, Proteus vulgaris; P. aeruginosa, Pseudomonas aeruginosa; P. fluorescens, Pseudomonas fluorescens; P. fragi, Pseudomonas fragi; S. cerevisiae, Saccharomyces cerevisiae; S. enterica, Salmonella enterica; S. Enteritidis, Salmonella Enteritidis;* S. Paratyphi A, *Salmonella* Paratyphi A*;* S. Typhi, *Salmonella* Typhi*;* S. Typhimurium, *Salmonella* Typhimurium*; S. marcenscens, Serratia marcenscens; S. putrefaciens, Shewanella putrefaciens; S. aureus, Staphylococcus aureus; S. pyogenes, Streptococcus pyogenes; Y. lipolytica, Yarrowia lipolytica; Y. enterocolitica, Yersinia enterocolitica.*

[b]*Abbreviations used: BA_{50}, bactericidal activity; IZ, inhibition zone; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MLC, minimum lethal concentration; SFE, supercritical fluid extraction/extract.*

[c]*Elgayyar, M., Draughon, F.A., Golden, D.A., Mount, J.R., 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot. 64, 1019–1024.*

[d]*Sipailiene, A., Venskutonis, P.R., Sarkinas, A., Cypiene, V., 2005. Composition and antimicrobial activity of celery (*Apium graveolens*) leaf and root extracts obtained with liquid carbon dioxide. Acta Hortic. 677, 71–77.*

[e]*Wannissorn, B., Jarikasem, S., Siriwangchai, T., Thubthimthed, S., 2005. Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia 76, 233–236.*

[f]*Kamble, V.A., Patil, S.D., 2008. Spice-derived essential oils: effective antifungal and possible therapeutic agents. J. Herbs Spices Med. Plants 14, 129–143.*

Table by Adam Kokotkiewicz, previously unpublished.

TABLE 4 Studies on Antioxidant Properties of *Apium graveolens* L. Essential Oil

References	Antioxidant Assays Applied (Evaluated Parameters Given in Parentheses)[a]	Results[a]	Comments
Wei and Shibamoto (2007)	Aldehyde/carboxylic acid (% inhibition); DPPH (% inhibition); MA/GC (% inhibition).	Aldehyde/carboxylic acid assay: inhibitory activity of celery oil at 0.5 mg/mL (96%) lower compared with ylang–ylang, parsley, and jasmine oils, higher activity compared with juniper berry, patchouli, and angelica seed; DPPH assay: inhibitory activity of celery oil at 0.2 mg/mL (55%) lower compared with jasmine, ylang–ylang and rose, higher activity compared with parsley, chamomile, juniper berry, patchouli, sandal wood, lavender, and angelica seed; MA/GC assay: inhibitory activity of celery oil at 0.5 mg/mL (23%) lower compared with parsley seed and rose, higher compared with ylang–ylang, juniper berry, patchouli, and jasmine.	Study conducted using commercially available essential oils; major constituents of celery seed oil: limonene (74.6%) and β-selinene (11.0%).
Kiralan et al. (2012)[b]	DPPH (% inhibition)	DPPH assay: 41.67% inhibitory activity of celery oil at ca. 29 mg/mL concentration (assuming 0.9 specific gravity of celery oil according to Rao et al. (2000)), effect comparable to myrtle oil, lower in compared with fennel, aniseed, parsley, and garlic oils.	Celery seed oil obtained by hydrodistillation (37.4% β-selinene, 16% limonene, details on chemical composition given in Table 1).
Alves-Silva et al. (2013)	DPPH (% inhibition, IC_{50}), FIC (% inhibition, IC_{50}), FRAP (TEAC), TBARS (IC_{50})	DPPH test: inhibition for celery oil (ca. 20–25% at 0.15–20 mg/mL range) comparable to coriander (ca. 20%) and significantly lower compared with bush-basil (>90% at 2.5–20 mg/mL range), IC_{50} value for celery oil (88.2 mg/mL) comparable to coriander (89.6 mg/mL), and significantly higher compared with bush-basil (0.31 mg/mL); FIC assay: dose-dependent inhibition, weak activity (<30%) for all oils at 0.15–0.5 mg/mL range, >80% inhibition for all oils at >10 mg/mL range, comparable IC_{50} values for celery and coriander oils (2.61 and 2.36 mg/mL, respectively), lower than IC_{50} for bush-basil (3.44 mg/mL); FRAP assay: no antioxidant activity of celery oil at concentration below 2.5 mg/mL; antioxidant activity at 20 mg/mL (ca. 0.25 mmol Trolox/L) significantly lower compared to bush basil (>2.5 mmol Trolox/L); TBARS test: dose-dependent inhibition (ca. 65, 70, and 80% at 0.5 mg/mL for celery, coriander, and bush-basil oils, respectively, >80% for all oils at 5–20 mg/mL range), highest IC_{50} value for celery oil (0.06 mg/mL), followed by coriander (0.01 mg/mL) and bush-basil (1.39×10^{-6} mg/mL).	Essential oils obtained from flowering aerial parts by microwave-assisted hydrodistillation, celery oil components: 76.6% limonene, 11.1% β-selinene (details on chemical composition given in Table 1).

The table summarizes the results of studies of antioxidant activity of celery oil. Data are given on the assays used, as well as the composition of essential oil (if available). In the case of studies involving many oils, antioxidant activity of celery oil is compared to volatile fractions of other plants.
[a]*Abbreviations used: DPPH, 2,2′-diphenyl-1-picrylhydrazyl; FIC, ferrous ion-chelating; FRAP, ferric reducing antioxidant power; IC_{50}, half maximal inhibitory concentration; MA/GC, malonaldehyde/gas chromatography; TBARS, thiobarbituric acid reactive species; TEAC, Trolox equivalent antioxidant capacity.*
[b]*Kiralan, M., Bayrak, A., Abdulaziz, O.F., Özbucak, T., 2012. Essential oil composition and antiradical activity of the oil of Iraq plants. Nat. Prod. Res. 26, 132–139.*
Table by Adam Kokotkiewicz, previously unpublished.

Given the obtained results, it can be assumed that celery essential oil is worth further examination as a natural food preservative. However, it order to define its usefulness as an antispoilage agent, antimicrobial and/or antioxidant activity of *A. graveolens* volatile oils of known composition need to be evaluated in specific food products. It is worth mentioning that due to the high nitrate content, celery has long been used in the meat industry as a natural curing agent (Sebranek et al., 2012). Thus, it would be of interest to examine possible synergistic effects between nitrate-rich celery powder and volatile oil with respect to meat preservation. However, the use of volatile oil as an ingredient inevitably gives food a strong flavor which is often unacceptable in the final product (Lis-Balchin and Deans, 1997; Sebranek et al., 2012). In fact, the flavor of *A. graveolens* is considered as a factor that limits the amount of celery powder that can be added to meat (Sebranek et al., 2012). Given that the effective antimicrobial concentration of essential oil may far exceed that sufficient for flavoring purposes (Lis-Balchin and Deans, 1997; Sebranek et al., 2012), the use of celery oil would probably be restricted to food products that are in harmony with its specific taste and aroma.

SUMMARY POINTS

- Essential oil is obtained mainly from celery fruits, commonly referred to as "celery seeds".
- Volatile oil is composed of monoterpenes (mainly limonene), sesquiterpenes (selinene), and phthalide derivatives (3-*n*-butylphthalide, sedanolide, sedanenolide).
- There is substantial variance in the composition of essential oil depending on celery variety, plant part, and country of origin.
- The distinctive flavor is due to the presence of phthalide derivatives.
- Celery seeds and essential oil are used as condiments and flavoring agents in food products.
- Antibacterial, antifungal, and antioxidant activity of celery volatile oil has been demonstrated in vitro.
- Its usefulness as a preservative in specific food products yet to be evaluated.

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Chapter 38

Cinnamon (*Cinnamomum zeylanicum*) Essential Oils

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INTRODUCTION

Cinnamon is among the earliest, most popular spices used by mankind. The commercial presentation of cinnamon is the dried inner bark of the tree, or the milled bark. The bark oil, bark oleoresin, and leaf oil are important value-added products from cinnamon. Bark oil is used in the food and pharmaceutical industries. Cinnamon leaf oil is cheaper than bark oil and is used in the flavor industry. Cinnamon oleoresin, obtained by solvent extraction of the bark, is used mainly for flavoring food products such as cakes and confectionary, soft drinks, and other beverages (Leela, 2008).

Cinnamon essential oil may be extracted by hydrodistillation, solvent extraction, and by microwave-assisted extraction. Yield and composition of the extracted essential oil are affected by the extraction method, as shown in Table 1. The composition of the essential oil is commonly determined throughout gas chromatography–mass spectrometry (GC–MS), when diverse volatile compounds are detected. Figure 1 shows a typical spectrogram obtained for cinnamon essential oil.

Cheng et al. (2006) reported that cinnamon essential oil main compounds consist of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated hydrocarbons, diterpene hydrocarbons, and oxygenated diterpenes. Osuna et al. (2005) reported a detailed classification according to the functional groups of the components isolated from cinnamon essential oil, which are listed on Table 2. In general, the major compounds present in the cinnamon essential oil are cinnamaldehyde, camphor, cinnamyl-acetate, caryophyllene, *trans* α-bergamotene, caryophillene oxide, linalool, geraniol, bornyl acetate, α-cubebene, γ-elemene, α-copaene, guaiol, and eugenol, among others (Jayaprakasha et al., 1997, 2000; Cheng et al., 2006). Figure 2 shows the chemical structures of the major compounds in cinnamon essential oil.

Regarding its physical characterization, Table 3 shows the analyses made to the cinnamon essential oils in different studies. Only the optical rotation showed a remarkable difference with respect the species of cinnamon, whereas specific gravity and refractive index showed similar ranges. Physical properties analyses are often conducted with the aim of establishing the quality, purity, and chemical stability of the essential oils (Gende et al., 2008).

BOTANICAL ASPECTS

The genus *Cinnamomum* (Laureaceae family) consists of 250 spices of trees and shrubs distributed in south-east Asia, China, and Australia. It is a small, evergreen tree, 10–15 m tall. The bark is widely employed as a spice, its leaves are ovate-oblong in shape, and 7–18 cm long. The flowers, arranged in panicles, have a greenish color and have a rather disagreeable odor. The fruit is a purple 1 cm berry containing a single seed (Leela, 2008). It is found in tropical rain forests, where it grows at various altitudes from highland slopes to lowland forests and occurs in both marshy places and on well-drained soils. However, in latitudes with seasonal climatic conditions, they become exceedingly rare (Jantan et al., 2008).

The most important cinnamon oils in word trade are those from *Cinnamomum zeylanicum*, *Cinnamomum cassia*, and *Cinnamomum camphora*. The cinnamon essential oil composition varies depending on the geographical origin of the spice and the processing conditions. Likewise, it has been detected slight differences between the composition of volatile oils from buds, flowers, and fruits from *C. zeylanicum*. Jayaprakasha et al. (2000) reported that the oil from buds contains higher amounts of mono and sesquiterpenes, and most characteristic compounds such as cinnamaldehyde are found in flowers and fruits and in lower amounts in buds, as shown in Table 4.

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TABLE 1 Extraction Methods to Obtain Cinnamon Essential Oil from Some Species

Specie	Extraction Method	Yield (%)	Reference
Cinnamomum zeylanicum	Steam distillation	0.04–0.40	Kaul et al. (2003)
Cinnamomum osmophleum	Hydrodistillation (Clevenger-type apparatus)	0.1–4.7	Lee et al. (2005)
C. osmophleum	Hydrodistillation	0.1–2.9	Cheng et al. (2006)
C. osmophleum	Hydrodistillation	0.08	Tung et al. (2008)
C. osmophleum	Hydrodistillation	0.82–4.69	Cheng et al. (2009)
C. zeylanicum	Microwave-assisted extraction	0.15–0.47	Reyes-Jurado (2013)

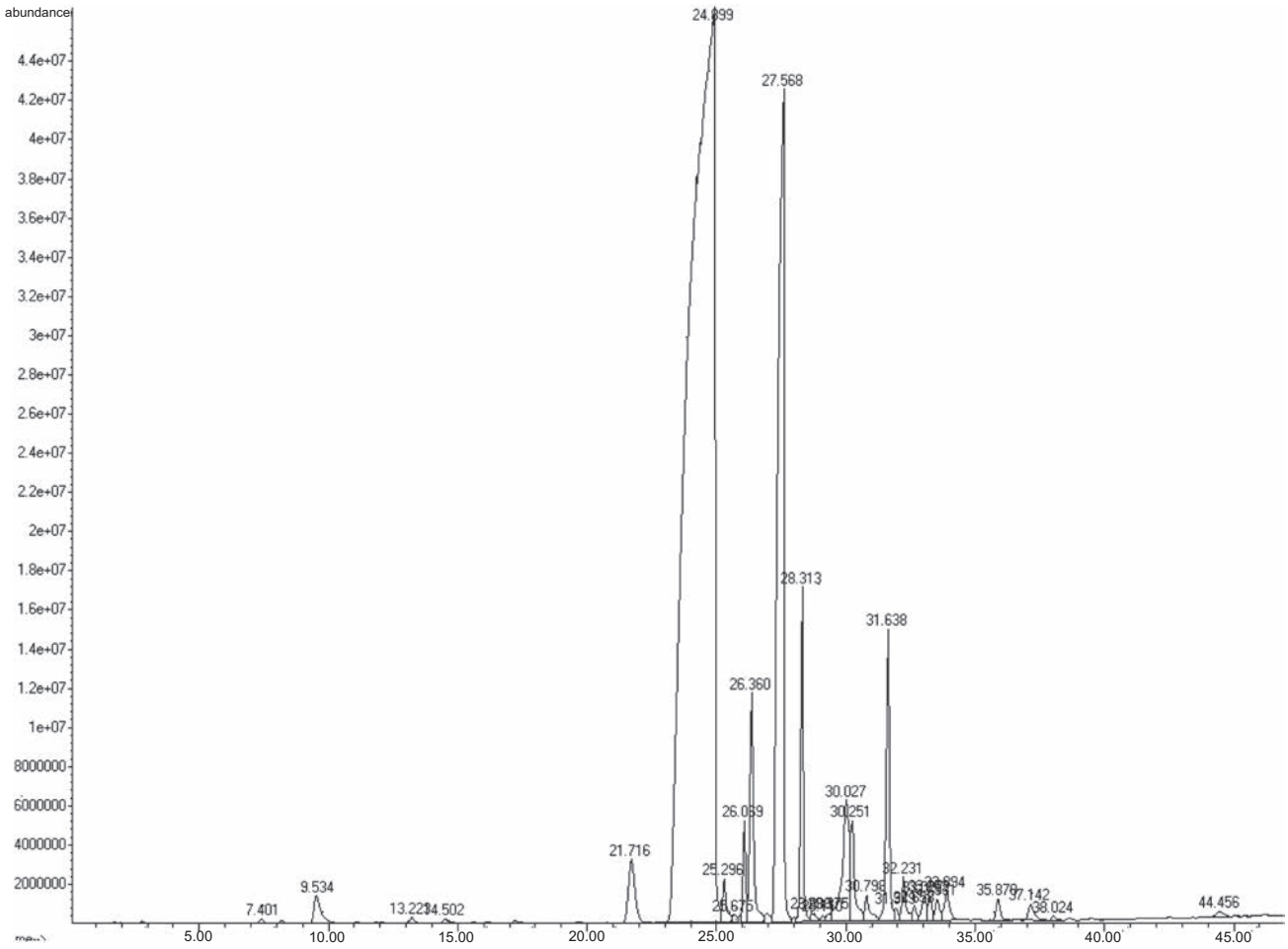

FIGURE 1 **Example of spectrogram of composition of cinnamon essential oil.** *Adapted from Reyes-Jurado (2013).*

USAGE AND APPLICATIONS

Throughout history, cinnamon essential oil has been used as an ingredient in many medicinal preparations; the oil from bark is an aphrodisiac, anthelmintic, and tonic. It is useful in the treatment of biliousness, parched mouth, bronchitis, diarrhea, itching, and heart and urinary disease; it is also carminative and expectorant, and is useful in hydrocoele, flatulence, headache, piles, etc. (Kirtikar and Basu, 1984). Several studies have demonstrated that leaf essential oils from *Cinnamomum osmophloeum* have excellent properties against termites, bacteria, mildew, mosquitoes, and other pathogens (Cheng et al., 2006). According to Verspohl et al. (2005), the essential oil from *C. cassia* has positively shown antidiabetic effects by

TABLE 2 Classification of Functional Groups and Components Isolated from Cinnamon Essential Oil

Monoterpenes	α-Terpineol
	Geraniol
	Phellandrene
	Borneol
	Cymene
	Decanal
	Furfural
	Citronellol
	Carvacrol
Alcohols	2-Phenylethyl
	Cinnamic
	Benzyl
	Cuminic
Sesquiterpenes	Limonene
	Linalool
Monoterpene hydrocarbons	Caryophyllene
	α-Pinene
Acids	*Trans*-cinnamic
	Benzoic
	Caproic
	Caprylic
	Formic
	β-Hydroxybutyric
	Isovaleric
	Lauric
	Myristic
	Propionic
	Salicylic
	Tannic
Esters	Benzylbenzoate
	Phenylethylbenzoate
	Methyl cinnamate
	Benzyl cinnamate
Aldehydes	Cinnamaldehyde
	Hydroxycinnamaldehyde
	Benzaldehyde
	O-methoxycinnamaldehyde
	Acetaldehyde
	Cumminic aldehyde
	Isovaleric aldehyde

Continued

TABLE 2 Classification of Functional Groups and Components Isolated from Cinnamon Essential Oil—cont'd

Phenols	Cinnamyl acetate
	Cinnamic aldehyde
	Eugenol
	Isoeugenol
	2-Vinylphenol
	Eugenol methyl ether
	Coumarin
Other compounds	Lignans
	Sterols

Adapted from Osuna et al. (2005).

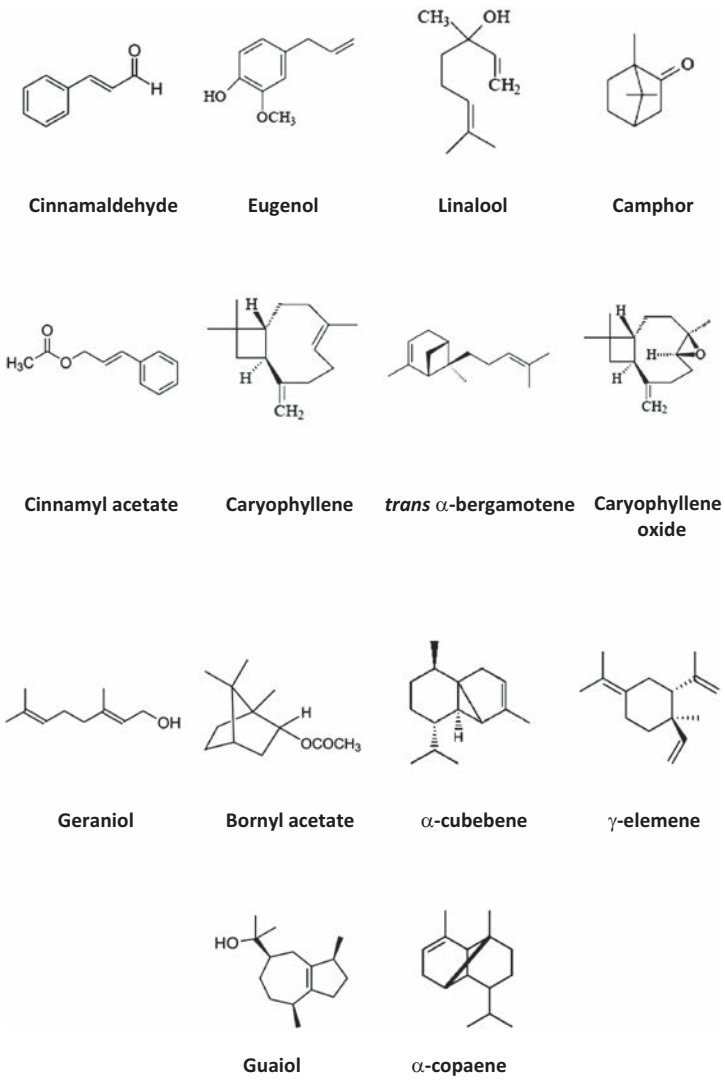

FIGURE 2 Chemical structures of the major compounds present in cinnamon essential oil.

TABLE 3 Physical Properties of the Essential Oil of Selected Species of Cinnamon

Property	*Cinnamomum cassia*	*Cinnamomum verum*	*Cinnamomum zeylanicum*
Density (g/mL)	1.055 to 1.070 (at 15 °C)	1.021 to 1.070 (at 20 °C)	1.061 (at 20 °C)
Refractive index	1.6000 to 1.6060 (at 15 °C)	1.5670 to 1.6140 (at 20 °C)	1.6073
Optical rotation (°)	1 to 6 (at 15 °C)	−1 to 0	–

Adapted from Leela, 2008; Gende et al. (2008).

TABLE 4 Major Compounds Detected in the GC–MS Analysis of Essential Oils From Different Parts of Cinnamon

Part of the Plant	Major Compounds
Buds	α-copaene, α-bergamotene, α-humulene, and δ-cadinene
Flowers	α-copaene, *trans*- α-bergamotene, caryophyllene oxide, tetradecanal, and α-cadinol
Fruit stalks	caryophyllene, (*E*)-cinnamyl acetate, α-humulene, d-cadinene, and t-cadinol
Fruits	(*E*)-cinnamaldehyde, β-caryophillene, and (*E*)-cinnamyl acetate

Adapted from Jayaprakasha (2003).

displaying an insulin-enhancing activity in vitro. Studies have shown that the essential oil and its constituents also possess antimicrobial, insecticidal, acaricidal, antityrosinase, antioxidant, and antimutagenic activities (Unlu et al., 2010).

The consumption of some compounds in cinnamon essential oils is restricted to light doses because of their toxicity. For example, cinnamic acid, a compound used in perfumery produces dermatitis, and when added to toothpaste, it promotes oral sensitivity. Moreover, it has been reported that eugenol is an irritant and a weak tumor promoter. More toxic compounds in the cinnamon essential oil are limonene, linalool, terpineol, geraniol, phelandrene, borneol, cymene, decanal, and furfural; however, its toxicity is associated with a dose higher than 3g/3 kg. The recommended dose for internal use is 1–2 g per day (Osuna et al., 2005).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Cinnamon has been granted generally recognized as safe status as a food additive by the Food and Drug Administration. It is useful as a food preservative to inhibit the growth of fungal species (Xing et al., 2010) and also owing to its antioxidant properties, antibacterial, insecticidal, and nematicidal activities; it has been widely used in the food industry. Also, it is employed in seasonings, sauces, bakery, confectionery, and drinks.

Antioxidant

Nowadays, natural antioxidants are in great demand, due to both consumer preference and health concerns associated with the use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

To estimate the antioxidant ability of essential oils, many different methods are used, and the value of antioxidant activity depends on the method of estimation. As it has been done with most synthetic and natural antioxidants, researchers have employed several in vitro and in vivo tests to assess the antioxidant activity of cinnamon essential oil.

The evaluation of extinction capacity of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a commonly used assay to determine the antioxidant activity. This method was utilized by Lin et al. (2009) to evaluate the antioxidant activity of 42 kinds of essential oils including cinnamon essential oil, which showed the strongest DPPH free-radical scavenging activity (91.4 ± 0.002%) among the essential oils tested. Furthermore, this strong antioxidant activity of cinnamon essential oil was confirmed through the photochemiluminescence assay that measures the antioxidant capability of lipid soluble substances; the capability reported was 133.9 ± 0.26 μmol trolox/g. Nevertheless, synthetic antioxidant BHA showed a stronger antioxidant activity than cinnamon essential oil.

Wang et al. (2010) applied three different in vitro assays including 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation radical ($ABTS^+$) radical scavenging ability, metal chelating activity, and reducing power in order to test the antioxidant activity of 45 essential oils including cinnamon essential oil. In the $ABTS^+$ assay, cinnamon essential oil showed the strongest antioxidant activity at a concentration of 1 mg/mL (96.45 ± 0.01%). Similar results were observed in the determination of reducing power assay; cinnamon essential oil showed the best reducing power among all the essential oils tested at a concentration of 10 mg/mL (119.42 ± 0.68% reducing power relative to BHA). However, cinnamon essential oil showed one of the weakest results in the metal chelating activity assay reporting 4.63 ± 0.07%. It was also determined that eugenol, the major compound in cinnamon essential oil used in this study, possessed higher antioxidant activity than the essential oil itself.

Another method to assess the antioxidant activity of essential oils is based in the inhibition of autoxidation of aldehyde to carboxylic or hexanoic acid in the presence of compounds exhibiting antioxidant. The inhibition of 2-hexenal oxidation by cinnamon essential oil was studied by Misharina et al. (2009), who reported an inhibition of 66 and 78% at concentrations of 3.33 and 16.5 μL/mL, respectively. This antioxidant activity was attributed to the main compounds found in the essential oil, the cinnamaldehyde and cinnamyc acetate. Moreover, the antioxidant activity was higher than the reported by ginger, nutmeg, laurel, marjoram, and fennel essential oils.

Antifungal

The strong antifungal activity of natural products containing phenolic compounds such as linalool, eugenol, menthol, cinnamaldehyde, thymol, and carvarcrol, as well as the misuse of toxic fungicides and its contribution to environmental pollution (Lee et al., 2005) has led to the increase of the application of essential oils from herbs and spices in the food industry as additives.

Early, extracts of cinnamon were evaluated as antifungal agent against *Aspergillus flavus* by López-Malo et al. (2007). Xing et al. (2010) evaluated in vitro and in vivo antifungal activity of cinnamon essential oil against *Rhizopus nigricans*, *A. flavus*, and *Penicillum expansum*. The minimum inhibitory concentration of cinnamon essential oil against the tested fungi was 0.64, 0.16, and 0.16% (v/v) for *R. nigricans*, *A. flavus*, and *P. expansum*, respectively. Because cinnamaldehyde was the major compound detected in the cinnamon essential oil used in this experiment (76.34%), the authors attribute the strong antifungal activity of the essential oil mainly to this compound. Regarding in vivo experiments, cinnamon essential oil was tested to control the postharvest decay of two kinds of fruits (lingwu long jujube and sans sugar orange) caused by the same fungi. Concentrations of 0.10, 0.25, 0.50, 1.0, 2.0, and 3.0% essential oil were directly inoculated to wounds on the fruits. The highest two concentrations provided a complete control of disease incidence caused by the three molds in both fruits tested; in addition, a concentration of 1.0% successfully controlled the disease incidence caused by *A. flavus* in lingwu long jujube.

The strong antifungal activity attributed to cinnamaldehyde has also been reported by other researchers. Lee et al. (2005) compared the chemical constituents of essential oils from various geographical provenances of *C. osmophleum* and investigated their antifungal activities against some tree pathogenic fungi; the essential oil from the cinnamaldehyde type at a dosage of 100 μg/mL showed an inhibitory effect against seedling pathogen *Rhizoctonia solani* of 85.7%, 100.0% against root pathogens *Ganoderma australe*, and *Fusarium solani* and 85.8% against leaf pathogen *Collectotrichum gloeosporioides*. A similar study was conducted by Cheng et al. (2006) who tested the antifungal activity of *C. osmophleum* essential oils from nine different provenances. Results also showed that the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type possessed the strongest antifungal activity by completely inhibiting *Trametes versicolor*, *Lenzites betulina*, and *Laetiporus sulphureus* at a concentration of 200 μg/mL.

In a study performed by Manso et al. (2011), the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) against *A. flavus* for cinnamon essential oil were determined using a direct contact antifungal assay and compared with the MIC and MFC for oregano essential oil. The MIC and MFC of cinnamon essential oil (100 and 200 ppm, respectively) were lower than the 400 and 800 ppm obtained for oregano essential oil.

Reyes-Jurado (2013) evaluated the antifungal activity of the vapor phase of cinnamon essential oil against molds. *Aspergillus niger* was inhibited with 500, 1000, and 2000 μL of cinnamon essential oil per liter of air in agar systems formulated at pH 3.5, 4.5, and 5.5, respectively. For the same pH values, concentrations of 300, 800, and 1500 μL of cinnamon essential oil per liter of air were the minimal inhibitory concentrations against *P. expansum*.

Antibacterial

The application of essential oils from herbs for antibacterial purposes in the food science field was developed many years ago. The leakage of cell contents such as lipids and proteins and the damage to cell membrane caused by lipophilic compounds present in the essential oils are some of the mechanisms of action suggested for their antibacterial activity (Mello et al., 2012).

Pathogenic bacteria are one of the most concerning issues during the production, handling, and processing of foodstuffs. The antibacterial effect of cinnamon essential oil against *Eschericha coli* O157:H7 and *Salmonella enteric*a in strawberry juice was tested and compared with the bactericidal activities from lemongrass and basil by Duan and Zhao (2009). Four concentrations of essential oils were tested: 0, 1, 3, 5, and 10 μL/mL of juice. Among the three essential oils tested, cinnamon essential oil showed the best bactericidal activity inactivating the growth of both bacteria with the lowest concentration used (1 μL/mL) in the in vitro test. On the other hand, the tests of the antimicrobial activity of the cinnamon essential oil in strawberry juice showed that the faster rate of inactivation of *E.* coli O157:H7 was reached with a concentration of 0.5 μL/mL or above. However, contrary to what was reported by other researchers, in this study the antimicrobial activity of cinnamon essential oil was attributed to eugenol, the major component detected.

The antibacterial activity of cinnamon essential oil was tested by Gende et al. (2008), who determined its MIC and minimal bactericidal concentration (MBC) against three different strains of *Paenibacillus larvae*. The lowest MIC value of cinnamon essential oil, 25–50 mg/L, was obtained for the Chapadmalal strain, followed by 50 mg/L for the Mechongué strain, and 50–100 mg/L for the Cobo strain. With respect to the MBC, the lowest value was 125–150 mg/L, registered for the strain Chapadmalal, followed by 150–200 mg/L registered for the other two strains. As reported for the antifungal activity of cinnamon essential oil, the authors also attribute its bactericidal activity to cinnamaldehyde, the main compound detected in this study.

The presence of cinnamaldehyde as the most abundant compound in cinnamon essential oil and as its main bactericidal compound was also reported by Bouhdid et al. (2010), who concluded that the cinnamon essential oil mechanisms of action against *Staphylococcus aureus* were the leakage of the intracellular potassium ion from the cells of the bacteria and a considerable reduction in metabolic activity and in its replication capacity.

Mello et al. (2012) tested the MIC and MBC of cinnamon essential oil against six selected pathogenic food-related bacteria. *Yersinia enterocolitica* resulted to be the most sensitive bacteria in the presence of cinnamon essential oil with MIC and MBC values of 0.075 and 1.25 mg/mL, respectively, followed by *Listeria monocytogenes* and *Bacillus cereus* that presented the same values of MIC and MBC (2.5 and 5.0 mg/mL, respectively). Finally, the highest MIC and MBC values were obtained for *St. aureus*, *E. coli*, and *Salmonella* typhimurium: 5.0 and 10.0 mg/mL, respectively. However, in this study, the authors indicated that the antibacterial activity of cinnamon essential oil was due to the presence of oxygenated compounds such as linalool, 1,8-cineole, neral, and geranial.

Packaging

As it has been mentioned in the present review, cinnamon essential oil possesses strong antimicrobial activity against both bacteria and fungi. This feature makes the essential oil ideal to be used as an additive in the food industry in different ways as in the packaging processes. According to Hosseini et al. (2009), active packaging is one of the innovative food packaging concepts that has been introduced as a response to the continuous changes in current consumer demands and market trends; edible films and coatings are used with foods to prevent moisture permeation, limit gas transport retard fat and oil migrations, retain volatile flavored compounds, and improve mechanical handling of foods.

Hosseini et al. (2009) evaluated the influence of the addition of cinnamon, thyme, and clove essential oils to a chitosan-based film in its physical, mechanical, and antimicrobial properties against Gram-positive (*L. monocytogenes* and *St. aureus*) and Gram-negative strains (*Salmonella* enteritidis and *Pseudomonas aureaginosa*). The inhibition zones produced by cinnamon essential oil were lower than those produced by clove and thyme essential oils at all concentrations tested. The authors attributed this weak antimicrobial effect to the chemical interactions between functional groups of chitosan and oxalic acid present in cinnamon essential oil. However, the addition of cinnamon essential oil improved the physical properties of the chitosan-based film, reducing its moisture content, the water vapor transmission rate, and its solubility in water with respect to the films added with clove and thyme essential oils in all the concentrations tested. In addition to that, the incorporation of cinnamon essential oil to the chitosan films increased its tensile strength. Therefore, the authors proposed to use cinnamon essential oils in films for coating highly perishable foods such as fish and poultry.

In order to take advantage of cinnamon essential oil's antimicrobial properties, Du et al. (2009) developed an edible apple film, with sensory properties such as flavor and aroma best fitted with cinnamon essential oil. The in vitro assays demonstrated that the films added with cinnamon essential oil inhibited the growth of pathogenic bacteria in the following order, *E.coli* O157:H7, *L. monocytogenes*, and *Sa. enterica*, both by direct contact and vapor phase diffusion methods. With respect to the mechanical properties of the apple edible films, the addition of cinnamon essential oil did not affect their water vapor transmission rate; however, the tensile strength of films was slightly reduced by the addition of the essential oil.

A gelatin coating enriched with cinnamon essential oil was tested as freshness maintainer of rainbow trout by Ghasem and Rezaei (2011). Fish fillets were immersed into gelatin solutions with cinnamon essential oil in concentrations of 1.0,

1.5, and 2.0% v/v. It was found that after 15 days of refrigeration storage the addition of 1.5 and 2.0% of cinnamon essential oil kept the total viable aerobic bacteria count below 7 log CFU/g, the limit proposed by the International Commission of Microbiological Specifications for Foods. Moreover, the addition of cinnamon essential oil to the gelatin coating decreased the lipid oxidation of fish during storage measured by means of the thiobarbituric acid reactive substances (TBARS) index. The TBARS value reported was much lower than the limits established (1–2 mg malonaldehyde/kg of fish sample). Additionally, after the sensory evaluation, fish fillets coated with gelatin and cinnamon essential oil were judged acceptable for food consumption, contrary to the samples without cinnamon essential oil.

The packaging effectiveness of cinnamon essential oil from barks and leaves along with the use of modified atmospheres and low-density polyethylene on the control of postharvest diseases of bananas (*Musa*, AAB) was tested by Ranasinghe et al. (2005); results showed that mature bananas harvested at the green stage could be stored for 21 days at 14 °C, at a relative humidity of 90%, and 28 days at 28 °C at the same relative humidity. Samples did not develop a postharvest disease caused by pathogen fungi and ripening was retarded because of the modified atmosphere created inside the packages. In addition, essential oils did not affect firmness of the samples, pH, titratable acids, carbohydrates, or reducing sugar content. Moreover, the addition of essential oils did not have an effect on the sensory attributes of bananas after 21 days of storage.

SUMMARY POINTS

- Cinnamon essential oil is obtained from the different species of the genus *Cinnamomum*.
- The main uses of cinnamon essential oil are in the pharmaceutical, confectionary, perfumery, and food industries.
- Cinnamon essential oil composition varies according its geographical provenance and the parts of the plant used.
- Cinnamon essential oil's main components are cinnamaldehyde, camphor, cinnamyl acetate, caryophyllene, *trans* α-bergamotene, caryophillene oxide, linalool, geraniol, bornyl acetate, α-cubebene, γ-elemene, α-copaene, guaiol, and eugenol.
- Cinnamon essential oil is used in the food industry as a flavoring, seasoning, and aroma enhancer agent.
- Biological activities such as antioxidant, antifungal, and antibacterial give cinnamon essential oil a broader application in food industry.
- Antioxidant activity of cinnamon essential oil is mainly attributed to eugenol, cinnamaldehyde, and cynammic acetate.
- Antimicrobial activity of cinnamon essential oil has been attributed to cinnmaldehyde in both fungi and bacteria.
- Addition of cinnamon essential oil in bio active films does not affect sensory characteristics of products and retards microbial growth in packaged foods.

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Chapter 39

Clove (*Syzygium aromaticum*) Oils

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List of Abbreviations

MIC Minimal inhibitory concentration
MBC Minimal bactericidal concentration
E. coli Escherichia coli
USA United States of America
GAP Good Agricultural Practices
ATP Adenosin triphosphate
LAB Lactic acid bacteria
Aw Water activity
MES Total mesophyllic count
TC Total coliforms counts
PSC Total psycrophillic counts
DBH Days before harvest

INTRODUCTION

Many consumers tend to be suspicious of chemical additives and demand for natural preservatives (Omidbeygi et al., 2007). As a consequence, the exploration of naturally occurring antimicrobials for food preservation receives increasing attention. Plant extracts have been used in the treatment of different diseases and illness. After penicillin was introduced as a broad spectrum antibiotic, phytotherapy was relegated to second place (Shiva-Ramayoni, 2007). However, the new and increased interest for natural and organic products has been responsible for the reinstatement of natural plant extracts as a subject for many research studies.

The most popular plant extracts that have antimicrobial activities are essential oils. These are obtained directly from the plant, without subsequent transformation. Currently, oil yield is low and this is still expensive. Nevertheless, the interest of the consumers makes them subject of several studies.

BOTANICAL ASPECTS

Clove is the name given to the aromatic dried flower buds of the tree *Syzygium aromaticum.* It belongs to the genus *Syzygium*, tribe *Syzygieae*, and subfamily *Myrtoideae* of the family *Myrtaceae*. It is classified in the order of *Myrtales*, which belong to superorder *Rosids*, under Eudicots of Dicotyledonae. Clove is an angiospermic plant and belongs to division of *Magnoliophyta* in the kingdom *Plantae*. The tree grows primarily in Indonesia, India, Madagascar, Zanzibar, Pakistan, and Sri Lanka.

The clove tree is an evergreen that grows to a height ranging from 8 to 12 m, having large leaves and sanguine flowers in numerous groups of terminal clusters. The flower buds are at first a pale color and gradually become green, after which they develop into a bright red, when they are ready for collecting. Cloves are harvested when they reach 1.5–2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals which form a small ball in the center. The chemical composition of clove essential oil varies among the different locations the plant has been cultivated from as well as the method used to extract the essential oil, among other factors (Shiva-Ramayoni, 2007). Nevertheless, a general

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composition could be informed as a reference and it is highly recommended, when possible, to state a detailed composition to assure repeatability of the results. Omidbeygi et al. (2007) showed the composition of clove essential oil (cultivated in Iran and extracted for 3 h by distilled water) to be mainly eugenol (63.37%), β-caryophyllene (15.94%), eugeyl acetate (13.14%), α-humelene (2.62%), and caryophyllene oxide (1.06%).

USAGE AND APPLICATIONS

Most traditional medicine therapies involve the use of plant extracts or their active components, which are already established as flavorings in food. However, certain essential oils also have added technical benefits that may be exploited.

The essential oil extracted from the dried flower buds of clove is used as a topical pain reliever and to promote healing. Traditionally, clove is used against intestinal parasites, skin infections, digestive upsets, and toothaches. It also finds use in the fragrance and flavoring industries.

Clove essential oil has also been used as an antioxidant agent. Farag et al. (1989) treated cotton seed oil with different essential oils, including clove. Tests indicated that the addition of clove to cottonseed oil, at concentrations ranging from 50 to 1200 ppm, do not affect the odor note while reducing oxidation. Therefore, clove could be recommended as natural antioxidants to suppress lipid oxidation.

The usage of clove, in a mix with anise and oregano oils, was studied as a natural growth promoting substance, instead of antibiotics (Ertas et al., 2005). Different levels of clove were added to a standard diet, to determine its effect on feed intake, daily weight gain, and feed conversion ratio compared with control and antibiotic groups. The feed intake was similar between the groups, but the highest daily weight gain was observed in the group in which essential oils were added (16% increased over the control group and 8% over the antibiotic group). The results showed that clove essential oil could be considered as a potential natural growth promoter for poultry.

Another possible application of essential oils in general, and clove in particular, could be as insect repellent. Trongtokit et al. (2005) made a comparative study on mosquito (*Aedes aegypti*) repellent activity of 38 essential oils under laboratory conditions using human subjects. Clove essential oil, applied undiluted, was effective and provided 2 h of complete repellency.

USAGE AND APPLICATIONS IN FOOD SCIENCE

The exploration of essential oils for food preservation receives increasing attention due to consumer preference and the increasing concern for the resistance that microorganisms have developed to conventional preservatives. Consumers demand fewer chemical residues in vegetables and legislation imposes lower limits. These facts generate the necessity of developing viable alternatives that reduce environmental risks and increase consumer confidence in the product (Zhang et al., 2007).

Essential oils are used in different foods as aromatizers or biopreservatives. Several studies have shown that essential oils presented also antibacterial, antifungal, antiparasitic, and insecticidal properties (Ponce et al., 2008, 2011). Some of the essential oils that have been tested in food are tea tree (*Melaleuca alternifolia*), rosemary (*Rosmari nusafficinalis*), peppermint (*Mentha piperita*), clover (*S. aromaticum*), lemon (*Citrus Limonum*), oregano (*Origanum vulgare*), etc. Many in vitro studies reported efficiency of essential oils as antimicrobials or natural antioxidants (Ponce et al., 2003; Moreira et al., 2007). Ponce et al. (2004) and Moreira et al. (2005) studied the in vitro antimicrobial activity of clove essential oil and determined the minimal inhibitory concentrations (MIC) and its effect on the antioxidant properties of vegetables. Studies have also been conducted in vivo, applying clove essential oil in lettuce seeds (Goñi, 2013), young lettuce plants while still in the greenhouse (Goñi, 2013; Goñi et al., 2013), and postharvest lettuce (Ponce et al., 2008; Ponce et al., 2010; Goñi, 2013; Goñi et al., 2013).

Effects on the Native Microflora of Organic Swiss Chard

Eugenol, carvacrol, and thymol are phenolic compounds present in cinnamon, clove, sage, and oregano, which present antimicrobial activity. The exact cause–effect relation for the action mode of phenolic compounds has not been determined, but Davidson (1993) indicates that they may inactivate essential enzymes, reacting with the cell membrane or disturbing material functionality. Zaika (1988) reported a significant antimicrobial activity of clove, where eugenol accounts for 95% of the fraction (Shelef, 1983). Ponce et al. (2003) evaluated the antimicrobial activity of clove (*S. aromaticum*) essential oil on the native microflora of Swiss chard grown by organic methods. The susceptibility of the native microflora to clove, as determined by the agar diffusion method, showed that clove produced an inhibition halo of 22.27 ± 2 mm in diameter, making it the oil with a significant inhibitory effect.

Ponce et al. (2003) evaluated the MIC and minimal bactericidal concentration (MBC) for native microflora of Swiss chard, using the optical density method. The MIC and MBC of clove essential oil were 0.049 and 0.093 mL/100 mL, respectively. These results indicate clearly that clove oil has the potential to become a technologically useful product as a sanitizing agent.

Inhibitory Parameters of Essential Oils to Reduce Food-Borne Pathogens

The technological applications of essential oils as natural sanitizing agents on foods require the establishment optimal conditions, such as sensitivity of a pathogen, optimal concentrations, and the contact time between the oils and the pathogen. The inhibitory parameters (MIC, MBC, and optimal time of contact) of clove essential oil to reduce a food-borne pathogen (*Escherichia coli* O157:H7) was evaluated by Moreira et al. (2005). The MIC and MBC values for clove were 0.25 and 0.3 mL/100 mL, respectively. In accordance, Hammer et al. (1999) reported similar MIC values (0.25 mL/100 mL) for clove against *E. coli*. On the other hand, to reach an adequate shelf life, food products require a reduced initial microbial load as soon as its control or inhibition during the production steps. To obtain the highest sanitizing agent performance is very important to optimize the contact time between pathogen and essential oils. Moreira et al. (2005) studied clove essential oil's inhibitory effect on *E. coli* survival during a 20-h period at 20 °C. The obtained results reported that at time zero, clove application produced one log reduction compared with control sample. After 15 min of clove application the counts were reduced 3.5 log with respect to the control, concluding that the optimal contact time between essential oil and pathogen was near 15 min and demonstrating that clove exerted a significant bactericidal and bacteriostatic action on pathogen growth. Also, Elgayyar et al. (2001) showed antimicrobial activity of clove against selected pathogenic and saprophytic microorganisms.

Clove Essential Oil Application on Lettuce Seeds

Clove essential oil was applied to lettuce seeds in order to reduce its microbial load without affecting the germination percentage (Goñi, 2013). Seeds are vehicles of microorganisms, some of them pathogenic, that remained hidden inside them; therefore are difficult to eliminate. Agricultural practices, like utilization of untreated fertilizers or contaminated water, are frequent causes of early contamination, even as early as in the seeds. Sprouting conditions (room temperature, high relative humidity, and plenty of nutrients available) are highly suitable to microbial growth, therefore increasing the risk of contamination (Peñas et al., 2009). It is important to reduced microbial counts before sprouting as during the days required to the seedling to developed, microbial growth can reach microbial counts as high as to 8–10 log (Peñas et al., 2009) or 5–7 log for *E. coli* O157:H7 (Stewart et al., 2001).

Several treatments have been proposed to eliminate these microorganisms from seeds. However, none of them has been able to eliminate all microorganisms present in the seeds or that can be applied to all kind of seeds (Scouten and Beauchat, 2002; Singh et al., 2003). Currently, washing the seeds with chlorinated water is the standard procedure, recommended especially by the United States Food and Drug Administration for seeds that will be used in the production of edible sprouts. However, clove application is a natural alternative to chlorine, with less toxicity and is agreeable with organic production and has a lower environmental impact.

Clove essential oil (1.5 mL/L) was solubilized in distilled water, corresponding to three times the MIC as reported by Ponce et al. (2010). Lettuce seeds washed with the clove essential oil solution (10 min, 100 rpm of agitation) were sampled after washing (day 0) and after sprouting (7 days, 20 °C, 8 h of photoperiod) to assess immediate and residual effect of the clove essential oil in the microbial load, respectively. Microbiological quality, germination percentage, and biomass parameters (weight and length) were also measured at day 0 and day 7.

Immediately after treatment, lettuce seeds washed with clove essential oil solutions presented significantly lower microbial counts that those washed only with water or those without essential oil for all the populations investigated, while not significant differences were found in the germination rate or the length or weight of the seedlings. Results obtained microbiological counts are presented in Figure 1. Reductions in total mesophyllic bacteria, total coliforms, and yeast and molds compared with the control were found (2.2, 1.5, and 1.3 log, respectively). Washing the lettuce seeds with clove essential oil also eliminated *E. coli* beyond detection (less than 2 log), as the pathogen was present in the untreated seeds and in the ones only washed with water.

After sprouting, lettuce seeds washed with clove essential oil solutions presented significantly higher microbial counts that those washed only with water or those without essential oil for all the populations investigated, while nonsignificant differences were found in the germination rate or the length or weight of the seedlings. The results for the biomass assay are presented in Table 1, while Figure 2 shows the results for the microbial analysis of the seedlings. After 7 days of

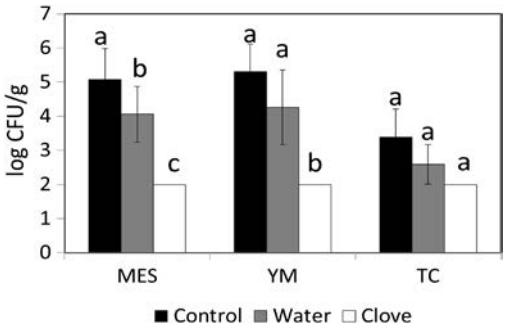

FIGURE 1 Microbial counts (log CFU/g) in lettuce seeds after being washed with a clove essential oil solution (day 0). MES, total mesophyllic count; PSC, total psychrophilic counts; YM, yeasts and molds; TC, total coliforms. Values represent least square means ± SD. Treatments: control: without treatment; water: seeds immersed in distilled water; clove: seeds immersed in 1.5 mL/L clover essential oil solution. Values represent least square means ± SD. Different letters in the same microorganism population (column) indicates significant differences according to Duncan multiple comparison test ($\alpha < 0.05$). *Unpublished data.

TABLE 1 Biomass Assay Parameters for Lettuce Seeds Treated With Clove Essential Oil (10 min, 100 rpm, 20 °C)

	Weight (g/50 Seeds)		Seedling Length (mm)
Treatment[a]	**Day 0**	**Day 7**	**Day 7**
Control	–	1.0380 ± 0.1081	55.2 ± 0.6
Water	0.3541 ± 0.0079	1.0144 ± 0.0099	55.4 ± 0.8
Clove	0.3521 ± 0.0091	1.0209 ± 0.1153	55.7 ± 0.6

[a]*Treatments: Control: without treatment; water: seeds immersed in distilled water; clove: seeds immersed in 1.5 mL/L clover essential oil solution. Values represent least square means ± SD. Different letters in the same microorganism population (column) indicates significant differences according to Duncan multiple comparison test ($\alpha < 0.05$). Unpublished data.*

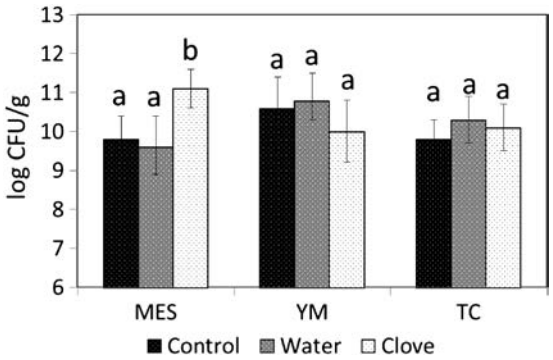

FIGURE 2 Microbial counts (log CFU/g) in lettuce seeds washed with clove essential oil solution after 7 days of sprouting (20 °C, 8 h photoperiod). MES, total mesophyllic count; PSC, total psychrophilic counts; YM, yeasts and molds; TC, total coliforms. Values represent least square means ± SD. Treatments: control: without treatment; water: seeds immersed in distilled water; clove: seeds immersed in 1.5 mL/L clover essential oil solution. Values represent least square means ± standar deviation. Different letters in the same microorganism population (column) indicates significant differences according to Duncan multiple comparison test ($\alpha < 0.05$).

germination, no significant differences were found between treated seeds and control seeds for total coliforms and yeast and mold counts (9.2 and 10.0 log, respectively). Moreover, total mesophyllic counts in treated seeds were significantly higher than in control seeds (11.2 and 9.2 log, respectively). In conclusion, clove essential oil did not produce a positive effect in the microbiological quality of sprouted seeds but did prove to be effective in reducing the initial microbial counts. These results may indicate that clove essential oil could be used as a biopreservative agent, reducing superficial microorganisms but not those protected inside the seed and without residual effect. More options could be explored in the application of essential oils as biopreservative in seeds, as not effect was found in the biomass parameters; in this sense application of the biopreservative solution during sprouting could be an alternative.

Preharvest Application of Clove Essential Oil Solutions in Lettuce Plants

Clove essential oil was applied as an early intervention method to improve the microbiological quality of lettuce at harvest. Butterhead lettuce plants growing in greenhouses with the addition of plastic mulching were used as row material (Goñi et al., 2013). Lettuce plants can be contaminated from the beginning of the production system and in those circumstances microorganisms get attached to the leaves or even they get internalized within the tissue, making its postharvest decontamination more difficult (Solomon et al., 2002; Artés et al., 2009). The risk of contamination increases in Butterhead lettuce, due to the morphology of the lettuce plant, in which leaves are in a close array establishing concentric rings, with external leaves covering the inner leaves (Goñi et al., 2010). Early intervention strategies have the advantage of reducing microbial counts from the start, in the greenhouse; and with the aid of the application of good agricultural practice consumers may receive safer produce, therefore reducing the incidence of food-borne illnesses (Tomás-Callejas et al., 2011).

Clove essential oil (1.5 mL/L) was diluted in distilled water and applied by spray in young lettuce plants still in the greenhouse. Clove was applied to lettuce plants at different dates before harvest, in a single dose (14, 10, 7, 4, and 0 days before harvest) and in successive doses (14, 10, 7, 4, and 0 days before harvest). Microbiological and sensorial analyses were performed in the lettuce plants after harvest to assess the effect of the preharvest application on native microflora (sampling 1 h after harvest at day 0) and during refrigerated postharvest storage (sampling after 5 days, 7–8 °C).

Essential oils are often used as flavoring in food products. Clove in particular has a distinct odor, often associated with deontological products, and it would be detrimental to the acceptance of the produce by consumers. Therefore a sensorial assay was also performed after harvest in treated and untreated lettuce plants to assess the effect of the preharvest application of clove on the general appearance, color, texture, odor, brightness, and enzymatic browning of the leaves (Table 2). No significant differences were found for the sensorial indices of lettuce treated with clove compared to control (plants without clove). These results are also in concordance with those previously presented by Ponce et al. (2011) of postharvest Swiss chard.

Preharvest application of clove essential oil in lettuce plants reduced microbial counts, compared with control, at harvest for total coliforms (in some monodosis and in successive applications), and for total mesophyllic bacteria (when applied right before harvest). Results are shown in Table 3. No significant reductions were found for psychrophilic bacteria or yeast and molds. Clove antimicrobial activity is usually related to the eugenol content (Chaieb et al., 2007) and its ability to permeate the cell membranes and interact with proteins. Eugenol permeability has been shown in several studies and has been associated to the increase the potassium and 12-O-tetradecanoylphorbol-13-acetate transport rate (Gill and Holley, 2006; Hemaiswarya and Doyle, 2009). Eugenol can also produce minor changes in fatty acid configuration (Di Pasqua et al., 2007). The hydroxyl group present in the eugenol structure could bond with the proteins affecting its configuration and functionality, therefore acting as an antimicrobial agent. In concordance with these affirmations, eugenol is an effective

TABLE 2 Scores for Lettuce Plants Treated With Clove Essential Oil During Preharvest

	Treatment	
Index	Control	Clove
General appearence[a]	8.07 ± 0.76 a	8.82 ± 0.96 a
Color[a]	8.17 ± 0.29 a	8.44 ± 0.46 a
Texture[a]	8.19 ± 0.36 a	7.98 ± 0.39 a
Flavour[a]	8.82 ± 0.86 a	7.03 ± 0.78 a
Brightness[a]	7.28 ± 0.71 a	7.86 ± 0.84 a
Enzymatic browning[b]	1.78 ± 0.82 a	1.87 ± 0.77 a

Data shown correspond to a single dose right before harvest, the remaining treatments presented similar results; therefore, data is not shown.
Data corresponding to plants treated with water are omitted in the table as no differences were found with control. DBH: days before harvest. Successive application of clove: 14 DBH, 10 DBH, 7 DBH, 3 DBH, 0 DBH. Values represent least square means ± SD. Different letters in the same index (line) indicates significant differences according to Tukey–Kramer multiple comparison test ($\alpha < 0.05$).
[a]*A nine point scale was used to asses these indices, where 9 represented excellent quality, 5 the acceptability limit, and 1 very poor quality.*
[b]*A five point scale was used for this index, where 1 represented total absence of, 3 the acceptability limit, and 5 represented excessive enzymatic browning. Unpublished data.*

TABLE 3 Microbial Counts at Harvest (log CFU/g) in Lettuce Plants Treated During Preharvest With Clove Essential Oil

	Clove Essential Oil 3 MIC			
Treatment	**MES**	**PSC**	**YM**	**TC**
Control	$6.88^{A} \pm 0.52$	$6.22^{A} \pm 0.72$	$5.92^{A} \pm 0.43$	$5.44^{A} \pm 0.48$
0 DBH	$5.13^{B} \pm 0.48$	$5.55^{A} \pm 0.66$	$5.43^{A} \pm 0.43$	$4.03^{B} \pm 0.51$
3 DBH	$6.06^{AB} \pm 0.55$	$5.51^{A} \pm 0.61$	$5.13^{A} \pm 0.51$	$4.23^{B} \pm 0.44$
7 DBH	$5.73^{AB} \pm 0.66$	$5.80^{A} \pm 0.73$	$5.14^{A} \pm 0.36$	$4.47^{B} \pm 0.41$
10 DBH	$5.99^{AB} \pm 0.47$	$5.23^{A} \pm 0.68$	$5.14^{A} \pm 0.40$	$4.91^{AB} \pm 0.39$
14 DBH	$5.80^{AB} \pm 0.51$	$5.73^{A} \pm 0.75$	$5.28^{A} \pm 0.52$	$4.95^{SB} \pm 0.48$
Successive[a]	$5.86^{AB} \pm 0.61$	$5.65^{A} \pm 0.81$	$5.42^{A} \pm 0.38$	$4.12^{B} \pm 0.39$

MES, total mesophyllic count; PSC, total psychrophilic counts; YM, yeasts and molds; TC, total coliforms. Values represent least square mean ± SD; DBH, days before harvest.
Data corresponding to plans treated with water are omitted in the table since not differences were found with control.
[a]*Successive application of clove (14 DBH, 10 DBH, 7 DBH, 3 DBH, 0 DBH). Different letters in the same microorganism population (column) indicate significant differences according to Tukey–Kramer multiple comparison test ($\alpha < 0.05$).*

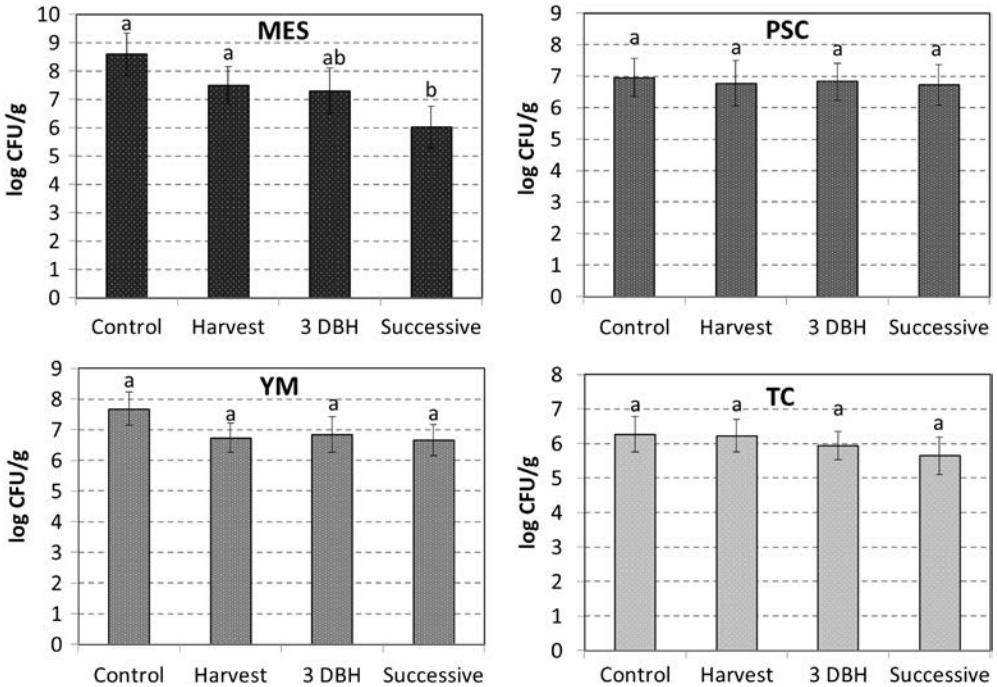

FIGURE 3 Microbial counts after postharvest storage (log CFU/g) in lettuce plants treated during preharvest with clove essential oil. MES, total mesophyllic count; PSC, total psychrophilic counts; YM, yeasts and molds; TC, total coliforms. Values represent least square means ± SD. * DBH: days before harvest ** Successive application of clove (14 DBH, 10 DBH, 7 DBH, 3 DBH, 0 DBH). Different letters in the same microorganism population (column) indicates significant differences according to Duncan multiple comparison test ($\alpha < 0.05$).

inhibitory agent against several enzymes, including ATPase which contributed to the inability of microorganisms to repair damage created to the membranes or generate energy (Gill and Holey, 2006).

Preharvest treatment of lettuce plants with clove essential oil did not produce significant reductions in the microbial counts present after 5 days of refrigerated storage, except for total mesophyllic bacteria counts (−2.3 log) after a single application right before harvest (day 0). No significant reductions were found in any of the other applications when comparing treated plants with control (Figure 3). These results may indicate a low residual effect of the clove essential oil, reducing its effectiveness during postharvest storage.

Different Methods for the Technological Application in Lettuce Leaves

It is crucial that proper measures are taken to ensure the safety and stability of vegetables during its shelf life. Postharvest application of essential oils may be limited by flavor because effective antimicrobial doses may exceed acceptable levels from a sensorial point of view (Moreira et al., 2007; Ponce et al., 2010). However, the possibility of reinforcing their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be a way to attain a balance between sensory acceptability and antimicrobial efficacy (Dimitrijevic et al., 2007). Most studies have evaluated their antimicrobial activity by in vitro assays. However, the information of their effect in actual food systems is still insufficient. Higher concentrations are needed to achieve the same effects in food systems, taking into account the sensorial acceptability of the products (Gill et al., 2002; Burt, 2004; Ponce et al., 2004). The greater availability of nutrients in foods compared with laboratory media may enable bacteria to repair damaged cells faster (Gill et al., 2002). Studies on actual foods show that some botanicals have the potential to be effective biopreservatives, although it is necessary to optimize their use and provide more details about their application. Many spices and herbs exert antimicrobial activity due to their essential oil fractions, applied in spray form or by immersion. Moreover, natural antimicrobials and antioxidants such as essential oils embedded in support material, such as lactose capsule, can be gradually released on the food surface, therefore, requiring smaller amounts of them to achieve the target shelf life (Shaikh et al., 2006; Champagne and Fustier, 2007). Ponce et al. (2011) investigated the antimicrobial effects of clove applied by spray, immersion, and embedded in lactose capsules on the microbiological quality of lettuce leaves. Clove was applied and treated lettuce leaves were stored for 7 days at 8 °C and 97 ± 1% relative humidity. The initial load of mesophilic, psycrotrophic, coliforms, lactic acid bacteria, and yeast and molds was evaluated, as well as the initial sensorial parameters and its evolution during the refrigerated storage. Application method was shown to be an important factor in determining the effectiveness of the essential oil. Clove at 1 MIC and applied embedded in lactose capsules presented a significant inhibition on mesophilic, psicrotrophic, and coliforms populations. Clove at 0.5 MIC applied by spray, immersion, and embedded in lactose capsules exerted lower inhibitory effects, with respect to 1 MIC, on the different microbial populations present on lettuce leaves. At the end of the storage, lettuce samples treated with clove at 1 and 0.5 MIC by spray were the only acceptable from a sensorial point of view. It is concluded that clove could control different microbial population present in lettuce.

Effects of Clove Oil on *Escherichia coli* O157:H7 in Blanched Spinach and Cooked Minced Beef

The effectiveness of essential oil application in actual food systems is the result of multiple factors, such as the food composition or the storage temperatures (Ponce et al., 2004). When foods are kept at proper refrigeration temperatures, the growth of microbial populations is arrested. However, in some food handling operations, such as food catering services and self-serve restaurants, food items may be accidentally exposed to abusive temperatures. Therefore, biopreservatives that could control food-borne pathogens when foods are exposed to abusive temperatures could make an important contribution. Moreira et al. (2007) evaluated the effectiveness of clove to control *E. coli* O157:H7 on actual food systems (blanched spinach and minced cooked beef) exposed to abusive refrigeration (8–10 °C) and environmental (20–22 °C) temperatures. Results indicated that highest concentrations of clove (3 and 4 MIC) were needed to restrict *E. coli* O157:H7 populations in blanched spinach and cooked minced beef, respectively. The antimicrobial action of clove was dependent on the oil concentration, food composition, and storage temperature. The inhibition of pathogen and native microorganisms was greater as temperature increased. Preliminary findings in actual foods suggest that clove could be used to control food-borne pathogens when foods are exposed to abusive temperatures. However, the oil concentrations required to produce a certain level of inhibition in actual foods were questionable due to the organoleptic impact. So, essential oils, in combination with other factors in obstacles technologies, are an alternative to control the pathogen growth minimizing undesirable changes in organoleptic characteristics.

Clove Essential Oil as Reducing Agent of Peroxidase Activity in Leafy Vegetables

Enzymatic browning in fruit and vegetable tissues can cause undesirable quality changes during handling, processing, and storage. Peroxidase is a plant enzyme associated with off-flavors in fruits and vegetables. Inactivation of the peroxidase enzyme is considered necessary to minimize the possibility of deterioration, and it is generally achieved by using physical or chemical treatments such as heating (blanching), lowering pH or water activity, or adding chemical additives (Ponce et al., 2004). Consumers prefer fresh produces that are prepared for convenient consumption, without the loss of quality attributes such as texture, flavor, and appearance. Thus, organically produced vegetables to be marketed fresh, would

require preservation technologies different from the conventional methods. The possible application of natural essential oils as natural antimicrobial and antioxidant agents in fresh vegetables organically grown may be an alternative to natural preservation technology. Ponce et al. (2004) evaluated the use of clove to reduce peroxidase activity in leafy vegetable extracts produced by organic methods. Clove was evaluated at 1, 2, and 4 MIC for its effect on peroxidase activity in five leafy vegetables extracts: Swiss chard, spinach, cabbage, romaine, and butter lettuces. The peroxidase activity of leafy vegetable extracts, butter lettuce, cabbage, and spinach showed the greatest resistance against the action of clove, while romaine lettuce extract showed the least resistance. The action of clove on chard and cabbage extracts indicated a significant antioxidant activity of clove (59% and 33% of reduction, respectively) compared with the control. Clove exerted a poor effect over the peroxidase activity on lettuce extracts (15% reduction). The antioxidant activity of clove was also compared with that of ascorbic acid, which exerted a significant antioxidant effect on all the vegetable extracts. The reductions of peroxidase activity in vegetable extracts were 87% in spinach, 74% in butter lettuce, 56% in romaine lettuce, and 35% in chard and cabbage. Although the antioxidant effects of clove were lower compared with ascorbic acid solutions, they would provide other benefits as antimicrobial agents (Ponce et al., 2003).

SUMMARY POINTS

- Clove essential oil has been used in phytotherapy for centuries and is now also used as a biopreservative and antioxidant in foods.
- Clove essential oil is a known antimicrobial agent, mainly due to its eugenol content.
- Clove essential oil is a viable alternative to chlorine as a sanitizing agent as it is nontoxic has low environmental impact.
- Clove essential oil acted as a superficial sanitizer in lettuce seeds, reducing its microbial counts after washing, but it did not maintain its effect 7 days after sprouting.
- Preharvest application of clove essential oil in young lettuce plants managed to reduce microbial counts at harvest.
- Preharvest application of clove essential oil did not reduce microbial counts after 5 days of refrigerated postharvest storage.
- Clove oil exerted a significant bactericidal and bacteriostatic action on survival and growth of *E. coli* O157:H7.
- Clove oil applied in lettuce leaves and embedded in lactose capsules presented a significant inhibition on mesophilic, psicrotrophic, and coliforms populations.

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Chapter 40

Coriander (*Coriandrum sativum*) Oils

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List of Abbreviations

ACE Angiotensin converting enzyme
BHA Butylated hydroxyl aniline
CEO Coriander essential oil
CoE Council of Europe
FDA Food and drug Administration
FEMA Flavor and extract manufacturers association
ppm Parts per million

INTRODUCTION

Coriander (*Coriandrum sativum*) is a medicinal and aromatic plant. It is extensively cultivated in south Asian countries, central Europe, and Mediterranean countries. The main commercial coriander producers include members of the former Soviet Union, Hungary, Poland, Romania, Czech Republic, Slovakia, Morocco, Canada, India, Pakistan, Iran, Turkey, Guatemala, Mexico, and Argentina. The dried fruits of coriander are widely used for flavoring sauces, meat products, and bakery and confectionery items. It is also a typical ingredient in several Asian curry powders (Lopez et al., 2008). The aroma and flavor of coriander are attributed to the essential oil present in the oil glands in the mericarp. Coriander essential oil (CEO) has many medicinal values. It is one of the 20 major essential oils in the world market and is obtained by steam distillation of the dried fully ripe fruits of *C. sativum*. The oil is a colorless or pale yellow liquid with a characteristic odor of linalool and a mild, sweet, warm, aromatic flavor. The organoleptic characteristics of the distilled oil tend to deteriorate during prolonged storage, especially if left exposed to light and air. Coriander oil is approved for food uses by the Food and Drug administration (FDA), Flavor and Extract Manufacturers Association (FEMA), and the Council of Europe (CoE). Coriander is a highly valued medicinal and aromatic plant; however, this chapter focuses on the uses of CEO as a food ingredient.

BOTANICAL ASPECTS

Coriander is a medicinal plant belonging to the family Umbelliferae. *Coriandrum sativum* is a herbaceous plant. It is cultivated as a summer or a winter annual crop and grows 25–60 cm (9–24 in) in height. It has thin, spindle-shaped roots and alternate leaves as shown in Figure 1. The stem of the plant is more or less erect and sympodial, monochasial-branched, sometimes with several side branches at the basal node. Each branch of the plant ends with an inflorescence. The color of the stem is green and may turn to red or violet during the flowering period. The stem of the mature plant is usually hollow and its basal parts can reach up to a diameter of up to 2 cm. The leaves are alternate, and the first are often gathered in a rosette and sometimes turn red or violet during the flowering period. The petals of the flowers are small and pinkish-white in color. Usually there are five petals and sepals in the flower with about five stamens. The plant starts flowering in June and yields round fruits consisting of two pericarps. The fruits are 2–8 mm in length. Coriander oil is obtained from the dried fruits. Coriander oil has been reported to contain approximately 30% terpene hydrocarbons and 70% oxygenated compounds. The major constituent of CEO is linalool. The typical compositional analysis of coriander oil has been shown to contain linalool, geraniol, terpinen-4-ol, α-terpineol, c-terpinterpinene, limonene, α-pinene, camphene, myrcene camphor, geranyl acetate, and linalyl acetate as shown in Table 1. These volatile constituents have their characteristic odor descriptors identified by gas chromatography coupled to olfactometry (Ravi et al., 2007).

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FIGURE 1 A representative picture of the coriander plant. The figure shows the mature and immature green leaves, with fruits and characteristic flower.

USAGE AND APPLICATIONS

The coriander plant is a highly valued medicinal plant. All parts of the plant are in use as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations. It is a potential source of lipids (rich in petroselinic acid) and an essential oil (high in linalool) isolated from the seeds and the aerial parts. Due to the presence of several of bioactive ingredients, a wide arrays of pharmacological activities have been ascribed. A review by Sahib et al. (2013) concluded the antimicrobial, antioxidant, antidiabetic, anxiolytic, antiepileptic, antidepressant, antimutagenic, antiinflammatory, antidyslipidemic, antihypertensive, neuroprotective, and diuretic properties of coriander may be ascribed to the bioactive components present in essential oil. Similarly, Ramadan et al. (2008) demonstrated that CEO has hypocholesterolemic properties in rats fed a cholesterol-rich diet.

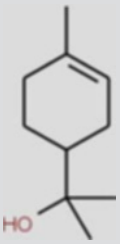
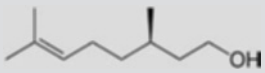
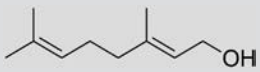
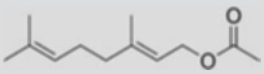
USAGE AND APPLICATIONS IN FOOD SCIENCE

Antioxidant and Food Preservation

Studies have shown that essential oils are better natural antioxidant and may be used as a substitute for the synthetic antioxidants in food preservation. Misharina and Polshkov (2005) showed that the mixture of essential oils from laurel and coriander possessed antioxidant properties and strongly inhibited the oxidation of the components of the fennel oil during various storage conditions. Similarly, Ramadan and Wahdan (2012) prepared blends (10% and 20%, w/w) of black cumin seed oil and coriander seed oil with corn oil. The results of their studies showed that oxidative stability of oil blends was better than its original oil, due to the consequent changes in the fatty acid and tocopherol profiles and minor bioactive lipids found in coriander and black cumin oils.

CEO containing camphor (44.99%), cyclohexanol acetate, limonene (7.17%), and α-pinene (6.37%) was evaluated in cake during 60 days storage at room temperature (Darughe et al., 2012). The results showed that 0.05%, 0.10%, and 0.15% CEO inhibited the rate of primary and secondary oxidation product formation in the cake and their effects were almost equal to butylated hydroxyl aniline at 0.02%. Organoleptic evaluation of cakes containing 0.05% CEO was not different from the control. These results showed that CEO could be used as natural antioxidants in lipid-containing foodstuffs.

TABLE 1 Chemical Composition of Coriander (*Coriandrum sativum*) Essential Oil

No	Compound	Structure	Concentration Ranges (%)	Odor Descriptors
1	α-Pinene		2.3–23.2	Woody, spicy, oily
2	Linalool		57.5–75.1	Pleasant, citrusy, grassy
3	Terpineol		0.08–5.3	Sweet
4	Citronellol		0.6–1.6	Rose, sweet-like
5	Geraniol		0.5–2.3	Sweet, rose-like
6	Geranyl acetate		8.9–24.5	Pleasant, floral rose

The volatile composition was studied with the help of gas chromatography with olfactometry. The quantification was based on the aromagram generated by superimposing sensory aroma descriptors of gas chromatoraphy effluent emerging at different intervals depending on their retention time.
Modified from Ravi et al. (2007).

The antioxidant activity of CEO has been studied and compared with other essential oils. Teixeira et al. (2013) showed that the antioxidant activity measured by the ferric reducing power assay revealed similar results to those obtained with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique for coriander and other essential oils. Coriander oils show very small antioxidant potential as compared with other essential oils. Contrary to these results, Chaudhary et al. (2013) showed that CEO showed a high angiotensin converting enzyme (ACE) inhibition with an IC_{50} value of 34.8 µg/mL and possess strong DPPH radical scavenging activity. Their finding suggests that CEO can be potential leads for the management of hypertension as an ACE inhibitor.

Food Flavoring Agent

Coriander oil is widely used as a flavoring agent in all types of foods and food products, such as alcoholic beverages, candies, pickles, meat sauce, and seasonings. The average usage levels in food products ranges from 0.1 to 100 ppm. Coriander and CEO are recognized as one of the most important products in the world and are of great significance in international trade market. It has been estimated that the annual world production of coriander oil has a value of about 50 million US dollars, which places it the world's second most important essential oil after orange oil (Lawrence, 1993). Burdock and Carabin (2009) reviewed the safety assessment of the CEO as a food ingredient. The authors concluded that CEO is safe because of the history of consumption of coriander oil without reported adverse effects, lack of its

TABLE 2 Maximum Approved Levels of Coriander Essential Oil in Different Foods

No	Food Type	Maximum Level (ppm)
1	Alcoholic beverages	121.2
2	Baked foods	62.0
3	Chewing gum	6.6
4	Frozen dairy	47.3
5	Gelatin, pudding	32.8
6	Hard candies	181.1
7	Meat products	68.4
8	Nonalcoholic beverages	8.9
9	Soft candies	46.9

Minimum level may be used depending upon the taste and perception of the end users. The values above are approved by FEMA.
Adopted from Burdock and Carabin (2009).

toxicity in limited studies, and lack of toxicity of its major constituent, linalool. The authors also revealed the maximum usage limit in food products (Table 2). The maximum levels of CEO approved by FEMA in foodstuffs are alcoholic beverages, 121.2 ppm; baked foods, 62.0 ppm; chewing gum, 6.6 ppm; confectionary frosting, 13.8 ppm; frozen dairy, 47.3 ppm; gelatin pudding, 32.8 ppm; hard candy, 7.5 ppm; meat products, 68.4 ppm; nonalcoholic beverages, 8.9 ppm; and soft candies, 46.9 ppm. CEO has also been approved for use in food by the FDA and the CoE. Similarly, the FDA has approved coriander oil as generally recognized as safe with no limitations cited for the use of coriander oil as a flavoring agent and adjuvant.

Antibacterial and Antifungal Applications

In the food industry, the quality and safety of prepared or processed foods are of prime importance. The microorganisms present in food can lead to spoilage and deterioration of the quality of food products, and if ingested by humans can cause infection and illness. Thus food manufacturers try their best to reduce or eliminate microorganisms from food products. It has been estimated that about one-third of the world's food production is lost annually on account of microbial spoilage or contamination (Alboofetileh et al., 2014). Essential oil derived from coriander plants has been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, fungi, and viruses. CEO and its various fractional distillates were effective antimicrobial agents particularly against *Listeria monocytogenes* because of the presence of long chain (C6–C10) alcohols and aldehydes. Mixing of different fractions showed additive, synergistic, or antagonistic effects against individual test microorganisms (Delaquis et al., 2002). Similarly, Matasyoh et al. (2009) obtained essential oil from the leaves by hydrodistillation evaluated for in vitro antimicrobial activity. The oil was dominated by aldehydes and alcohols which accounted for 56.1% and 46.3% of the oil, respectively. The extracted oil was screened for antimicrobial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus* spp.) and Gram-negative (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosae*) bacteria and a pathogenic fungus, *Candida albicans*. Only *P. aeruginosae* showed resistance to CEO, while other tested bacteria were highly affected.

Rattanachaikunsopon and Phumkhachorn (2010) studied the 12 essential oils for antimicrobial activities against several strains of *Campylobacter jejuni*, a pathogen causing food-borne diseases worldwide. The authors showed that CEO exhibited the strongest antimicrobial activity against all the tested strains. The antimicrobial potency of coriander oil against *C. jejuni* on beef and chicken meat at 4 and 32 °C was also studied. It was found that the oil reduced the bacterial cell load in a dose-dependent manner; however, the type of meat and temperature did not influence the antimicrobial activity of the essential oil. This study clearly indicates the potential of CEO to serve as a natural antimicrobial compound against *C. jejuni* in food.

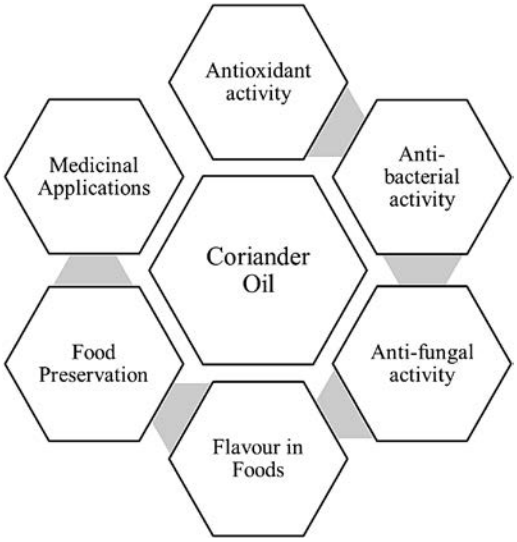


FIGURE 2 Graphical representation of applications of coriander essential oil. The antioxidant potential of the coriander oil is linked with the antibacterial and antifungal activities that help us to use it in food flavoring and preservation.

In another study, the essential oils extracted from coriander and other plants were evaluated for their antimicrobial activity against 11 different bacterial and three fungal strains belonging to species reported to be involved in food poisoning and food decay. These include *S. aureus, E. coli, Salmonella enterica, L. monocytogenes, Bacillus cereus, C. albicans*, and *Aspergillus niger*. Coriander essential oil showed the best antibacterial activity in all, while thyme and spearmint oils better inhibited the fungal species (Lixandru et al., 2010). The antifungal activity of essential oils of some species of family Apiaceae was tested against the fungus *Aspergillus flavus*. Coriander oil was the most effective oil against fungal growth and aflatoxin production at all concentrations studied (Abou El-Soud et al., 2012). The authors recommended that an amount of 1000 ppm as a food additive is protecting the spices from biodeteriorating fungi as well as from aflatoxin contamination.

It is clear from the above mentioned literature that the antioxidant, antibacterial, and antifungal activities of the coriander oil (Figure 2) are linked to its uses in food flavoring and preservation as well as medicinal applications. Thus using CEO for the above applications is recommended.

SUMMARY POINTS

- CEO is obtained from dried seeds or leaves using distillation.
- Coriander oil is rich in linalool, limonene, α-pinene, camphene, geranyl acetate, and linalyl acetate.
- Coriander oil is commercially used in food industries as a flavoring agent with or without limitation.
- The essential oil also contributes to the storage stability of the food products.
- Coriander essential oil possesses strong potential against food-spoiling microorganisms.
- The dietary uses of the CEO are therefore helpful in maintaining good health.

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Chapter 41

Costmary (*Chrysanthemum balsamita*) Oils

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List of Abbreviations

EO Essential oil
dw Dry weight
fw Fresh weight
t Traces

INTRODUCTION

The sweet, minty–lemony leaves of costmary (*Tanacetum balsamita* L.) have been used in various dishes, as flavoring, in the production of ale, and for various medicinal applications. However, compared with the medieval period the uses of costmary remarkably has decreased and currently *T. balsamita* L. is considered as "a forgotten medicinal plant". On the other hand, investigations of costmary secondary metabolites during last decades provided new knowledge on their composition, concentrations, and activities. Costmary yields yellowish essential oil, which according to the content of the main constituents may be distinguished into four main chemotypes (Hassanpouraghdam et al., 2008c). Although the reports on bioactivities of costmary essential oils are rather scarce, their main constituents such as carvone, α-thujone, camphor, which are common to many aromatic and medicinal plants, have been shown to possess antimicrobial and other activities. Therefore, selected subspecies and cultivars of costmary may possess potential to warrant increasing their cultivation and uses in foods and other products.

BOTANICAL ASPECTS

The botanical taxonomy of costmary, which belongs to the *Asteraceae* family (*Asterales* order, *Asteridae* subclass, and *Anthemidae* tribe) is rather complicated. In the Index of Garden Plants (Griffiths, 1997) it is assigned to the species of *T. balsamita* L.; however, such synonyms for species as *Tanacetum balsamitoides* Schultz Bip.; *Balsamita major* Desf.; *Balsamita vulgaris* Wild.; *Chrysanthemum balsamita* (L.) Baill.; non L.; *C. balsamita* L. f. (Boiss.) B. Boivin; *Pyrethrum majus* (Desf.) Tzvele; *Pyrethrum tanacetum* DC.; *Pyrethrum balsamita* (L.) Willd, as well as the varieties *B. major* Desf. var. *tanacetoides* (Boiss.) Moldenke; *C. balsamita* L. var. *tanacetoides* Boiss.; *P. balsamita* (L.) Willd. var. *tanacetoides* Boiss. may be found in various literature sources (Robert W. Freckmann Herbarium; Hassanpouraghdam et al., 2008c). For instance, *T. balsamita* is represented in the flora of Iran by two subspecies, namely *T. balsamita* subsp. *Balsamitoides* and T. *balsamita* subsp. *balsamita* (Yousefkadi et al., 2009). The English name "costmary" stems from "costus of Saint Mary", it also has other, less frequently used common names such as alecost, balsam herb, costmarie, mace, balsamita, mint-geranium, and bible leaf. In other languages, it is also associated with the Virgin Mary, most probably because it is sometimes has been used to cure women's diseases. It is unclear whether the plant called "balsamita" described by Columella in 70 AD is the same. According to Heinrich Marzell, it was first mentioned in 812 AD in a plant catalog; in the Middle Ages, the plant was widely associated with the Virgin Mary's name, being called Our Lady's Sage, Our Lady's Seasoning, The Virgin's Leaves, Mary's Bedstraw, Mary's Leaf, Mary's Mint (Marzell, 2000), and was known in France as *Herbe Sainte-Marie* (Grieve, 1984).

Costmary is a large perennial plant growing up to 2 m high with oval serrated leaves, which when young are covered with silvery hairs, later smooth, with edges with rounded teeth. It is flowers in August–October in clusters of small yellow button-shaped blossoms. The flowers are hermaphrodite (have both male and female organs) and are pollinated by insects. Costmary is suitable for light (sandy), medium (loamy), and heavy (clay) soils and prefers well-drained soil. It can grow

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in acid, neutral, and alkaline soils, in semishade (light woodland) or no shade; however, when grown in the shade, it goes strongly to leaf but will not flower (Grieve, 1984).

The origin of the plant in different sources is indicated as Mediterranean, Asian, or both; it has been grown in Europe and Asia since the Middle Ages. It has also spread to other continents, for example, as a weed of roadsides in eastern North America (Fernald, 1954). Costmary is cultivated in Iran, Turkey, Romania, Germany, Italy, Spain, and England (Hassanpouraghdam et al., 2008c).

USAGE AND APPLICATIONS

Costmary has been used for medicinal and food applications since antiquity. The plant was used for ancient herbals and was widely grown in Elizabethan knot gardens. It was referred to by Nicholas Culpeper and others as the "balsam herb". Due to the aroma and taste of its leaves, costmary was much used to give a spicy flavoring to ale, giving it its other name, alecost. The fresh leaves were also used in salads and in pottage. Bundles of costmary and lavender were tied "to Iye upon the toppes of beds, presses, etc., for sweet scent and savor" (Grieve, 1984).

Costmary leaves possess strong mint-like aroma and astringent taste. On a domestic scale they have been used to flavor different dishes including fatty minced meat, game, poultry, lamb, fish, curd, potato, soups, sauces, beverages, and cakes. Essential oil of costmary together with some other volatile products was tested in creation of flavoring compositions for confectionery (Bylaitė et al., 2000).

Costmary was widely grown from medieval times in herb gardens until the late nineteenth and early twentieth centuries for medical purposes. Nowadays it has mostly disappeared in Europe, but is still widely used in south-west Asia. Costmary (*Herbe Sainte-Marie*) was at one time employed medicinally in France, having somewhat astringent and antiseptic properties, and had a place in British Pharmacopceia until 1788, chiefly as an aperient, its use in dysentery being especially indicated (Grieve, 2014).

Costmary is seldom used in herbal medicine, though it does have a beneficial effect upon the digestive system (Stuart, 1987). The leaves have antiseptic, astringent, digestive, and laxative properties (Grieve, 1984; Holtom and Hylton, 1979; Bown, 1995). They have been used internally as an aperient in the treatment of dysentery, and as a remedy for liver and gall bladder complaints (Bown, 1995). Externally, they have been used as a salve to treat burns and insect stings (Bown, 1995; Stuart, 1987). However, they are considered to be virtually obsolete in modern herbalism (Grieve, 1984; Bown, 1995).

The plant was traditionally used for its insecticidal properties, as a strewing herb (Huxley, 1992), and in pot pourri for the ability of the dried leaves to retain their fragrance (Bown, 1995). It was also used in medieval times as a place marker in bibles. *C. balsamita* oil is a fragrance agent (CAS Number 85251-67-8; ESIS EC# (EINECS) 286-479-9) with recommended usage levels of up to 0.2% in the fragrance concentrate. Dried leaves added in the bath (sometimes also adding yeast) as ingredient of the "St. John's water" (cold macerate in water of a few species, prepared on St. John's night, on June 24). It has been used in Italy not only for cosmetic and cosmeceutical purpose (as in the Marches), but also as sedative, antitussive, carminative, diuretic, and even for aromatizing food in homemade omelettes.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Costmary Essential Oil Yield and Composition

Steam or hydrodistillation of costmary aerial parts yields yellowish essential oil. The yield of oil depending on plant origin, anatomical part, harvesting period, and other factors was reported in the range of 0.05 in stems to 1.34% in flowers (Table 1) and in some cases even higher, up to 2.2% (Juknevičiene et al., 1973). In the period of vegetation until intensive blooming in August 8 the yield from costmary leaves was in the range of 0.18 (May 25) to 1.14 (July 25, bud formation), while during full blooming the yield decreased both from leaves and flowers (Bylaitė et al., 2000). Earlier, Strobel et al. (1987) monitored oil content in costmary leaves from May to December: it increased from 0.02% in May to 0.2% fresh weight in the second half of July and afterward decreased to 0.04% in December. The content of the oil in blossom was reported to be higher (Bylaitė et al., 2000; Juknevičiene et al., 1973; Paris et al., 1976) or lower (Jaimand and Rezaee, 2005) than in the leaves. Nitrogen (N) and potassium (K) treatment combinations were shown to affect costmary growth characteristics and essential oil accumulation, so that the highest oil content (0.54%) and yield (11 mL m^2) were obtained using $N_{200}K_{200}$ treatment (Hassanpouraghdam et al., 2008a).

Composition of costmary oils from different countries was reported in several articles. Aerial parts or in some cases leaves, flowers, and stems were analyzed separately and in a total of 186 constituents were listed as identified in costmary oils. The variations in qualitative and quantitative oil composition are remarkable (Table 1); however, in summarizing all the

TABLE 1 Percentage Composition of Costmary Essential Oil

Component	Leaves[a]	Flowers[a]	Aerial Parts[b]	Aerial Parts[c]	Aerial Parts[d]	Leaves[e]	Flowers[e]	Stems[e]	Aerial Parts[f]	Aerial Parts[g] EO	Aerial Parts[g] HE AW	Aerial Parts[h]	Flower-Heads[i]	Aerial Parts[j]	Flowers[k]
α-Pinene	0.12–0.25	0.13–0.15		2.3	0.1					tr					0.04
β-Pinene	0.12–0.19	0–0.14		1.0	1.2					0.16					
Myrcene	t–0.23	tr-0.12		0.1		1.1	1.2	1.1		0.25					
limonene			0.1	7.5	0.1					0.82			2.7		2.57
1,8-cineole			0.7	3.3	2.7				2.59	0.42	3.51	1.34	1.74	4.4	0.63
limonene + 1,8-cineole	2.88–5.42	2.82–3.75													
trans-β-ocimene						1.7	0.2	1.7							
Linalool oxide					11.5										
2H-Pyran-3(4H)-one					9.7										
Terpinolene						5.4	2.0	6.0		tr					
α-Thujone	10.79–16.10	9.04–10.78	11.7						24.6	15.61	6.22	21.3	11.04	20.8	
β-Thujone	1.09–2.21	1.03–1.06	1.2						2.68	2.10	0.55	2.21	1.1	3.2	0.19
Chrysanthenone											1.76				
trans-p-Mentha-2,8-dien-1-ol	0.87–2.05	1.25–1.40	0.4						1.4			3.00	1.51	1.8	
cis-p-Mentha-2,8-dien-1-ol	0.45–0.92	0.48–0.50	1.2						0.71			1.1	0.67	0.8	
p-Mentha-1(7),5-dien-1-ol										1.23				0.4	
trans-p-Mentha-1(7),8-dien-1-ol			1.0						1.44			1.77	1.29		
cis-p-Mentha-1(7),8-dien-1-ol									1.67			1.82	1.55	1.5	

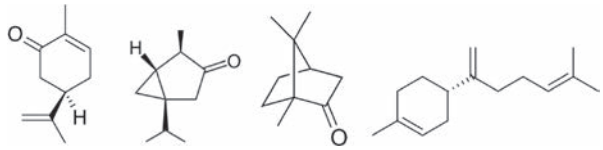
Continued

TABLE 1 Percentage Composition of Costmary Essential Oil—cont'd

Component	Leaves[a]	Flowers[a]	Aerial Parts[b]	Aerial Parts[c]	Aerial Parts[d]	Leaves[e]	Flowers[e]	Stems[e]	Aerial Parts[f]	Aerial Parts[g] EO	Aerial Parts[g] HE AW	Aerial Parts[h]	Flower-Heads[i]	Aerial Parts[j]	Flowers[k]
cis-p-Mentha-1,8-dien-1-ol														2.1	
trans-p-Mentha-2-en-1-ol	0.87–2.05	1.25–1.40	t												
Z-Sabinene hydrate			0.1			1.2	0.7	1.3		tr					
3.9-Epoxy-*p*-mentha-1,8-diene														1.4	
trans-Verbenol	tr–0.17	tr–0.16	1.0						0.3				0.29		
Camphor				1.1	7.5	9.3	2.8	9.5							
Pinocarvone	tr–0.18	0.22–0.28	0.5			27.1	34.2	28.0	0.36	1.00	0.64				
cis-Dihydrocarvone	0.3–0.78	0.52–0.54								1.83	0.97		0.64		
trans-Carveol	tr–0.33	0.13	0.5							0.35	1.25		1.01	1.9	
cis-carveol	0.19–0.38	0.20–0.33	0.2						1.16	0.98	2.19			1.2	0.36
cis-Piperitol			1.0									0.82	0.54		
trans-Piperitol												1.26			
trans-Chrysanthenol					22.3										
Carvone	51.83–68.01	59.5–61.67	52.4[l]	68.0					49.11	43.54	74.92	42.53	52.01	51.0	51.51
trans-Carvone oxide						1.0	0.4	1.0		0.28	0.68				
Carvone oxide	0.20–0.54	0.18–0.20												0.5	
Chrysanthenyl acetate	0.40–0.79	0.50–0.85	0.4		19.7				0.32	0.51	0.10		0.41	0.4	
Bornyl acetate						47.7	55.2	49.2							0.46
γ-Muurolene									1.76						
β-cubebene												2.21	0.46		4.72

α-cedrene													1.11		
4-*epi*-Cubebol	0.23–1.10	0.51–0.90													
β-Bisabolene	1.26–4.45	1.01–1.07		1.2					4.44	4.53	tr	10.56		1.8	
Germacrene D			3.3	0.8	0.1					4.24		0.39		0.6	
E-β-Farnesene										0.16			3.91		0.39
γ-Gurjunene													1.74		
cis-Calamenene	0.36–1.50	0.73–0.85								1.02		0.43			
δ-Cadinene	0.42–1.53	0.85–1.00	0.6		0.3				0.46	1.09	tr		1.14		
epi-Cubebol			1.5	0.1						1.30					
cubebol			2.4	0.3											
Viridiflorol			0.2									1.05	0.45		
τ-Muurolol	0.34–2.19	2.16–3.77	1.4	0.8						1.66					
Z,E-Farnesol													1.16		
δ-Cadinol			0.1										2.18		
α-Cadinol			0.2		1.5				0.64				1.88		
Selin-11-en-4-ol	0.14–0.69	1.03–1.24	0.8							1.12	tr				
Methyl isocostate	tr–0.43	0.62–1.08													
Methyl 4-hydroxy-4,5-dihydroisocotate	0.13–0.43	0.80–1.80													
Essential oil	0.18–1.15	1.15–1.34	0.38	0.25	0.25	0.25	0.15	0.05	0.58			0.57	0.75	0.48	

[a] *Bylaitė et al. (2000)*.
[b] *Başer et al. (2001)*.
[c] *Monfared et al. (2002)* (spp. *balsamitoides*).
[d] *Bagci et al. (2008)* (spp. *balsamita*).
[e] *Jaimand and Rezaee (2005)* (spp. *balsamitoides*).
[f] *Hassanpouraghdam et al. (2009)* (spp. *balsamitoides*).
[g] *Gallori et al. (2001)* (*Brugmansia suaveolens; EO = essential oil, HE AW = hexane extract of aromatic water*).
[h] *Hassanpouraghdam et al. (2008b)* (*hydroponically grown*).
[i] *Hassanpouraghdam et al. (2009)* (*culture-grown*).
[j] *Yousefzadi et al. (2009)*.
[k] *Bestmann et al. (1984)*.
[l] *99.8% R- and 0.2% S+ isomer.*



(R)-carvone α-Thujone Camphor β-bisabolene

FIGURE 1 Main essential oil constituents in costmary essential oils.

published data, four chemotypes were suggested (Figure 1): carvone, camphor, camphor–thujone, and camphor-α-thujone (Hassanpouraghdam et al., 2008c). In addition, bornyl acetate (47.7–55.2%)–pinocarvone (27.1–34.2%) chemotype of *T. balsamita* spp. *balsamitoides* was reported by Jaimand and Rezaee (2005). It is interesting noting that neither carvone nor α-thujone was reported for this subspecies.

Early chromatographic studies of costmary oils date to the 1960s; however, carvone chemotype oil containing 69–82% of carvone was described even earlier, by Voigt et al., in 1938, as it is indicated by Bestmann et al. (1984). Later Göckeritz (1968) defined two chemotypes of *B. major* according to the dominant terpene in the essential oil: camphor-type, *B. major* (L.) Desf. subsp. *majus* (Desf.) Asch. with 72–91% of camphor it the total oil content and carvone-type, *B. major* (L.) Desf. var. *tanacetoides* (Boiss.) Fiori. Camphor-chemotype was also described by Zielinska–Sowicka and Wolbis (1970). Bestmann et al. (1984) suggested distinguishing a third chemotype, with camphor and thujone as prevailing components in the essential oil. The oils of costmary from France (Paris et al., 1976) and Lithuania (Bylaitė et al., 2000; Jukneviciene et al., 1973) contained carvone as a dominating compound, constituting 52–80% of the total oil depending on the growth stage of the plant. The differences in the percentage content of carvone at different harvesting time were not considerable except for May 25 when it was the lowest (Bylaitė et al., 2000). The concentration of this constituent increased with the increase of the total oil content until bud formation with some exception on July 10, when it was slightly reduced compared with previous harvesting on June 25. It was shown that it is possible to obtain up to 8 g of natural carvone from 1 kg of dried costmary herb. Strobel et al. (1987) showed that the composition of the oil from *C. balsamita* leaves changes more distinctly during the season than the oil from flower head.

The second major compound in the analyzed *B. major* was α-thujone, the content of which exceeded 10% in most cases. It is worth noting that the percentage content of α-thujone, on the contrary to that of carvone, was highest on May 25 (16.1%). However, its concentration also was the highest during bud formation, for example, 1262 mg/kg in leaves. Other constituents exceeding 1% in costmary oil are also listed in Table 1: limonene, 1,8-cineole, β-thujone, *trans-p*-mentha-2, 8-dien-1-ol, β-bisabolene, T-muurolol, and selin-11-en-4-ol (only flowers). The differences in percentage composition between leaves and blossoms were not considerable, except for sesquiterpenes, especially sesquiterpene alcohol T-muurolol, the content of which was considerably higher in blossoms than in leaves at flowering period on July 25 and August 8 (Bylaitė et al., 2000). The absolute amount of sesquiterpenes in costmary flowers was approximately two times higher than in the leaves at the same growing phase. It is interesting to note that the highest percentage content of all identified sesquiterpenes in leaves was much higher at early growth phase (May 25) compared with later harvesting periods. For instance, the major sesquiterpene β-bisabolene on May 25 constituted 4.45%, compared with 2.33% on June 10 and 1.26% on June 25.

Other published studies focused on particular components of costmary, for example, five compounds were identified in *o*-dihydroxyphenolic fraction of *C. balsamita* L. var. *tanacetoides*, which included caffeic, chlorogenic, and ferrulic acids (Tămaş et al., 1982). Todorova and Ognyanov (1989) isolated seven C9 β-hydroxylated or esterified germacranolides from the flowers of *B. major* population cultivated in Bulgaria and found these lactones differ from the presently known eudesmanolides in a *B. major* population cultivated in Poland.

Bioactivities of Costmary Essential Oil and Its Main Constituents

Although Haslinger described the antibiotic properties of *Tanacetum balsamita* oil against Gram-positive and Gram-negative microorganisms in 1955 (Korzybski et al., 1967), the reports on this topic are rather scarce. Lukic et al. (1965) and Bestmann et al. (1984) studied the insecticidal activity of costmary essential oil. They identified and quantified more than 20 constituents in the essential oil of *C. balsamita* and found each of them to possess insecticidal properties against aphids. The distillate of *n*-hexane extract of dried flowers of *T. balsamita* applied at 2000 μg/mL showed a broad spectrum of activity when tested against 14 selected microorganisms (Kubo and Kubo, 1995); however, the active principles in the distillate were identified as 8 α,β-unsaturated aldehydes, while the terpenes were not reported. Antioxidant activity of costmary was demonstrated (Bandonienė et al., 2000); however, it was reported that costmary extracts and their fractions were weak antioxidants in rapeseed oil although some fractions were active in scavenging synthetic free radicals.

Several active compounds were detected in these fractions, using high-performance liquid chromatography with the online radical-scavenging detection and after multistep fractionation of these fractions, four radical-scavenging constituents, namely 5-*O*-caffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, 5,7,40-trihydroxy-30,8-dimethoxyflavone, and 5,7,30,40-tetrahydroxy-3,8-dimethoxyflavonol were isolated and their properties were assessed (Pukalskas et al., 2010). Cytotoxicity of the essential oil from *T. balsamita* subsp. *balsamita* in human fetal skin fibroblast and monkey kidney (Vero) expressed in IC_{50} values was 2500 ± 1.9 and 1250 ± 1.4 μg/mL, respectively (Yousefkadi et al., 2009).

Carvone was found to be the most abundant constituent in many previously published articles on costmary essential oil, therefore, it is reasonable to review the results on bioactivities of pure carvone or the plants, containing carvone as a major compound. Bioactivities of carvone include allergenic, antiacethylcholinesterase, antiseptic, central nervous system stimulant, cancer-preventive, carminative, flavor, insecticide, insectifuge, motor depressant, nematicide, perfumery, pesticide, sedative, trichomonicide, and vermicide (Hassanpouraghdam et al., 2008c).

(R)-carvone, which prevails in costmary (Başer et al., 2001), showed significant bactericidal activities, particularly against *Enterococcus faecium* and *Escherichia coli* above a concentration of 10 μL/mL. A fungicidal efficiency of (R)-carvone against *Aspergillus niger* was also noted by Naigre et al. (1996). Bactericidal activity expressed as the percentage of the sample in the assay mixture that resulted in a 50% decrease in colony forming units relative to a buffer control for R-carvone was 0.45 ± 0.004 (*E. coli*), 0.41 ± 0.004 (*Salmonella enterica*), 0.031 ± 0.005 (*Campylobacter jejuni*), and >0.67 (*Listeria monocytogenes*) (Friedman et al., 2002). In another study MICs were 53.3 mM for *Staphylococcus aureus* and 106.5 mM for *E. coli, Bacillus cereus* and *Candida albicans* (Van Zyl et al., 2006). Carvone and some other known antimicrobially active oxygenated terpenes, were found to cause significant K^+ leakage from *E. coli* cells (Griffin et al., 2005), however, (+)-carvone was remarkably weaker agent disintegrating the outer membrane and causing the release of outer membrane-associated material from the cells of *E. coli* O157:H7 and *Salmonella typhimurium* comparing to phenolic antimicrobials carvacrol and thymol (Helander et al., 1998). Among the tested 21 oxygenated monoterpenes, carvone and six other terpenes had potent inhibitory effects against most of the tested fungal species in the in vitro mycelial growth assays with 31 plant pathogen fungi, while camphor was less active (Kordali et al., 2007). Carvone was tested against *C. albicans* and at the dose of 10 μg it created 31.7 ± 2.08 mm diametre inhibition zone (Bader et al., 2007). Camphor and 1,8-cineole exhibited no inhibition effects on the growth of the tested bacteria using a disc diffusion method (in vitro) against 63 bacterial strains, belonging to 37 different genera and 54 species (plant, food and clinic origins) (Kotan et al., 2007). In vitro bioactivity evaluation of the isolated oil components revealed that both the optical isomers of carvone were active against a wide spectrum of human pathogenic fungi and bacteria tested (Aggarwal et al., 2002). High antifungal activity of carvone was reported in vitro on *Fusarium subglutinans, Fusarium* cerealis, *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium oxysporum*, *Fusarium sporotrichioides*, *Aspergillus tubingensis*, *Aspergillus carbonarius*, *Alternaria alternata*, and *Penicillium* sp. (Morcia et al., 2012). Treatment with carvone resulted in a significant decrease in the numbers of *L. monocytogenes* and *E. coli* O157:H7 in a raw meat dish in Turkish and Kurdish cuisine *çiğ köfte*. The results showed that carvone can be used for the inactivation of theses pathogens in such type of foods (Dikici et al., 2013). MIC of carvone (optical isomer not indicated) determined by the disc diffusion method was from 0.003 against *A. niger* to 0.33 mg/mL against *E. coli* (Hussain et al., 2010). However, R-carvone caused *L. monocytogenes* biofilm enhancement on food-processing surfaces, rather than inhibition (Leonard et al., 2010; Table 2).

Antimicrobial tests showed high activity of the *Anethum graveolens* EO containing 50.1% of D-carvone against the mold *A. niger* and the yeasts *Saccharomyces cerevisiae* and *C. albicans* (Jirovetz et al., 2003). The MICs of *Carum carvi* EO containing carvone (23.3%), limonene (18.2%), germacrene D (16.2%) and *trans*-dihydrocarvone (14.0%) were in the range of 170.2 (*Xanthomonas campestris* pv. *phaseoli*) to 7280 μg (*Pseudomonas cichorii*). Among numerous tested strains the activity was particularly high against the genera *Clavibacter, Curtobacterium, Rhodococcus, Erwinia, Xanthomonas, Ralstonia*, and *Agrobacterium* which are responsible for plant or cultivated mushroom diseases worldwide, while a lower activity was observed against bacteria belonging to the genus *Pseudomonas* (Iacobellis et al., 2005). L-Carvone was reported to demonstrate the highest nematicidal activity of the 13 tested terpenes with EC_{50} value (24 h) of 115 μg/mL (Ntalli et al., 2010). The EO of *C. carvi* containing carvone and limonene as the main components possessed stronger antifungal (19 species) and antibacterial (7 species) potential than did citronella oil with dominating components citronellal and geraniol (Simic et al., 2008). The oil of *Mentha spicata* L. subsp. *spicata* containing 48.4% of carvone showed great potential for its antimicrobial activities against *E. coli, C. albicans, Candida tropicalis*, and moderate activities against *S. aureus* (Şarer et al., 2011). The carvone-rich oils displayed promising activity against the citrus pathogens (*Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Alternaria citrii, Botrytis cinerea*, and *Penicillium digitatum*); moreover, the oils characterized by high concentrations of S-carvone were less effective than those containing the R-enantiomer (Combrinck et al., 2011). Significant antimicrobial activity against *Shigella flexneri, Klebsiella pneumoniae, Salmonella*, and *E. coli* demonstrated dill seed EO containing up to 52% of carvone, whereas no inhibitory effects were observed for this oil against

TABLE 2 Antimicrobial Activities of Costmary Essential Oil and Carvone

Microorganism	Essential Oil[a] DZ/MIC	Carvone[a] DZ/MIC	Ampicilin[a] DZ	Nystatin[a] DZ	Essential Oil[b]	Amicasine 30e[b]	Penicillin 10[b]
	10 µg/disc	10 µg/disc	10 µg/disc	30 µg/disc	DZ		
Bacillus subtilis	31 ± 0.3/1.87	19 ± 0.4/3.8 (25.0)	19 ± 0.4		13	17	15
Bacillus pumulis	34 ± 0.2/0.93	23 ± 0.22/1.9 (12.5)	15 ± 0.3				
Enterococcus faecalis	10 ± 0.2/15		11 ± 0.3				
Staphylococcus aureus	20 ± 0.3/7.5	21 ± 0.3/1.9 (12.5)	13 ± 0.3		17	18	8
Staphylococcus epidermidis	35 ± 0.5/0.93	16 ± 0.2/3.8 (25.0)	19 ± 0.5				
Escherichia coli	12 ± 0.3/15	13 ± 0.4/7.5 (50)	12 ± 0.2		15	18	9
Klebsiella pneumoniae	10 ± 0.4/15	10/>15 (100)					
Pseudomonas aeruginosa			19 ± 0.4				
Aspergillus niger				16 ± 0.4			
Candida albicans	12/10	10 ± 0.3/>10 (100)		18 ± 0.5			
Saccharomyces cerevisiae	16/5	11 ± 0.5/>10 (100)		18 ± 0.2			
Salmonella typhimurium					13	19	8
Candida globrata					15	19	–
Candida tropicalis					14	20	–

DZ = diameter of zone includes diameter of disc (6 mm); MIC = minimum inhibitory concentration, mg/mL.
[a]*Yousefkadi et al. (2009)*.
[b]*Bagci et al. (2008)*.

*St*aphylococcus *pyogenes* and *S. aureus* (Jianu et al., 2012). EO of *M. spicata* containing 67% of carvone showed antibacterial activity against *S. aureus* and *E. coli* (Scherer et al., 2013). Carvone was recognized as a one of the active component of caraway EO, which exhibited medium antimicrobial activity: a significant negative correlation was observed between MIC and carvone content (Seidler-Lozykowska et al., 2013).

Toxicity of R-carvone in human kidney epithelial cells was $IC_{50} = 140.9 \pm 9.6\,\mu M$, antimalarial activity $IC_{50} = 57.3 \pm 9.6\,\mu M$, antioxidant activity in DPPH radical reaction $IC_{50} > 659.9\,\mu M$ (Van Zyl et al., 2006). In other work, radical scavenging activity in DPPH radical system was reported as $IC_{50} = 19.4\,\mu g/mL$, while inhibition of linoleic acid oxidation was 51.7% (Hussain et al., 2010). De Carvalho and Da Fonseca (2006) concluded that several applications of carvone as fragrance and flavor, potato sprouting inhibitor, antimicrobial agent, building block, and biochemical environmental indicator, along with its relevancy in the medical field, justify the interest in this monoterpene.

CONCLUSION

Costmary has been used as a food, food flavoring, and medicinal plant since ancient times. Extensive studies of phytochemicals biosynthesized in costmary during last few decades resulted in the identification and quantification of several compounds possessing various bioactivities. However, the uses of costmary preparations are rather limited, because developments of broader applications require more systematic studies focused on antimicrobial, antifungal, and health beneficial activities of costmary oils and their valuable constituents. In conclusion, costmary may be considered as a forgotten plant with some potential in the future for development of functional ingredients for foods and other products.

SUMMARY POINTS

- Costmary has a long history and food and medicinal application, however its importance nowadays remarkably diminished.
- Costmary oil contains several valuable phytochemicals, in particular R-carvone.
- Costmary oil possesses antimicrobial, antioxidant, and many other activities.
- Costmary oil has potential in the development of functional food ingredients.

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Chapter 42

Cumin (*Cuminum cyminum* L.) Oils

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List of Abbreviations

DPPH 2,2-diphenyl-1-picrylhydrazyl
GRAS Generally recognized as safe
MFC Minimum fungicidal concentration
MIC Minimum inhibitory concentration
CEO *Cuminum cyminum* essential oil
ZDI Zone diameter of inhibition

INTRODUCTION

Cumin, *Cuminum cyminum* Linn (Apiaceae family), is commonly known as *Jeera* in Hindi vernacular. It is indigenous to south-west Asia and the eastern Mediterranean region (Lim, 2013), with 90% of the world's production from India (Kumar et al., 2011). Cumin as whole, in powdered form, or as an essential oil is widely used as a spice for its strong characteristic flavor in various ethnic cuisines around the world, for flavoring foods, beverages, liquors, and is used in medical preparations (Iacobellis et al., 2005). Cumin seeds possess a strong aromatic odor and a sharp burning taste that draws out the natural sweetness of many dishes and thus is used for seasoning curries, soups, pickles, cheese, enchiladas, tacos, salsa, meat, breads, barbecue sauces, snack foods, cakes, and is also used as a flavoring agent in the food industry (Kumar et al., 2011; Nadeem and Riaz, 2012; Sahana et al., 2011; Peter, 2003; Donna and Antony, 1993). Cumin is used widely in traditional medicine to treat flatulence, digestive disorders, diarrhea, and in the treatment of wounds (Peter, 2001). In ancient medicine, cumin seeds, containing 3–4% volatile oil and about 15% fixed oil (Sowbhagya et al., 2008), were used to treat toothache, diarrhea, and epilepsy, and currently *C. cyminum* is considered as an alternative antimicrobial agent, safe for human applications.

BOTANICAL ASPECTS

Cumin is a thin, aromatic herbaceous, subglabrous dicotyledonous annual plant, about 50 cm high, with a slender branched stem (Lim, 2013; Bahraminejad et al., 2012). The leaves are alternate, dissected, sparsely hairy, bluish green in color, and bear thread-like leaflets. They are about 5 cm long, pinnate or bipinnate with 2–3 pinnasitect and filliform ultimate segments 3 to 6 × 0.05 cm. The petioles have tapered crusted wings, sheathing the stem at the base (Nadeem and Riaz, 2012). The flowers are small, white or pink in color, diploid allogam hermaphrodite, are born in umbels each containing 5–7 umbellets, and have five unevenly segmented calyx that are linear lanceolate and petals are oblong ovate (Sahana et al., 2011). The fruit is yellowish green or brown in color, schizocarp, single seed containing ellipsoid to lateral fusiform nondehiscent achene, 3–6 mm long, and the surface bears five longitudinal pale ridges with alternate less distinct ribs bearing numerous short hairs (Peter, 2003). The main stem is 3–5 cm in diameter, gray or dark green color, divided into five secondary branches which are further divided into 2–3 subbranches all of which grow to the same height forming a canopy (Lim, 2013). The whole cumin seeds and the powder of the seeds are shown in Figure 1.

USAGE AND APPLICATIONS

Cumin and value added products from cumin such as alcohol and water extract and cumin seeds as rich sources of essential oils possess many nutraceutical properties with many biological activities such as antiallergic, antioxidant, antiplatelet

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FIGURE 1 *Cuminum cyminum* (A) whole and dry seeds, and (B) seed powder. *Photographed by the corresponding author, Dr Shyamapada Mandal.*

aggregation, hypoglycemic (Sowbhagya, 2013), antidiabetic (Lee, 2005) antihyperlipidemic (Kode et al., 2005; Shirke and Jagtap, 2009), antiinflammatory (Srivastava, 1989), anticarcinogenic (Gagandeep et al., 2003), immunomodulatory (Chauhan et al., 2010), and relaxant properties (Boskabady et al., 2005).

The relaxant effect of *C. cyminum* makes it a powerful bronchodilator (Prasad et al., 2009), as demonstrated in guinea pig tracheal chains, with stimulation of β-adrenergic receptors, inhibition of histamine H1 receptors, or anticholinergic properties of this plant proposed as the possible mechanisms (Boskabady et al., 2005).

The fruit of *C. cyminum* is rich in estrogenic isoflavonoids, luteolin, and apigenin and are effective in a variety of menopausal disorders, such as osteoporosis and cardiovascular disorders (Shirke and Jagtap, 2009). Cumin oil, due to the presence of monoterpenes like anethofuran, carvone, and limonene, inhibited benzopyrene-induced stomach tumorigenesis, 3-methylcholanthrene-induced uterine cervix tumorigenesis, and 3-methyl-4-dimethyaminoazobenzen- induced hepatomas in mice through augmentation of cytochrome P450 reductase and cytochrome b5 reductase (Johri, 2011).

The flavonoid glycoside, 3′,5-dihydroxyflavone 7-O-β-d-galacturonide-4′-O-β-D-glucopyranoside, isolated from cumin produced a significant enhancement of antituberculosis drug, rifampicin, when codosed, that was attributed to a permeation-enhancing effect of cumin (Johri, 2011; Sachin et al., 2007). Extracts of *C. cyminum* showed hepatoprotective activity in a profenofos-intoxicated group with reduced levels of liver function test (Kumar et al., 2011). The volatile oils from the fruits of *C. cyminum* showed antiinflammatory activity as a dose-dependent inhibition of carrageenan-induced rat paw edema (Shivakumar et al., 2010).

The seed extract of *C. Cyminum* imparted corrosion inhibition activity attributed to the presence of aromatic ring, aldehyde, and isopropyl groups and its role as a natural corrosion inhibitor on mild steel surfaces was investigated by gravimetric, potentiodynamic polarization, electrochemical impedance spectroscopy (Singh et al., 2012).

Sergio et al. (2013) demonstrated *C. cyminum* essential oil (CEO) as an active agent against *Mycobacterium tuberculosis*, with minimum inhibitory concentrations (MICs) of 6.25–12.5 μg/mL (Figure 2), suggesting the use of the agent in ethno-medicine as an alternative remedy for symptoms of tuberculosis as well further phytochemical and pharmacological studies for obtaining the CEO compound effective against *M. tuberculosis*.

USAGE AND APPLICATIONS IN FOOD SCIENCE

The CEO is effective on the inhibition of growth and reduction in numbers of serious food-borne bacteria, such as *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* as well as fungi like *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, capable producing aflatoxins, thus protect the foods by maintain quality and increasing shelf life.

Nutritional Value

The nutritional value of cumin seeds per 100 g includes 460 kcal energy, 6.0 g water, 44.6 g carbohydrate, 18.0 g protein, 23.8 g fat, 7.7 g ash (9.5% acid-insoluble ash), 10.5 g dietary fiber (total 59.0%, of which 48.5% is insoluble and 10.5% is soluble, while the spent residue after oil and nonvolatiles extraction contains 62.1% total, of which 51.7% and 10.4% are insoluble and soluble fiber, respectively), 0.730 mg vitamin B1 (thiamin), 0.380 mg vitamin B2 (riboflavin), 2.5 mg vitamin B3 (niacin), 0.435 mg vitamin B6, 17 mg ascorbic acid, 3.33 mg vitamin E, vitamin A activity of 127 retinal equivalents,

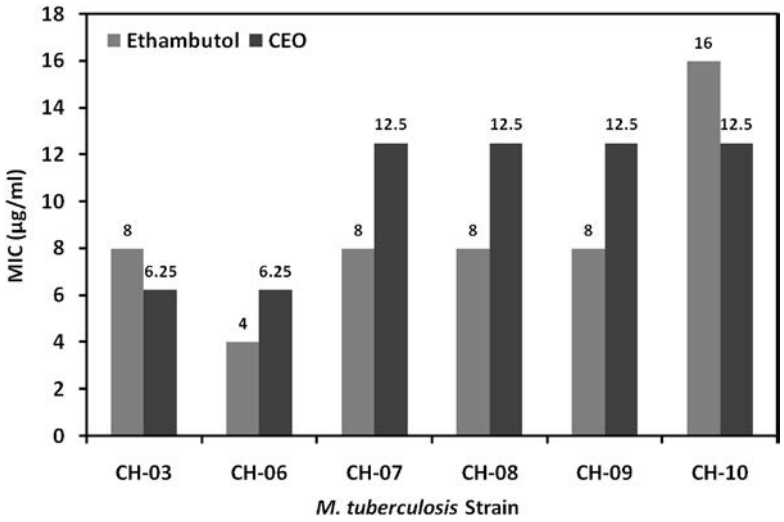

FIGURE 2 Minimum inhibitory concentrations (MICs) (µg/mL) of *Cuminum cyminum* essential oil (CEO) and ethambutol (conventional antituberculosis antibiotic) against drug-resistant *Mycobacterium tuberculosis* strains. *Data table adapted from Sergio et al. (2013). http://dx.doi.org/10.4236/abc.2013.35052.*

0.9 g calcium, 450 mg phosphorus, 160 mg sodium, 2100 mg potassium, 47.8 mg iron, 4.8 mg zinc, other trace elements, 2.5% volatile oil, and 9% moisture (Donna and Antony, 1993; Nadeem and Riaz, 2012; Sahana et al., 2011).

Antispoilage Activity

Cumin spice and its essential oils have been used as preservatives owing to their antioxidant and antimicrobial property to improve the shelf life of foods, and also as a safer alternative to synthetic antioxidants (Singh et al., 2005). Also, CEO is used as fumigant and additive in the storage of foodstuffs owing to its high antifungal and antibacterial activity against various pathogenic microorganisms (El-Lakwah et al., 2001).

Storage life of strawberries was enhanced with CEO by inhibition of fungal infection compared with controls and was found to decrease postharvest decay and an increase in some overall qualities of strawberry fruits (Asghari et al., 2009). Cumin oil caused 100% inhibition of fungal deterioration of stored pigeon pea seeds, predominantly *A. flavus* and *Aspergillus niger*, indicating judicious use of cumin oil as botanical preservative for pigeon pea seeds against spoilage by fungi during storage (Pandey et al., 2007).

The microencapsulation and stability under storage of cumin oleoresin in combination with wall materials was found to provide most efficient protection as studied from the time required for a constituent to reduce to 50% of its initial value using a blend of 4/6:1/6:1/6 of gum arabic, maltodextrin, and modified starch (Kanakdande et al., 2007). Dietary *C. cyminum* powder (1.14%), as a feed additive for growth performance and disease resistance during first-feeding of Mozamique tilapia (*Oreochromis mossambicus*), provided the best survival rates when challenged with *Streptococcus iniae* infection, and thus the agent can be used as an alternative to antibiotics in controlling streptococcal disease in tilapia culture (Yilmaz et al., 2013).

Antibacterial Activity

CEO decreased formation of biofilm by *Klebsiella pneumoniae*, an important virulence factor, and also enhanced the activity of ciprofloxacin against multidrug resistant *K. pneumoniae* (Derakhshan et al., 2010). In another study, inhibition of *K. pneumoniae* was reported with CEO through cell elongation, repression of capsule expression, and inhibition of urease activity; the antibacterial properties of cumin essential oil being mostly attributable to the cumin aldehyde (Derakhshan et al., 2008).

Extract of *C. cyminum* exhibited significant antibacterial properties through inhibition of *Staphylococcus aureus*, *Bacillus amyloliquefaciens*, and *Pseudomonas aeruginosa* (Singh and Singh, 2011). CEOs showed activity against *M. tuberculosis* strains with MIC values in the range of 6.25–25 µg/mL showing resistance to five first-line antituberculosis drugs, streptomycin, isoniazid, rifampicin, ethambutol, and pirazinamide (Sergio et al., 2013). Abushama et al. (2013) showed the antibacterial activity of CEO with 1:100 dilutions was high against *S. aureus*, *E. coli*, and *K. pneumoniae*, with zone diameter of inhibition (ZDI) values of 16, 16, and 15 mm, respectively.

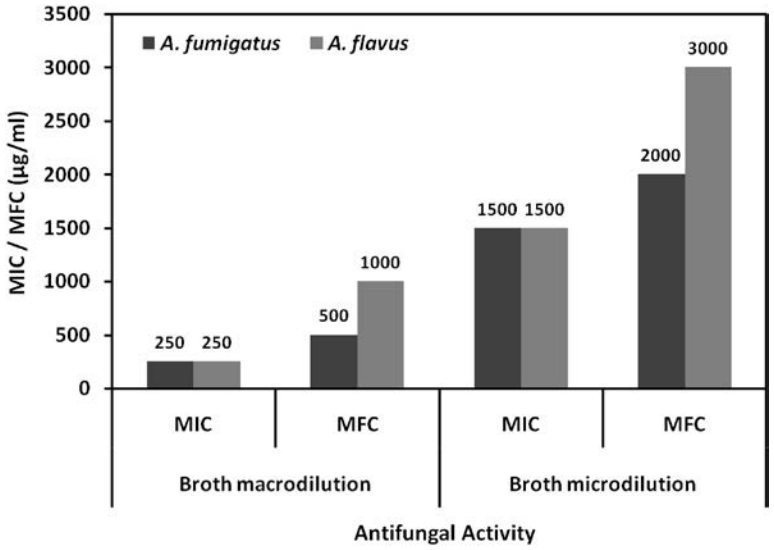

FIGURE 3 Anti-*Aspergillus* susceptibility of *Cuminum cyminum* essential oil broth macro and microdilution techniques. *Data table partly converted to figure, Khosravi et al. (2011).*

Antifungal Activity

Rhizobacteria such as *Bacillus lentus*, *Castellaniella denitrificans*, *Pseudomonas cedrina*, *P. aeruginosa*, *Lasinibacillus fusiformis*, *Tetrathiobacter kashmeriansis*, and *L. fusiformis* isolated from cumin plants possessed antifungal activity against *Fusarium oxysporum*, *Verticillium dahliae*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* causing wilt, blight, and powdery mildew in cumin plants and thus shown to have potential biocontrol activity (Kumari and Deshwal, 2012). The essential oil extracts of cumin demonstrated antifungal activity against *Candida albicans* causing hydroxyapatite dissolution leading to dental caries (Pai et al., 2010). Ridawati et al. (2011) showed strong antifungal activity of cumin oil against the yeasts *Candida parapsilosis*, *Candida orthopsilosis*, *Candida metapsilosis*, and *Candida etchellsii* with MIC values of 0.028–0.042% and minimum fungicidal concentration values 0.09–0.14%. The antifungal effect of CEO with dilution (1:100 v/v) was high against *A. niger* and *C. albicans* with ZDI of 18 mm for each, followed by *F. oxysporum* with ZDI of 14 mm, suggesting the possible usage of CEO as an industrial antifungal agent (Abushama et al., 2013). The anti-*Aspergillus* activity of CEO, as reported by Khosravi et al. (2011), is shown in Figure 3.

Antioxidant Activity

Cumin seed extracts showed antiradical (34.25–39.25%) and antioxidant (8.25–11.24 mg/mL) activity, as determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and phosphomolybdenum complex methods, respectively, thus decreasing the risk of serious health problems (Al-Juhaimi and Ghafoor, 2013). Cumin can be used as a potential source of natural antioxidants in foods. El-Ghorab et al. (2010) showed the antioxidant activity of cumin essential oil was 85.44% and obtained 2.52% (w/w) yield of volatile oil in cumin with cuminal, γ-terpinene, and pinocarveol as the major components, and 4.08% (w/w) yield of nonvolatile oil. Antioxidant activity of cumin showed 6.61 mmol of trolox/100 g of dry weight and 0.23 g of gallic acid equivalent/100 g of dry weight as the total phenolic contents (Shan et al., 2005).

Cumin oils and extracts have significant antioxidant activity due to the presence of monoterpene alcohols, linalool, carvacrol, anethole, estragol, flavonoids, and other polyphenolic compounds through their ability to quench hydroxyl radicals, DPPH radicals, and lipid peroxides using the ferric thiocyanate assay method in the linoleic acid system, Fe^{2+} ascorbate-induced rat liver microsomal lipid peroxidation, soybean lipoxygenase-dependent lipid peroxidation, and ferric reducing ability (Johri, 2011). Thus, the CEO and cumin extracts minimize rancidity of foods, restrict the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life.

Cumin Chemistry

Profiling of cumin essential oils showed that the major compounds were 8–17% cuminal, 22–27% β-pinene, 1.3–1.75% β-myrcene, 23–39% ρ-cymene, 11–27% γ-terpinene, and 1.0–5.5% ρ-mentha-1,4-dien-7-ol (Ramasamy et al., 2013). Cumin fruit is composed of 10% fixed oil and proteins, 2.5–4.5% volatile oil comprising 30–50% cuminaldehyde and

TABLE 1 The Main Chemical Compounds Identified in Essential Oil of *Cuminum cyminum*

Origin	Major Components	References
Chinese cumin	Cumin alcohol (24.4%), 2-caren-10-al (20.9%), cuminal (18.4%), and γ-terpinen (10.6%)	Zhang et al. (2011)
Chinese cumin	Cuminal (22.76%), cumin alcohol (21.76%), ρ-cymene (14.33%), β-pinene (11.06%), 2-care-10-al (8.56%), and γ-terpinene (7.05%)	Wang et al. (2009)
Chinese cumin	Cuminal (45.75%), α-proyl-benzenemethanol (14.76%), and 2-ethylidene-3,5-heptadienal (12.91%)	Wang et al. (2006)
Tunisian cumin	γ-terpinen (25.58%), 1-phenyl-1,2 ethanediol (23.16%), cuminaldheyde (15.31%), β-pinene (15.16%), and ρ-cymene (9.05%)	Bettaieb Rebey et al. (2012)
Mashhad, Iran	Cuminaldehyde (30.2%), ρ-cymene (14.1%), γ-terpinene (12.8%), safranal (9.4%), and β-pinene (6.4%)	Oroojalian et al. (2010)
Iranian cumin	α-pinene (29.2%), limonene (21.7%), 1, 8-cineole (18.1%), linalool (10.5%), and α-terpineole (3.17%)	Mohammadpour et al. (2012)
Sudanese cumin	2-Caren-10-al (29.64%), benzaldehyde, 4-1-methyethyl (16.58%), and 2-J-pinene (12.06%)	Abushama et al. (2013)

small quantities of α-pinene, phellandrene, cuminic alcohol, hydrated cuminaldehyde, and hydro cuminine are also present (Kumar et al., 2011). In another report, cumin oil was shown to contain various volatile components such as α-pinene (0.5%), myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), ρ-menth-3-en-7-ol (0.7%), ρ-mentha-1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β-bisabolene (0.9%), β-pinene (13.0%), ρ-cymene (8.5%), β-phellandrene (0.3%), ᴅ-terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol (2.8%), β-farnesene (1.1%), small quantities of α-phellandrene, α-terpinene, cis and trans-sabinene, myrtenol, α-terpineol, phellandral, and volatile components including tannins, oleoresin, mucilage, gum, protein compounds, and malates (Nadeem and Riaz, 2012). Bettaieb et al. (2010) reported the major components of the oils as bornyl acetate (23%), α-terpinene (34%), and γ-terpinene (51%) in roots, stems, leaves, and flowers, respectively of *C. cyminum* with total phenolics content ranging from 11.8 to 19.2 mg of gallic acid equivalents per gram of dry weight (26% quercetin in roots, 51% vanillic acid in flowers, *p*-coumaric, rosmarinic, *trans*-2-dihydrocinnamic acids, and resorcinol in stems and leaves) while the flower part exhibited strongest antioxidant activities as a DPPH radical scavenger, lipid peroxidation inhibitor, and reducing agent, with IC_{50} values of 4, 32, and 8 μg/mL, respectively.

The yield of CEO was 1%, as reported by Mohammadpour et al. (2012), while the essential oil yield of cumin seeds from a local market in India was reported to be 2.33% (Sowbhagya et al., 2008). The main components of CEOs are different too, as has been recorded by authors from different countries (Table 1). The composition of CEO depends on many factors, such as plant part, harvest time, extraction method, type of cultivar, geographic origin, and storage conditions (Leopold et al., 2005). Kan et al. (2007) reported that the essential oil in the cumin seeds at different harvesting times comprised cumin aldehyde (19.9–23.6%), ρ-mentha-1,3-dien-7-al (11.4–17.5%), and ρ-mentha-1,4-dien-7-al (13.9–16.9%) and that the cumin seeds should be harvested at the ripe stage for ideal volatile oil yield and composition.

Safety and Toxicity

Cumin seeds and their oil have gained generally regarded as safe (GRAS) 2340 and GRAS 2343 status under the regulatory system (Parthasarathy et al., 2008) and side effects of any of the whole or processed cumin, or its essential oil have not been reported (Sahana et al., 2011).

SUMMARY POINTS

- Cumin (*Cuminum cyminum* L.), as a whole, in powdered form, or as an essential oil is widely used as a spice for its strong characteristic flavor in various ethnic cuisines around the world mainly for flavoring foods, beverages, liquors, and is used in medical preparations.
- Cumin is a thin, aromatic herbaceous, subglabrous dicotyledonous annual plant, about 50 cm high, with a slender branched stem.

- Cumin possesses many nutraceutical properties with many biological activities like antiallergic, antioxidant, antiplatelet aggregation, and hypoglycemic, antidiabetic, antihyperlipidemic, antiinflammatory, anticarcinogenic, immunomodulatory, and relaxant properties.
- Cumin has been used as preservative to improve the shelf life of food material, and as a safer alternative to synthetic antioxidants due to the presence of monoterpene alcohols, linalool, carvacrol, anethole, estragol, and flavonoids.
- The major compounds present in cumin oil include cuminal, β-pinene, β-myrcene, ρ-cymene, γ-terpinene, and ρ-mentha-1,4-dien-7-ol.
- No side effects with cumin usage have been reported and it has GRAS status.
- Cumin is one of the most important medicinal plants and one of the important seed spices belonging to the Umbellifreae family. It shows significant antimicrobial activity against many bacteria and fungi and has potential medicinal value.

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Chapter 43

Curry Leaf (*Murraya koenigii*) Oils

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INTRODUCTION

Curry leaf or curry bush (*Murraya koenigii* (L.) Spreng., Rutaceae family, 2n = 18) is an evergreen, semideciduous, ornamental tropical tree of 4–6 m in height with many uses including as a spice and for medicinal purposes. It is found in wild and cultivated forms in India up to 1650 m altitude and in other countries. The plant parts have diversified uses. Fresh and dried leaves and their essential oil are employed as spice condiment in daily food preparations and processed vegetarian and nonvegetarian foods. Dehydrated leaves, powder, and paste improve texture, taste, nutrient status, shelf-life, and the antioxidant value of rice, potatoes, *chapatti*, *sandesh*, *dokhla*, meat, and chicken patties. Curry leaves consumed with pepper reduce cholesterol. As a food-additive, leaves fried with *Aspergillus*-contaminated cooking oil reduce aflatoxin production. Green leafy porridge made with rice and coconut milk serves as antidiabetic functional food. Nutritious leaves are useful in correcting calcium deficiency. Leaves cooked in coconut oil improve hair color and growth. Hyaluronidase inhibitors isolated from the curry bush are used in skin-lightening and texture improving creams. The sweet fruits are eaten fresh. The fruit juice serves as dietary supplement or health drink for diabetes. Decorative articles are made from the root wood and the twigs as brush strengthens teeth and gums (Ajay et al., 2011; Harish et al., 2012; Vandana et al., 2012).

BOTANICAL ASPECTS

The genus *Murraya* (12–15 species) is distributed in Asia, Africa, Australia, and New Caledonia. *Murraya koenigii* is native to tropical Asia spreading from the Indian subcontinent to Sri Lanka and extending to Myanmar, Indonesia, China, and Hainan. It is cultivated in south-east Asia, Australia, United States, Nigeria, Kenya, Tanzania, and Indian Ocean islands. *Murraya koenigii* is a spreading tree with a 15–40 cm diameter trunk. The main stem and branches (10–50 per tree) are covered with dark grey to brownish bark with longitudinal striations encasing white-coloured wood. Spirally arranged leaves (Figure 1) are imparipinnately compound, exstipulate, 10–35 cm long bearing eight to 31 leaflets. Green-coloured, 1–8.5 cm long, 0.5–5.5 cm broad, short-petioled leaflets are alternate, glabrous, gland-dotted, ovate to ovate-lanceolate with obtuse to rounded bases, serrated margins, and notched apexes. Flowering and fruiting occurs from March to August. The inflorescence, a terminal, branched cyme, bears 60–90 sweetly scented, white to light green, round to oblong, short-pedicelled, funnel-shaped, bisexual, ebracteate, 1.4–1.6 cm long, 1–1.2 cm broad, actinomorphic, pentamerous, hypogynous flowers. Pubescent, persistent, inferior, green-coloured calyx with ovate teeth is five-lobed. White-colored, inferior, lanceolate to oblanceolate, 5–7 mm long, glabrous, gland-dotted corolla is five-petalled. Polyandrous, inferior androecium has 10 dorsifixed 4–6 mm long stamens. Five to six millimeters long gynoecium has capitate, short-styled, sticky stigma, and superior ovary. Self and cross (geitonogamy, xenogamy) pollinations aided by honey bees and butterflies occur. Ripe, one or two-seeded, 1.0–2.5 cm long, 0.3–1.2 cm broad, shiny, glandular, ovoid or subglobose, purplish–black fruits (weight 800–900 mg, volume 850–900 µL) with thin pericarp and wisteria-blue pulp are borne in clusters of 30–80. Spinach green-colored, ovate-oblong seeds are 11 mm long, 8 mm broad with 400–450 mg weight, and 400–460 µL volume (Ajay et al., 2011; Rajeswara Rao et al., 2011a; Harish et al., 2012; Vandana et al., 2012).

Curry bush grows in well-drained, black or red soils. It is propagated through freshly collected or less than 3-month-old scarified seeds, stem or root cuttings, and root suckers. Morphotypes, chemotypes (Rajeswara Rao et al., 2011a,b), and the variety *Suvasini* (rich in β-caryophyllene, 36.3%, and β-gurjunene, 24.5%) are cultivated. Individual trees planted in soil or in large pots are grown in home gardens for family use. Commercial plantations are raised by sowing 200 kg seeds/acre during rainy season with 2.0 m × 15–20 cm spacing. Curry bush is fertilized with 10 t farmyard manure, 150–250 kg

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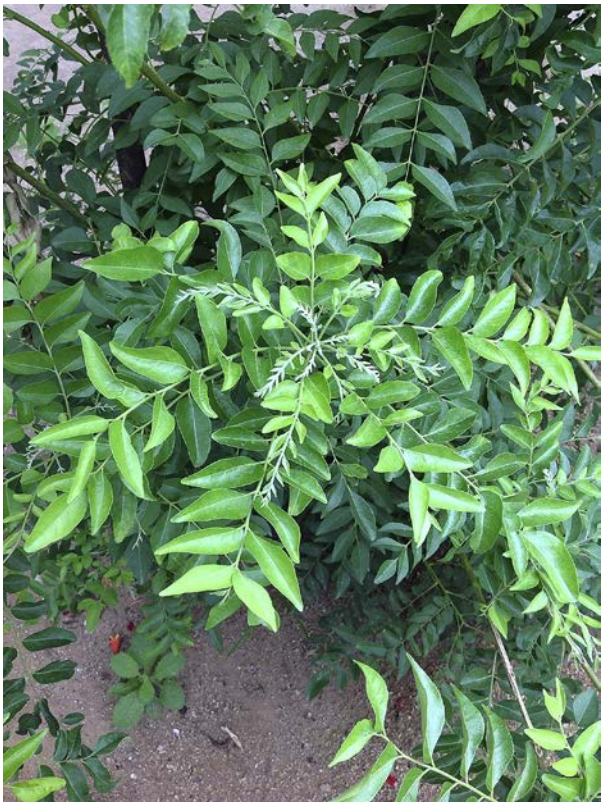

FIGURE 1 Close-up view of *Murraya koenigii*.

nitrogen, 60–80 kg each of phosphorus and potassium/acre/year; manually weeded two to three times and irrigated at 20-day intervals. Ten species of arbuscular micorrhizal fungi that aid in mineral nutrition inhabit the roots (Rao et al., 2000). Trees are coppiced 7–8 months after planting and a second harvest performed 3–4 months later. From the second year, three to four harvests are performed for 10 years. First year biomass yield ranges from 7 to 9 t/acre and 20–24 t/acre from the second year. In another method, trees planted at 3.0×1.5 m spacing are pollarded at 1.2 m height four times per year. A power-operated mechanical stripper has been developed to substitute manual harvesting.

USAGE AND APPLICATIONS

Different plant parts and their preparations are used in indigenous medicine systems as a tonic, stimulant, carminative, and stomachic. Infusion of roasted leaves is administered to stop vomiting, while leaf decoction along with bitters acts as febrifuge and antiperiodic. Green, tender leaves that are spicy, bitter, cooling, and weakly acidic are eaten to stop dysentery. Leaf and root bark pastes are applied to treat eruptions and bites of poisonous insects and animals (Nigam and Purohit, 1961). The fruit juice relieves kidney pains. Ground root bark relieves body aches (Walde et al., 2005). Green leaves and other parts are used to treat piles, inflammation, itching, cuts, bruises, and edema (Rajeswara Rao et al., 2011a,b). Aqueous and solvent extracts of plant parts containing triterpenoid alkaloids, carbazole alkaloids, carbazole carboxylic acids, steroids, coumarins, furocoumarins, flavonoids, triterpenes, sterols, saponins, phenolic, and other compounds exhibited pharmacological activities. Mahanimbine at 50–100 mg/kg body weight displayed antidiabetic activity in streptozotocin-induced diabetic Wistar rats. Kurrayam and koenimbine from seeds controlled castor oil-induced diarrhea in rats. Ethanol leaf extract healed wounds of male albino rats (Harish et al., 2012). Diabetic patients fed with curry leaves for 15 days showed a reduction in fasting and postprandial blood glucose levels (Ajay et al., 2011). Aqueous extract containing tannins and carbazole alkaloids and acetone extract of bark exhibited hepatoprotective activity. Alcohol extract of stem bark and root extract reduced carrageenan-induced inflammation (Vandana et al., 2012).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Fresh or dried leaves and their essential oil are the chief commercial products of curry leaves. Farm-fresh leaves transported in trucks are marketed on the same or the next day. Families and restaurants preserve them under ambient conditions or in

refrigerators. Essential oil trading is limited to the producing countries and a small quantity is exported to countries where immigrants from the producing countries have settled.

On steam or hydrodistillation, tender branches, leaves, stem, flowers, fruits, fruit pulp, and seeds yield strongly fragrant essential oils responsible for their characteristic flavor (Wong and Chee, 1996; Ramalakshmi et al., 2000; Walde et al., 2005, 2006; Rajeswara Rao et al., 2011a,b). The essential oils can be isolated by microwave distillation or solvent and supercritical fluid extractions. More than 100 aromatic constituents have been identified in the essential oils of Bangladesh, Borneo, China, India, Malaysia, Nigeria, and Sri Lanka. Their important components are listed in Table 1. Detailed essential oil profiles of fresh leaf, dry leaf, tender stem with rachis, and mature fruit produced in Hyderabad, India, are presented in Table 2, a chromatogram of the leaf essential oil is shown in Figure 2, and the structures of its important compounds are shown in Figure 3. Petiole essential oil was rich in α-pinene and α-copaene; flower essential oil in α-pinene, camphene, limonene, (Z)-β-ocimene, and β-caryophyllene; fruit essential oil in α-pinene, β-pinene, *p*-cymene, β-phellandrene, borneol, and caryophyllene oxide; the fruit pulp essential oil in α-pinene, (Z)-β-ocimene, and α-copaene and the seed essential oil in α-pinene, β-pinene, and γ-terpinene (Tables 1 and 2). The essential oils of flowers, fruits, fruit pulp, and seeds have future potential as flavoring agents. Leaf essential oil is strongly aromatic with a spicy note, light-yellow to yellow-colored, a specific gravity of 0.8589 (25 °C), a refractive index of 1.4706 (25 °C), 36° 14′ optical rotation (25 °C), an acid value of 1.17, and a saponification value of 8.18 and 52.81 after acetylation (Nigam and Purohit, 1961). Leaf essential oils of different countries and diverse locations in India were rich in α-pinene, sabinene, β-pinene, δ-3-carene, limonene, β-phellandrene, (Z)-β-ocimene, lavandulol, terpinen-4-ol, geraniol, α-copaene, isocaryophyllene, γ-elemene, β-caryophyllene, β-gurjunene, aromadendrene, α-humulene, α-selinene, bicyclogermacrene, and β-bisabolene (Tables 1 and 2). Monoterpenoid (α-pinene, sabinene, β-pinene, 3-carene, β-phellandrene, (Z)-β-ocimene), sesquiterpenoid (α-copaene, β-caryophyllene, β-gurjunene, α-humulene), mono and sesquiterpenoid (β-caryophyllene, β-phellandrene)-rich chemotypes have been identified in leaf essential oils offering opportunity for the food industry to select desirable chemotypes to suit consumer preferences (Rajeswara Rao et al., 2011a,b).

Leaf essential oil is used as an alternative to the leaves or as a stand-alone product. Fresh leaves (as such or fried with chopped onion and other spices), powder, and their essential oil promote appetite, digestion and are employed as spice and condiment for flavoring and seasoning soups, curries, curry powder blends, chutneys, *vada*, *upma*, *khadi*, *sambar*, *rasam*, pickles, sausages, buttermilk preparations, egg preparations, snacks, fish, meat dishes, and ready-to-eat and modern processed food items. The essential oil finds application in the soap, cosmetics, and aromatherapy industries. The essential oil is stored in stainless-steel, aluminum, or glass containers. The essential oil should not contain free or dissolved water before storing it. The presence of water, air, and long exposure to heat or sun light spoils the essential oil quality and shortens shelf-life. Prolonged storage of the essential oil results in changes in chemical composition as the terpenes polymerize to form long-chain compounds that impart a disagreeable odor. Young leaves yield better quality essential oil. Cross-flow drying, steam, and hot-water blanching of the leaves prior to distillation resulted in partial loss of low-boiling monoterpenes, while high-boiling sesquiterpenes were retained in the essential oil (Ramalakshmi et al., 2000). Prolonged distillation at high temperatures results in partial loss of odor imparting constituents.

The essential oil exhibited antioxidant, insecticidal, antibacterial, antifungal, and pharmacological activities. Curry leaf oleoresin, essential oil, methanol, and water extracts displayed antioxidant activities of 83.2%, <10%, 16.7%, and 11.3%, respectively, at 100 ppm concentration (Rao et al., 2007). The oleoresin increased the shelf-life (17.5 days) of Brussels sprouts and cauliflower by minimizing their weight loss and retaining total solids and reducing sugars (Subbanna et al., 2009). Insects, bacteria, and fungi damage food crops and stored food grains resulting in yield and quality losses, and often makes them unfit for human consumption. Herbal pesticides that control these pests are becoming popular globally. Leaf essential oil deterred oviposition and inhibited adult emergence of cowpea bruchid, *Callosobruchus maculatus*, on cowpea seeds, thereby protecting them in storage (Priyani et al., 2003). Stored greengram and chickpea seeds were similarly protected from another species of cowpea bruchid, *Callosobruchus chinensis*, by essential oil fumigation at ≥340 ppm concentration (Namrata et al., 1997). The essential oil protected peas from seed-borne pathogens *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum* f. sp. *pisi*, *Macrophomina phaseolina*, and *Rhizoctonia solani* (Sharma et al., 2003) and controlled damping-off of tomato caused by *Pythium aphanidermatum* and *Pythium debaryanum* (Pandey and Dubey, 1994).

The leaf essential oil exhibited moderate-to-strong antifungal and fungitoxic activities against *Alternaria solani*, *Aspergillus fumigatus*, *A. niger*, *Candida albicans*, *Candida blanki*, *Candida cylindracea*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Cochliobolus miyabeanus*, *Helminthosporium* species, *Macrophomina phaseolina*, *Microsporum gypseum*, *Penicillium notatum*, *R. solani*, *Saccharomyces cervisiae*, and *Trichophyton rubrum* at different concentrations. It displayed moderate-to-strong antibacterial activity against *Bacillus subtilis*, *Cryptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pasteurella multicida*, *Proteus vulgaris*, *Pseudomonas aeruginosa*,

TABLE 1 Important Constituents (Highest Percentage) of the Essential Oils of Petiole, Leaf, Flower, Fruit, Fruit Pulp, and Seed of *Murraya koenigii*

Constituent	Petiole	Leaf	Flower	Unripe Fruit	Fruit Pulp	Seed
Essential oil	NA[a]	0.1–0.8 (Fresh wt.[b])	NA	1.25 (Dry wt.)	0.5–0.6 (Dry wt.)	1.2 (Dry wt.)
β-Thujene	–[c]	5.8	–	–	–	–
α-Pinene	24.2	71.4	9.1	48.1	21.8	51.6
Camphene	–	1.8	14.9	–	–	–
Sabinene	–	66.1	–	–	–	–
β-Pinene	6.9	70.0	–	10.1	4.6	12.6
Myrcene	–	3.2	–	5.9	3.3	7.3
α-Phellandrene	7.3	6.8	–	2.7	5.0	–
δ-3-Carene	–	54.2	–	–	–	–
α-Terpinene	–	4.5	–	–	–	–
Limonene	–	15.9	37.2	3.5	–	–
β-Phellandrene	–	50.2	3.7	23.1	–	–
(Z)-β-Ocimene	–	28.5	34.1	13.9	1.3	–
(E)-β-Ocimene	–	12.7	18.0	7.4	39.2	2.5
γ-Terpinene	–	7.4	6.7	3.0	5.5	9.3
(Z)-Linalool oxide	–	0.1	–	4.0	1.4	–
(E)-Linalool oxide	–	0.1	–	–	–	5.8
Linalool	–	4.3	8.0	–	–	1.2
Lavandulol	–	8.9	–	–	–	–
Terpinen-4-ol	–	9.9	–	8.0	–	–
Geraniol	–	11.4	–	–	–	–
α-Copaene	8.9	44.3	–	5.0	10.3	6.0
β-Elemene	–	7.1	–	–	–	–
Isocaryophyllene	–	12.1	–	–	1.2	–
γ-Elemene	–	10.1	–	–	–	–
β-Caryophyllene	–	53.9	24.2	8.5	1.8	1.1
β-Gurjunene	–	25.5	–	–	–	–
Aromadendrene	–	10.7	–	–	–	–
2-Phenylethyl benzoate	–	–	2.1	–	–	–
α-Humulene	–	26.7	5.1	–	–	–
Dehydroaromadendrene	–	–	–	8.0	–	–
β-Selinene	–	6.5	–	–	–	–
α-Selinene	–	10.4	2.2	–	–	–
Bicyclogermacrene	–	9.9	2.6	–	–	–
γ-Muurolene	–	9.1	–	–	–	–

TABLE 1 Important Constituents (Highest Percentage) of the Essential Oils of Petiole, Leaf, Flower, Fruit, Fruit Pulp, and Seed of *Murraya koenigii*—cont'd

Constituent	Petiole	Leaf	Flower	Unripe Fruit	Fruit Pulp	Seed
β-Bisabolene	–	9.8	–	–	–	–
δ-Cadinene	–	1.5	4.4	–	–	–
Caryophyllene oxide	–	6.4	–	10.3	–	–
Viridiflorol	–	5.5	–	–	–	–
α-Cadinol	–	7.3	–	7.3	–	–
Tridecanoic acid	–	0.3	–	8.2	–	–
(Z,E)-Farnesol	–	0.1	–	5.7	–	–
Ethyl palmitate	–	–	3.5	–	–	–
Palmitic acid	–	–	3.3	–	–	–
2-Naphthlenemethanol	–	4.8	–	–	–	–
Eudesmane	–	5.3	–	–	–	–

NA[a], not available; wt.[b], weight; –[c], absent or present in <1%, Compounds α-thujene, *p*-cymene, 1,8-cineole, α-terpinene, α-terpineol, α-caryophyllene, γ-cadinene, germacrene-D, bicycloelemene, spathulenol, *epi*-cubenol, humulene epoxide, β-cubebene, α-farnesene, 2-furyl methyl ketone, α-eudesmol, etc. represented <2.0%, as well as several other trace compounds are not listed.
Nigam and Purohit (1961), Wong and Chee (1996), Ramalakshmi et al. (2000), Walde et al. (2005, 2006) and Rajeswara Rao et al. (2011a,b).

Staphylococcus aureus, and *Streptococcus pneumoniae* at different concentrations (Goutam and Purohit, 1974; Kamble and Patil, 2008; Ray and Srivastava, 2008; Manvi and Renu, 2010; Ajay et al., 2011; Thilagavani et al., 2011). Complete inhibition of *Listeria innocua* growth was achieved with microwave and hydro-distilled essential oils at 400 and 600 µg/mL minimum inhibitory concentrations, respectively, signifying their potential as antimicrobial agents (Erkan et al., 2012).

The leaf essential oil displayed nematicidal activity against root-knot nematode *Meloidogyne incognita* (Pandey et al., 2008), larvicidal activity against malarial mosquito *Anopheles stephensi* (Srivastava et al., 2009), analgesic, antiinflammatory activities (Dash et al., 2004), and cytotoxicity against tumor cell lines MCF7 and P388 (Thilagavani et al., 2011). At 1000 ppm concentration it controlled *Epidermatophyton floccosum* and *Microsporum gypseum* that cause dermatomycosis (Tiwari et al., 2003).

As leaf essential oil can be extracted by solvents, the leaf solvent extracts contain essential oil and other compounds. Antioxidant, antibacterial, antifungal, and insecticidal activities displayed by leaf solvent extracts are briefly described. The leaf extracts exhibited significant antioxidant activity (Harish et al., 2012; Vandana et al., 2012). Leaf extract fumigation containing volatile essential oil constituents protected stored maize seeds from rice weevil *Sitophilus oryzae*, red flour beetle *Tribolium castaneum*, and saprophytic, pathogenic, aflatoxin producing fungus *Aspergillus flavus*. Bacterial contamination of Malaysian yellow alkaline noodles with *Bacillus pumilus*, *Clavibacter agropyri*, *Corynebacterium urealyticum*, *Corynebacterium jeikeium*, *Cryptococcus neoformans*, *Enterobacter cloaceae*, *Ps. aeruginosa*, *Serratia marcescens*, and *Staphylococcus sciuri* was controlled by ethanol extract. Methanol extract protected fish from *Streptococcus agalactiae* at 0.39 mg/mL concentration. The solvent extracts were effective against *Bacillus cereus*, *B. subtilis*, *E. coli*, *K. pneumoniae*, *Micrococcus luteus*, *Ps. aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*. Several stored spices examined for *A. flavus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Trichothecium* species revealed minimum contamination in curry bush signifying its potential as an antifungal agent. The leaf extracts displayed antifungal activity against *Penicillium italicum* causing blue mould-rot of mandarin oranges, *Cercosporidium pesonatum* causing tikka disease of groundnut, food spoilage, aflatoxin producing species of *A. flavus*, *A. niger*, *Aspergillus parasiticus*, and other fungi *C. albicans*, *Candida kefyr*, *Candida parapsilosis*, *C. tropicalis*, *Colletotrichum falcatum*, *Pe. notatum*, and *Rhizoctonia solani* (Saxena et al., 2002; Pramila and Dubey, 2003; Sharma et al., 2009; Ajay et al., 2011; Harish et al., 2012; Vandana et al., 2012).

TABLE 2 Chemical Composition (%) of the Essential Oils of Fresh Leaf, Dry Leaf, Tender Stem with Rachis, and Mature Fruit of *Murraya koenigii* Grown in Hyderabad, India

Constituent	Fresh Leaf	Dry Leaf	Tender Stem	Mature Fruit
Essential oil (fresh weight %)	0.20	0.18	0.02	0.13
(Z)-3-Hexenol	–[a]	0.1	–	–
α-Thujene	–	0.1	–	–
α-Pinene	2.1	1.7	–	19.0
Sabinene	0.4	0.1	–	4.6
β-Pinene	0.1	0.1	–	2.0
Myrcene	2.5	2.4	–	–
α-Phellandrene	1.2	3.9	–	–
p-Cymene	3.0	1.8	0.1	15.9
β-Phellandrene	46.5	52.7	0.2	18.4
(Z)-β-Ocimene	3.5	2.2	–	–
(E)-β-Ocimene	–	0.1	–	–
(Z)-Linalool oxide	0.2	0.1	–	2.2
Terpinolene	1.2	0.6	0.9	–
(Z)-*p*-Menth-2-en-1-ol	0.9	1.0	0.2	–
(E)-*p*-Menth-2-en-1-ol	0.1	–	–	–
(Z)-*p*-Menth-2,8-dien-1-ol	0.5	0.6	0.2	–
(Z)-β-Terpineol	–	–	0.2	–
Isopulegol	0.6	0.5	–	–
Borneol	0.2	0.1	–	23.3
Lavandulol	4.3	0.8	4.1	2.6
Terpinen-4-ol	0.5	0.4	3.1	–
p-Cymene-8-ol	1.2	1.1	2.6	–
Myrtenal	–	–	0.5	–
α-Terpineol	0.2	0.2	–	–
(Z)-Piperitol	0.2	0.2	–	–
(E)-Piperitol	0.2	0.3	0.1	–
Nerol	0.1	0.1	0.6	4.5
Citronellol	0.3	0.2	2.8	–
Neral	–	–	0.8	–
Piperitone	0.1	0.1	–	–
Geraniol	–	–	0.8	–
Geranial	–	–	3.2	–
Linalyl acetate	–	–	2.6	–
Unknown compound	0.2	0.1	0.1	4.2
Bornyl acetate	0.5	0.4	0.2	–
Unknown compound	–	0.1	19.8	–

TABLE 2 Chemical Composition (%) of the Essential Oils of Fresh Leaf, Dry Leaf, Tender Stem with Rachis, and Mature Fruit of *Murraya koenigii* Grown in Hyderabad, India—cont'd

Constituent	Fresh Leaf	Dry Leaf	Tender Stem	Mature Fruit
Citronellyl acetate	0.4	0.1	3.1	–
Neryl acetate	0.2	0.1	1.5	–
α-Cubebene	–	–	4.0	–
Geranyl acetate	0.2	0.1	7.0	–
α-Copaene	0.1	0.1	–	–
β-Elemene	0.8	0.1	2.1	–
α-Caryophyllene	0.3	0.1	1.4	–
α-Gurjunene	–	–	0.2	–
β-Caryophyllene	9.9	15.3	3.3	–
Selina-3,6-diene	0.2	0.1	0.7	–
β-Gurjunene	0.1	0.1	–	–
Aromadendrene	–	0.1	0.1	–
α-Humulene	2.4	3.2	0.8	–
γ-Gurjunene	0.1	0.1	–	–
γ-Muurolene	0.2	0.1	1.2	–
Germacrene-D	1.1	0.8	0.3	–
(Z)-β-Guaiene	–	0.1	–	–
α-Selinene	1.6	1.4	0.5	–
α-Muurolene	0.1	–	0.4	–
γ-Cadinene	0.1	0.1	–	–
δ-Cadinene	0.1	0.2	0.3	–
Cada-1,4-diene	–	–	1.2	–
Elemol	0.4	0.3	0.2	–
Spathulenol	0.5	0.3	–	–
Caryophyllene oxide	2.9	1.7	1.4	–
Humulene epoxide-I	0.5	0.3	0.3	–
Humulene epoxide-II	0.5	0.3	0.2	–
1,10-di-*epi*-Cubenol	0.1	–	–	–
γ-Eudesmol	0.3	0.2	0.2	–
τ-Cadinol	0.2	0.1	0.2	–
α-Muurolol	0.1	0.1	–	–
Selina-11-en-4α-ol	1.4	0.8	1.0	–
α-Cadinol	–	–	0.6	–
β-Bisabolol	0.2	0.1	0.4	–
Phytol	0.1	0.3	0.2	–

–[a], not detected.

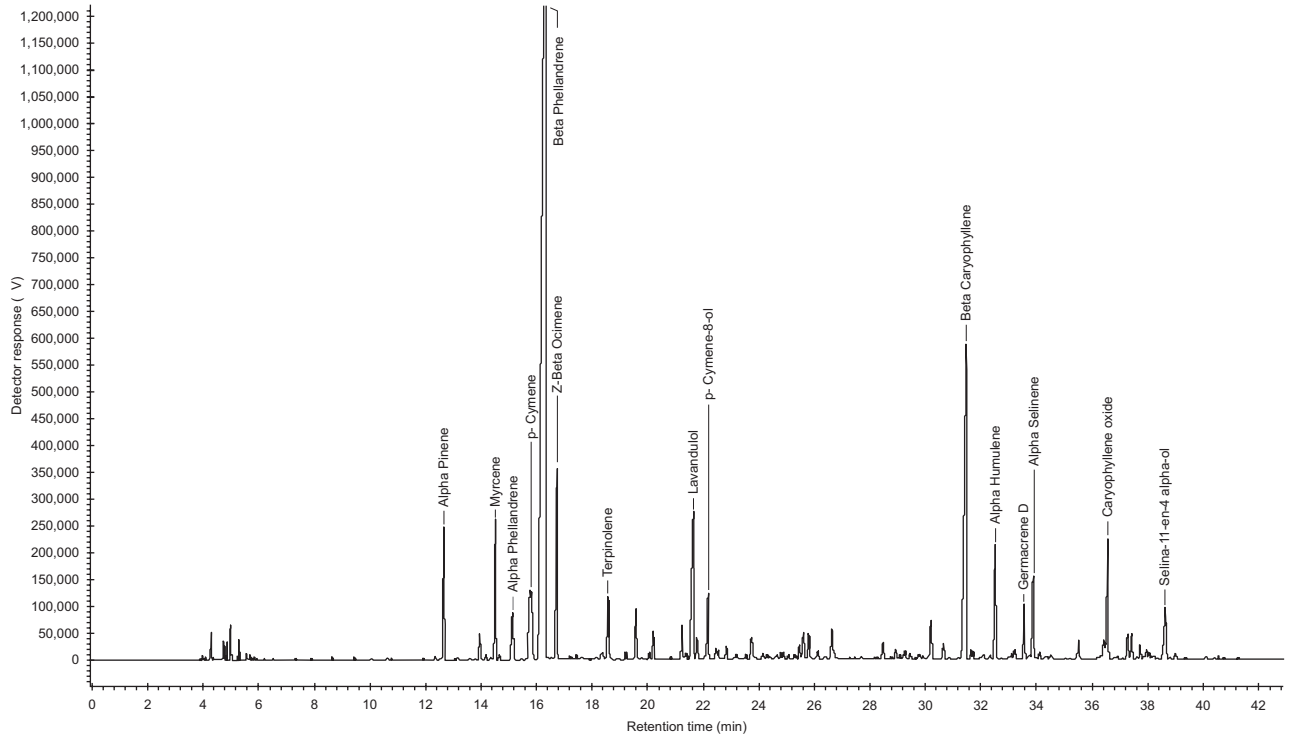

FIGURE 2 Gas chromatogram of *Murraya koenigii* leaf essential oil produced in Hyderabad, India. X-axis: Retention time (min). Y-axis: Detector response (μV). Body of the chromatogram: Identified constituents' names of important chromatographic peaks from left to right: α-Pinene, Myrcene, α-Phellandrene, *p*-Cymene, β-Phellandrene, Z-β-Ocimene, Terpinolene, Lavandulol, *p*-Cymene-8-ol, β-Caryophyllene, α-Humulene, Germacrene D, α-Selinene, Caryophyllene oxide, Selina-11-en-4 α-ol.

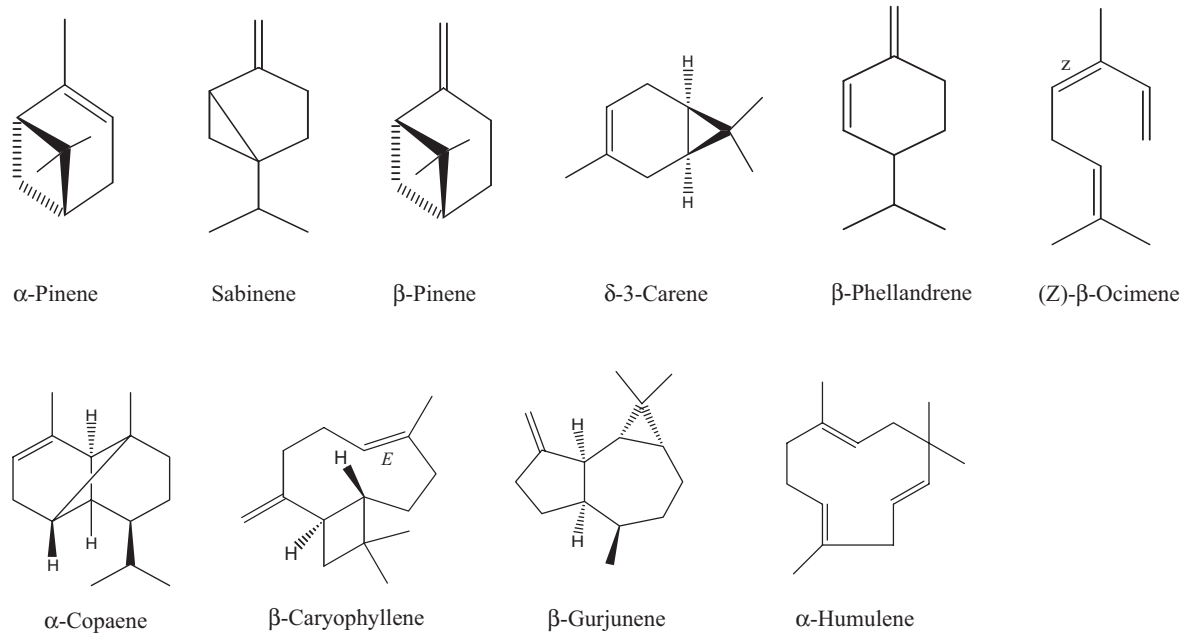

FIGURE 3 Structures of the important compounds of *Murraya koenigii* leaf essential oil. Names of individual compounds given below each structure from left to right: First row: α-Pinene, Sabinene, β-Pinene, δ-3-Carene, β-Phellandrene, (Z)-β-Ocimene. Second row: α-Copaene, β-Caryophyllene, β-Gurjunene, α-Humulene.

SUMMARY POINTS

- Curry leaf is a multipurpose, tropical tree primarily grown for its leaves and leaf essential oil, although all other parts have diversified uses.
- The leaves, flowers, fruits, fruit pulp, and seeds yield strongly aromatic essential oils containing more than100 flavor constituents.
- Essential oils can be isolated from the plant parts by steam, hydro, or microwave distillations, or solvent and supercritical fluid extractions.
- Several chemotypes identified in the leaf essential oils offer opportunity for the food industry to select desirable chemotypes to satisfy consumer preferences.
- Leaf essential oil is used as flavoring and seasoning agents in daily food preparations, processed and canned vegetarian and nonvegetarian food products, and in the soap, cosmetics, and aromatherapy industries.
- Leaf essential oil exhibited antioxidant, antifungal, antibacterial, insecticidal, and pharmacological activities.
- Essential oils of the flowers, fruits, fruit pulp, and seeds have future potential as food flavoring and seasoning agents.
- Solvent extracts of the leaves containing essential oil displayed antioxidant, antifungal, antibacterial, and insecticidal properties.
- In spite of their multifarious uses, curry leaf products have not attracted the attention of global consumers and global food industry. Their use is limited to the producing countries and in countries where immigrants from the producing countries have settled.

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Chapter 44

Curry Plant (*Helichrysum* sp.) Oils

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INTRODUCTION

Madagascar's flora is one of the richest in the world and has many medicinal and aromatic plants. The essential oils that are extracted are products with high added value that could contribute to economic island development. Among these aromatic and medicinal plants, we will focus on the genus *Helichrysum*. Varietal differences occur worldwide, with more than 600 species (Bigovic et al., 2010).

The genus *Helichrysum* (from the Greek *helios*, meaning sun, and *chrysos*, meaning gold) belongs to the Asteraceae family which incudes many species rich in essential oils and aromatic compounds. The Asteraceae family, also known as the Compositae family, includes 1600–1700 genera and 24,000–30,000 species (Funk et al., 2005). All species are well distinguished by the arrangement of the florets and the fruit. Plants belonging to this family may appear on different forms, as it includes herbs, succulents, lianas, epiphytes, trees, or shrubs. They are located everywhere, except Antarctica (Funk et al., 2005).

The uses of several members of the Asteraceae family are in medicine, as ornaments, and for trade. Among species we can cite those of commercial impact as the food crops *Lactuca sativa* (lettuce), *Cichorium intybus* (chicory), *Cynarascolymus* (globe artichoke), *Smallanthus sonchifolius* (yacon), and *Helianthus tuberosus* (Jerusalem artichoke).

Seeds of some species are used for the production of cooking oil such as the seeds of *Helianthus annuus* (sunflower) and *Carthamus tinctorius* (safflower) (Gao et al., 2010). Figure 1 illustrates some flowering plants which belong to the Asteraceae family.

Several researchers have focused on the chemical composition of the essential oil of some species belonging to this family. These volatile compounds may be applied in edible, medicinal, and herbal plants which make them safe when used in food products. Essential oils and their constituents have been largely employed as flavoring agents in foods and much of them exhibited a large antimicrobial activity (Alzoreky and Nakahara, 2002).

Raala et al. (2011) studied the content and composition of the essential oils of five Asteraceae species from Estonia, namely *Chamomilla recutita*, *Chamomilla suaveolens*, *Matricaria perforate*, *Anthemistinctoria*, and *Leucanthemum vulgare*. The oil yields ranged from trace amounts up to 0.2%. The chemical composition was established using gas chromatography (GC)–flame ionization detector and GC–mass spectrometry (MS) methods. The identified compounds varied significantly from one species to another. The main constituents of the essential oils of the studied Asteraceae species were as follows: *C. recutita*: bisabolol oxide A (39.4%), bisabolone oxide A (13.9%), (Z)-En-yne-dicycloether (11.5%), bisabolol oxide B (9.9%), α-bisabolol (5.6%), and chamazulene (4.7%); *C. suaveolens*: (Z)-En-yne-dicycloether (37.2%), geranylisovalerate (22.9%), (E)-β-farnesene (15.6%); *Anthemistinctoria*: α-eudesmol (10.2%), γ-cadinol (8.7%), γ-cadinene (4.0%); *M. perforata*: (Z,Z)-matricaria ester (77.9%), (E)-β-farnesene (3.5%), matricaria ester isomer (3.5%), and matricaria lactone (3.0%); and *L. vulgare*: (E)-β-farnesene (7.3%), hexadecahydrocyclobuta[1,2:3,4]dicyclooctene (5.3%), decanoic acid (4.9%), and γ-eudesmol (4.5%).

BOTANICAL ASPECTS

The genus *Helichrysum* (Asteraceae family) is abundantly prevalent throughout the world (Europe, Africa, Australia, North America) and is represented by more than 600 species. One hundred and fifteen of them are located in Madagascar; most are endemic, and some are used in folk medicine. Essential oils and extracts are obtained from the whole plant or from several parts of the plant.

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FIGURE 1 Flowering plants (Asteraceae family) (Funk et al., 2005).

This chapter will study of essential oils of seven Malagasy species, namely *Helichrysum bracteiferum* (DC) H. Humbert, *Helichrysum gymnocephalum* (DC), H. Humbert, *Helichrysum selaginifolium* Vig. Humboldt, *Helichrysum hypnoides* (DC) Vig. Humboldt, *Helichrysum cordifolium* (DC), and *Helichrysum faradifani* Sc Ell. We will start with a botanical description of each one, then focus on the chemical composition of their essential oils. The botanical description and embranchment is summarized in Table 1. It is important to note that both species *H. bracteiferum* and *H. gymnocephalum* are often confused because of their great morphological similarity. They both share the same common name Rambiazina, but they can be differentiated according to the size of their leaves. The first, with small leaves is classified as the male and called *lahy*, while the second, with broader leaves, is classified as the female and called *vavy* (Rasoanaivo and De La Gorce, 1998).

TABLE 1 Botanical Description of *Helichrysum gymnocephalum*

The Reign	Plant
Class	Dicotyledons
Sub-class	Asteridae
Order	Asterales
Family	Asteraceae
Genus	*Helichrysum*
Species	*gymnocephalum*

***Helichrysum bracteiferum* (DC) H. Humbert**, vernacular name is Rambiazina, is a shrub of 1–3 m high, with ultimate branches covered with dense brown tawny tomentum formed with small fine hairs. Leaves are sessile, attenuate at the base, and small (20–30 × 4–5 mm). Flowers are among two or three, all contained in flower heads, and are all homogamous. The fruits, called Achenes, are papillose (Humbert, 1962). This species is found in several places of Madagascar, particularly the siliceous rock in the middle of the ericoid vegetation peaks in the massif of Tsaratanana (North), the massif of Andrangovalo (East Lake Alaotra), or the massif of Ankaratra (south of Antananarivo).

***Helichrysum selaginifolium* Vig. and Humboldt** is a branching shrub ericoid,1–1.5 m in height, or it is a suffrutescent plant of 10–60 cm in places subject to periodic fires. It has very slender twigs (ultimate branches of 1–1.5 mm in diameter), prepared, and tomentose. The leaves are narrowly deltoid-acuminate (long May to September, mm wide and 0.5 mm at the base). Heterogamous flower heads are very small and grouped in globular clusters of 10–20, many of which are female. Flowering lasts from August to October. The achenes are generally papillose.

***Helichrysum hypnoides* (DC) R. Vig. and H. Humboldt** is an extremely branching ericoid shrub, 10–80 cm tall, with thin but highly lignified stems (brittle) and covered with a fine tomentum greyish white cobweb blanc. The leaves are sessile, lanceolates, and very small (3–5 mm long and 0.5–1.5 mm wide at the base). Heterogamous flower heads are all or most solitary, or two or three on top of twigs or partially close in small terminal clusters of three to 15 and are arranged in heads (several are female). The petals are red or brownish in color, especially at the top. Flowering lasts from March to October. The achenes are papillose.

***Helichrysum faradifani* Sc. Ell.**, vernacular name *ahibalala* (grasshoppers's grass), is a shrub measuring up to 60 cm in height and is abundant on the whole island of Madagascar. It is particularly present in rocky or sandy places. This species is used in traditional medicine. Flowering lasts all year.

***Helichrysum cordifolium* DC**, common name Fotsiavadika Tsimanandra, is a very rower suffrutescent plant (0.5–1.2 m high and sometimes up to 2–3 m, supported on the bushes). It has juvenile branches covered with loose tomentum. There are about 15 flowers, one to five of which are female. Flowering lasts all year following localities. It is found in forest edges, in ravines, etc. It is located in the center of the island and there are also a few specimens in the Comoros (probably introduced as a medicinal plant).

We will next focus on the botanical description of four species growing in Greece, namely *Helichrysum orientale, Helichrysum heldreichii, Helichrysum italicum* subsp. Microphyllum, and *Helichrysum doeryeri.*

- ***Helichrysum orientale* (L.)** is a non aromatic species with flat leaves; 20–60 mm at base and 7–10 mm in diameter.
- ***Helichrysum heldreichii* Boiss** is rare and endemic to West Crete. This species grows on vertical cliffs. The leaves are linear and long (more than 30 mm).
- ***Helichrysum italicum* (Roth) G. Don fil** is a Mediterranean species. It is a shrub with yellow flowers, with a height of 50–70 cm. This species grows on dry cliffs and sandy soil. *H. italicum* consists of two subspecies: subsp. *microphyllum* (Willd.) Nyman (Sardinia, south of Corsica), and subsp. *Italicum* (Tuscany, Corsica). Some phenotypic differences occur between the subspecies, for example, the height, ramification type, and size of the leaves (Bianchini et al., 2003).
- ***Helichrysum italicum* (Roth) G. Don in Loudon ssp. *microphyllum* (Willd.) Nyman** is an aromatic shrub. The leaves are very small (10 mm in length) with revolute margins. This species grows on cliffs and in rocky places of the islands in the Mediterranean region (Roussis et al., 2000). This species is also located in Tuscan Archipelago Islands, in Italy.
- ***Helichrysum doeryeri* Rech. Fil** is rare and endemic to east Crete. This species grows on mountain cliffs. It is a perennial plant characterized by very short stems and lower leaves. The capitulars are usually large with white outer bracts and form small bundles. Roussis et al. (2000) published the first analysis of essential oils in the Greek *Helichrysum* species which is described in the section related to the chemical composition of *Helichrysum* essential oils (see Usage and Applications).

FIGURE 2 *Helichrysum italicum* essential oil in a clear glass vial.

In addition to Madagascar, Greece, and Italy, other *Helichrysum* species have been found, such as ***Helichrysum cymosum* (L.)**, an aromatic perennial herb with yellow flowers and characteristic odors, widely distributed in southern tropical Africa. This species is used in traditional medicine to treat respiratory ailments and wound infections (Van Vurrenet al., 2006).

Libya is rich in ***Helichrysum stoechas*** species, but there is not sufficient botanical information concerning this species.

In Iran, 19 species of the genus *Helichrysum* were found, eight of them were endemic ***Helichrysum oligocephalum DC***, spread over the entire country, and were most common in the west of Iran. As far as we know, there is only one report describing the phytochemical composition of the essential oil of *H. oligocephalum* (Ebrahim Sajjadi et al., 2009).

Usage and Applications

Several studies have reported that there is great phenotypic variability in different species of the genus *Helichrysum*. There are also the problems of intra- and interspecific variability, all leading to significant variation in chemical composition. Some studies have analyzed the chemical composition of various *Helichrysum* species located in several places in the world (Madagascar; Turkey; Italy, Libya, Greece, Iran). The obtained essential oils varied significantly.

This variability may be attributed to the climatic conditions, harvested time, location, and geographic conditions. Afoulous et al. (2011) used *gymnocephalum* leaves harvested in July 2008; however, this plant was harvested in March 1997 by Möllenbeck et al. (1997) and during November–December 1994 by Cavalli et al. (2001). Therefore, it is important to note that despite the significant quantitative changes in *H. gymnocephalum* essential oil composition, 1,8-cineole remains the major component of *Helichrysum* essential oil. Roussis et al. (2000) showed that variability in the chemical constituents of the essential oil of *Helichrysum* species was detected according to the period of anthesis (before and after anthesis). Figure 2 shows a picture of a vial of *H. italicum* essential oil.

1,8-cineol has known antimicrobial properties (Pattnaik et al., 1997) and may possibly contribute to the antimicrobial effect of the *H. cymosum* oil. Table 2 shows the major components found in the different tested essential oils.

As we mentioned above, essential oils obtained from aerial parts of various *Helichrysum* species present a mixture of volatile compounds. Their chemical profiles vary with seasons, geographical conditions, plant parts used, the harvesting time, and even method of isolation. These secondary metabolites are known by their biological activities. In fact, many studies focused on the ability of essential oils to inhibit the development of food spoilage organisms, especially food-borne

TABLE 2 Major Compounds of *Helichrysum* Essential Oil

Helichrysum Species and Localization	Major Compounds of *Helichrysum* Essential Oil	References
Helichrysum gymnocephalum (Madagascar)	1,8-cineole (17.2–14.6%), borneol (16.2–10.4%), (E)-caryophyllene (12.7–9.9%), β-pinene (8.2–6.4%), and eugenol (2.2–13.5%)	De Medici et al. (1992)
	1, 8-cineole (20.4–14.1%), 3-ethyl-2,5-dimethylhexan-1,3-diene (17.2–2, 8%), α-farnesene (12.7–1.8%), β-pinene (3.1–10, 6%), (E)-caryophyllene (1.2–9.5%) and α-humulene (0.6–13.2%)	Theron et al. (1994)
	1, 8-cineole (66.7%)	Möllenbeck et al. (1997)
	1, 8-cineole (59.7%)	Cavalli et al. (2001)
	1, 8-cineole (47.4%), bicyclosesquiphellandrene[a] (5.6%), γ-curcumene[a] (5.6%), α-amorphene[a] (5.1%), and bicyclogermacrene[a] (5%)	Afoulous et al. (2011)
Helichrysum cymosum (L.) Less. (Tanzania)	*Trans*-caryophyllene (27.02%), caryophyllene oxide (7.65%), *p*-cymene (7.55%), Δ-3-carene (6.84%), and α-fenchene (6.25%)	Bougatsos et al. (2004)
Helichrysum fulgidum (L.) (Tanzania)	Caryophyllene oxide (12.45%), β-pinene (8.72%), spathulenol (7.88%), t-muurolol (7.31%), β-bourbonene (7.11%) and camphor (5.35%)	Bougatsos et al. (2004)
Helichrysum selaginifolium (Madagascar)	β-pinene (38.2%), α-pinene (16.3%), caryophyllene (7.5%), 1,8-cineole (7.1%)	Cavalli et al. (2001)
Helichrysum bracteiferum (Madagascar)	1,8-Cineole (27.3%), β-pinene (11.9%), α-pinene (5.9%), α-humulene (10.1%), and (E)-caryophyllene (7.1%)	Cavalli et al. (2001)
Helichrysum cordifolium (Madagascar)	(E)-caryophyllene (55.6%)	Cavalli et al. (2001)
Helichrysum faradifani (Madagascar)	(E)-Caryophyllene (34%) and linalool (16.1%)	Cavalli et al. (2001)
Helichrysum hypnoides (Madagascar)	(E)-Caryophyllene (35%) and 1,8-cineole (13.4%)	Cavalli et al. (2001)
Helichrysum orientale (Greece)	Nonacosane (11.1%)	Roussis et al. (2000)
Helichrysum heldreichii (Greece)	(E)-caryophyllene (38.5%) and caryophyllene derivatives (a total of 21.5%)	Roussis et al. (2000)
Helichrysum italicum subsp. *microphyllum* (Greece)	β-selinene (17.2%) and γ-curcumene (13.7%)	Roussis et al. (2000)
Helichrysum doeryeri (Greece)	Four eudesmol isomers (31.4%)	Roussis et al. (2000)
Helichrysum cymosum (southern tropical Africa)	α-pinene (12.4%) and 1,8-cineole (20.4%)	Van Vurren et al. (2006)
Helichrysum cymosum (Tanzania)	*Trans*-caryophyllene (27.02%), caryophyllene oxide (7.65%), *p*-cymene (7.55%), *Δ*-3-carene (6.84%), and *α*-fenchene (6.25%).	Bougatsos et al. (2004)
Helichrysum stoechas (Libiya)	α-pinene (59%), limonene (16,7%), and α-bisabolol (9.6%)	Sobhy and El feky (2007)
Helichrysum oligocephalum (Iran)	Thymol (14.4%)	Ebrahim Sajjadi et al. (2009)
Helichrysum graveolens (Bieb.) (Turkey)	α-cubebene (10.5%), β-caryophyllene (9.4%), caryophyllene oxide (8.2%) and azulene-octahydro (7.5%)	Bagci et al. (2013)

[a]*Newly identified compounds.*

TABLE 3 The Application Fields of the Major Components of *Helichrysum* Essential Oils

Major Components	Application	References
1,8-cineole	Antimicrobial properties against *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus*, *Enterococcus faecalis* and aroma characteristics, such as fresh, herb, spice	Ayala-zavala et al. (2009)
	Antibacterial properties in vitro or in food models	Burt (2004)
	Antioxidant activities	Wang et al. (2008)
α-pinene	Antibacterial properties in vitro or in food models	Burt (2004)
	Antimicrobial agent against *Sarcina* spp.	Holley and Patel (2005)
	Antioxidant activity	Wang et al. (2008) and Dai et al. (2013)
	Antibacterial, antifungal, antiinflammatory, insecticidal, and antioxidant properties, also traditionally used as a flavoring agent and antimicrobial material in food	Tantaoui-Elaraki et al. (1993)
β-pinene	Antibacterial properties in vitro or in food models	Burt (2004)
	Antioxidant activities	Wang et al. (2008)
(E)-Caryophyllene	Antimicrobial and antioxidant activities	Mimika-Dukic et al. (2004)
Trans-caryophyllene	Antioxidant activity	Legault and Pichette (2007)
	Antibacterial activity	Rahman et al. (2008)
thymol	Antibacterial properties in vitro or in food models with an important minimal inhibitory concentration against pathogens such as *Escherichia coli*, *Salmonella typhimurium*, *S. aureus*, *Listeria monocytogenes*, and *B. cereus*	Burt (2004)
	Antimicrobial properties against *B. cereus*, *Clostridium botulinum*, *E. faecalis*, *E. coli*, *S. aureus*, *L. monocytogenes*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium corylophilum*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus*, *Salmonella* sp., and aroma characteristics such as spice, citrus, and wood	Ayala-zavala et al. (2009)

pathogens (Burt, 2004). Nowadays, there is a great interest in natural products to provide solutions for food protection against microorganisms, agriculture products, preservation of raw materials, and crop products against any chemicals substances that are toxic and damage health (Ayala-Zavala et al., 2009).

To have great idea about some biological activities, we developed Table 3 which shows an overview of the fields of application of the major compounds found in *Helichrysum* essential oils.

Usage and Applications in Food Science

Species belonging to the genus *Helichrysum* have various preservative capacities, mainly antimicrobial and antiseptic effects on food products. Particularly, leaves of *H. gymnocephalum* have been used as tea (Boiteau and Allorge-Boiteau, 1993). Plants that belong to the genus *Helichrysum* are often used for food, and the leaves are cooked and eaten in Africa (Mathekga et al., 2000). Figure 3 shows a photo of *H. gymnocephalum*.

The species *H. italicum* is known as "immortal of Italy". This plant is very aromatic, and is used in food because of its flavour, close to curry. However, this plant is not used in curry, which is an Indian blend of spices, but its scent is very similar. Its leaves pleasantly perfumed for all kinds of food preparation. In fact, its aroma of curry marries well with south Asian culinary habitudes, and Middle Eastern grilled meats, fresh cheeses, salads.

Helichrysum essential oil does not tolerate long cooking. When used with potatoes, it gives a taste of the Maquis to the preparation. The essential oil aroma of several *Helichrysum* species goes well with garlic dressing. Also, fresh or dried

FIGURE 3 Photo of *Helichrysum gymnocephalum*.

leaves, as well as essential oil, can flavor many savory dishes such as rice, vegetables, poultry, meat, and fish and it goes well with marinades for the barbecue.

Mancini et al. (2011) used GC and GC–MS to study the chemical composition of the essential oil of *H. italicum* (Roth) Don subsp. *italicum* collected in the National Park of Cilento and Diano Valley, Southern Italy. Forty four compounds of 45 constituents were identified in the oil, and the oxygenated sesquiterpenes were preponderant. The essential oil was evaluated for its possible in vitro phytotoxic activity against germination and early radical elongation of radish and garden cress. The essential oil contributed to the significant inhibition of the radical elongation of radish when applied at the highest doses. This example elucidated the use of *H. italicum* (Roth) Don subsp. *Italicum* essential oil in agriculture.

Bougatos et al. (2004) investigated the chemical composition of two essential oils obtained from the aerial parts of *H. cymosum* and *Helichrysum fulgidum* from Tanzania using GC and GC–MS. Sixty-five compounds, representing 92.4% and 88.2%, respectively, of the two oils, were identified. *Trans*-caryophyllene, caryophyllene oxide, β-pinene, *p*-cymene, spathulenol, and β-bourbonene were found to be the main components. Moreover, the oils were tested against two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228), four Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC13883), and *Pseudomonas aeruginosa* (ATCC 227,853). Essential oils were also tested against the pathogenic fungi *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 13801), and *Candida glabrata* (ATCC 28838). Results showed that the oil of *H. fulgidum* exhibited significant antimicrobial activity, while the oil of *H. cymosum* did not exhibit any antimicrobial activity.

Afoulous et al. (2011) showed that the essential oil of *H. gymnocephalum* had poor antioxidant activity against two tests: 2,2-diphenyl-1-picrylhydrazyl (IC_{50} value >1000 mg/L) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($IC_{50} = 1487.67 \pm 47.70$ mg/L).

The antimicrobial activity of the essential oils of four *Helichrysum* species growing in Greece (*H. orientale, H. heldreichii, H. italicum* subsp. *Microphyllum*, and *H. doeryeri*) were investigated. *H. italicum* subsp. *microphyllum* exhibited the strongest antibacterial activity against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *Es.coli*, and *En.cloacae*. While only the oil of *H. oriental* significantly inhibited *Klebsiella pneumonia* (Roussis et al., 2000).

Van Vurren et al. (2006) studied the antimicrobial and toxicity activities of the essential oil of *H.cymosum.* The antimicrobial activity of the essential oil was determined using the *p*-iodonitrotetrazolium chloride (INT) microplate method, where the essential oil was tested against 10 pathogens. The result showed that the minimum inhibitory concentration ranged from 1 to 8 mg/mL. However, Bougatsos et al. (2004) reported that essential oil of *H. cymosum* was inactive against all pathogens tested (six bacteria and three pathogenic fungi).

Sobhy and EL Feky (2007) reported the antimicrobial activity of essential oil of *H. cymosum* against bacteria and fungi such as *S. aureus*, *S. epidermis*, *Es. coli*, and *C. albicans*.

SUMMARY POINTS

- *Helichrysum* essential oil is obtained from the different species of the genus *Helichrysum* which belongs to the Asteraceae family.
- The principal fields of use of *Helichrysum* essential oil are pharmacology, medicine, and the food industry.
- The chemical composition of *Helichrysum* essential oil varies according its geographical provenance, the period of essential oil isolation, and the parts of the plant used, harvested time, as well as the method of extraction.
- The main components of *Helichrysum* essential oil are 1,8-cineole, borneol, (E)-caryophyllene, β-pinene, eugenol, 3-ethyl-2,5-diméthylhexan-1,3-diene, α-farnesene, α-humulene, bicyclosesquiphellandrene, γ-curcumene, bicyclogermacrene, α-amorphene, *trans*-caryophyllene, caryophyllene oxide, *p*-cymene, spathulenol, *β*-bourbonene, camphor, α-pinene, linalool, and thymol.
- Biological activities such as antioxidant, antifungal, and antibacterial activity contribute to the application of *Helichrysum* essential oil in the food industry and agriculture.
- The antimicrobial activity of *Helichrysum* essential oil has been attributed to 1,8-cineol for both fungi and bacteria.
- *Helichrysum* has many food uses, such as in poultry and salads etc.

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Chapter 45

Dill (*Anethum graveolens* L.) Oils

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INTRODUCTION

Microorganism contamination and food spoilage during storage has been a major concern in the food industry. One of the major causes of food damage and contamination is some of the pathogenic fungi species. Production of the reactive oxygen species (ROS) in the lipid peroxidation of the lipid components in food is also another source of food deterioration. The rate of destruction increases with high temperature and high moisture content in a humid environment. To avoid such deterioration, synthetic preservatives have been used. However, using synthetic preservatives in foodstuffs for long periods may cause problems like carcinogenicity and other toxicities. Therefore, food industries are interested in using safe and natural preservatives like the essential oils of the plants in foodstuffs instead of synthetic preservatives, which is still usually used.

Volatile oils of plants and their major constituents may have antimicrobial, antifungal, and antioxidant activity. Therefore, they have the ability to control food contamination. Since most of the volatile oils extracted from plants generally have been recognized as safe compounds, they are a good source of natural and safe preservatives to use in the food industry.

Dill is a medicinal herb and has a pleasant spicy aroma. The essential oil seeds and leaves of dill are extensively used to flavor foods and beverages.

Studies shows that dill and its essential oil have antimicrobial, antifungal, and antioxidant activity, therefore they are suggested as natural and safe preservatives to replace synthetic ones.

BOTANICAL ASPECTS

Anethum graveolens L. (Syn. *Anethum sowa* Roxb. ex Flem., *Peucedanum graveolens* (L.) Benth. & Hook. f.) (Mozaffarian, 2007) and with common names of dill, dill seed, garden dill, dilly, dill weed, European dill, American dill, common dill, and Danish dill, is a member of the Apiaceae (Umbelliferae) family. The most common name of this plant "dill" refers to dilla, an Old Norse word meaning "to lull," which applies to the herb's calming effect on crying babies. The ancient Romans believed dill had strengthening properties; therefore, it was generously added to the gladiator's food (Small and Deutsch, 2001). Dill is an aromatic annual herb, and although it is native to the eastern Mediterranean region and western Asia, it has been widely cultivated throughout the world, with naturalization having occurred in some parts of Europe and North and South America.

A map of most of the areas in which dill is cultivated is shown in Figure 1. However, cultivation of dill is not restricted to these areas and it is likely to grow in more areas than those identified on the map.

Dill typically grows to 40–120 cm tall on cylindrical, thin, hollow stems, and has a slender, spindle-like taproot with few branches. The branched stems, clad with shiny, delicate blue-green leaves that are alternate, multipinnate (pinnately divided into fine thread-like segments). The filiform leaves are petioled 15–20 mm × 0.5 mm and leaf bases are sheathed up to 15 × 8 cm. The leaves have a distinctive slightly sharp, spicy, persistent flavor (Figure 2).

The numerous tiny yellowish flowers lack sepals and are arranged in compound terminal umbrella-like clusters (Figure 3), which may be up to 5–15 cm wide. The brown fruit (seed) that follows the flower is a double achene (or schizocarp), with mericarps that are oblong, laterally compressed and prominently ribbed 3.5–4 mm × 2–3 mm (Figure 4) (Mozaffarian, 2007).

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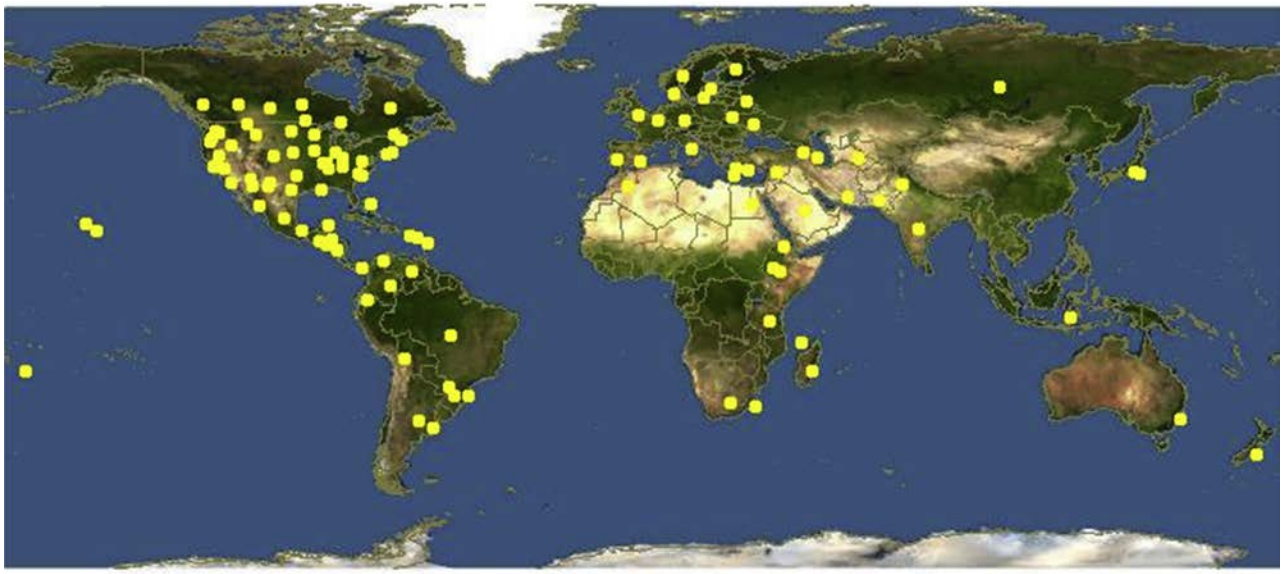

FIGURE 1 Map of most areas in which dill (*Anethum graveolens* L.) is cultivated. The cultivated areas are shown by yellow dots. *This figure was published on the Internet.*

FIGURE 2 The branched stems of *Anethum graveolens* L. *This figure was published on the Internet.*

FIGURE 3 The flowers of *Anethum graveolens* L. *This figure was published on the Internet.*

FIGURE 4 The fruit (seed) of *Anethum graveolens* L. *This figure was published on the Internet.*

TABLE 1 Main Chemical Constituents of the Essential Oil Derived from Dill (*Anethum graveolens* L.)

	Composition (%)				
Compound	(Osman et al.)	(Hassanzadeh et al.)[a]	(Singh et al.)[b]	(Mahmoodi, et al.)	(Sharopov, et al.)
α- terpinene	8.35	–	–	–	–
α- phellandrene	7.50	27.80	–	–	8.0
β- phellandrene	6.00	–	–	–	–
limonen	13.00	15.53	16.6	19.89	6.9
ρ-cymene	6.50	29.61	–	–	1.1
carvone	20.40	0.15	55.2	36.09	51.7
dihydrocarvone	12.00	–	–	–	–
Z-dihydrocarvone	–	–	2.6	6.59	1.6
E-dihydrocarvone	–	–	2.8	7.36	14.7
coumarin	6.50		–	–	–
thymol			–	6.5	–
dill apiole			14.4	16.83	0.4

[a]*Leaves.*
[b]*Seeds.*

All parts of this plant are aromatic, the fruits so more than the leaves. Yellow flowers bloom in midsummer (August to September) (Mozaffarian, 2007). Fruits (seeds) and leaves of this plant are the main parts that are used. Dill seeds have a warm, pungent, sharp, tangy flavor and a fragrance that resembles caraway. The main components of the aerial parts of the plant are the essential oil and phthalides. Seeds of the plant also contain fixed oil as well as furanocoumarins and hydroxycoumarins (Anonymous, 2007).

There are many reports about the composition of the essential oils extracted from leaves, flowers, and seeds of *A. graveolens*. The major components of the oil are reported to be carvone, limonene, dihydrocarvone, carvacrol, *p*-cymen, α-phellandrene and dill apiole Table 1 (Anonymous, 2007; Mahmoodi et al., 2012; Small and Deutsch, 2001; Hassanzadeh et al., 2000; Sharopov et al., 2013; Singh et al., 2005). The results obtained from different studies are not entirely corresponding to each other. These variations in the essential oil components of the plant are not unusual and are due to factors and conditions such as seasons, climate, different areas of each country, time of collection, etc. (Asili et al., 2008; Emami et al., 2007).

USAGE AND APPLICATIONS

Dill is a medicinal herb that has been used for more than 2000 years. The fruits (seeds) are carminative, stomachic, digestive, and tranquilizing, and have been traditionally used for treating stomach ailments, colic, hiccups, bad breath, flatulence, and hemorrhoids.

Although in different parts of the world dill seeds are still being used for these purposes, because of the lack of scientific proof for this plant's medical effectiveness it has not been accepted in modern Western medicine. Dill seeds' volatile oil is an important part of gripe water, which is a mixture used to treat infants' hiccups and colic. It also has galactogogue properties and promotes milk flow in nursing mothers. It has been reported that chewing dill seeds clears up bad breath or halitosis (Anonymous, 2007; Hoffman, 1996; Small and Deutsch, 2001; Volak and Stodola, 1998). Dill seed oil is active against harmful intestinal bacteria including *Escherichia coli* (Vokk et al., 2011) and against paramecia and helminthes (Anonymous, 2007). Dill seed preparations applied externally in poultices and compresses can also be used to treat bruises, blocked milk ducts and overloaded breasts in women who are breast feeding, and gum infections. The leaves can also be used for the same purposes. Dill essential oil rubbed onto the skin can stop feelings of nausea (Hoffman, 1996; Volak and Stodola, 1998). Being rich in vitamin C can explains why dill was once used to treat scurvy, a disease caused by a lack of vitamin C. It is an herb that has been used in Indian herbal and Ayurvedic medicine for its digestive properties. Specific uses are for fever, jaundice, and intestinal and genitourinary tract infections; it has also been used in rheumatic affections and other swelling of joints (external and in Charaka prescription together with paste of linseed and castor oil and milk) as an anti-inflammatory and analgesic agent. Dill herb is used for prevention and treatment of diseases and disorders of the gastrointestinal tract, kidney and urinary tract, for spasms and sleep disorders (Anonymous, 2007; Khare, 2004). Dill seeds with cotton seed kernel and cumin have also been used as a galactogogue (Khare, 2004). German Commission E has accepted the herb's efficacy in treating loss of appetite, liver and gallbladder complaints, inflammation of the pharynx, common cold cough, bronchitis, fevers, and colds (Khare, 2004).

Due to the pleasant spicy aroma of dill, this plant is widely used in food and the food industry in order to flavor foods and beverages. Seeds and leaves of dill are essential for flavoring and seasoning of salads, dips, pickled vegetables, seafood dishes, soups, sour cream, meats, sauces, and cream cheese (Small and Deutsch, 2001). The food industry uses the essential oil, extracted from both dill leaves and seeds, in candies, pickles, chewing gums, etc. as flavoring agent (Shyu et al., 2009; Tian et al., 2012).

As mentioned previously, studies have shown that dill essential oils have significant antifungal effect during the storage of foods. Although there must be further research to standardize the safety of this oil, it is clear that using this oil as antifungal agent increases food safety. Its use is also economical in comparison to the use of commercial compounds.

USAGE AND APPLICATIONS IN FOOD SCIENCE

In food industry, spoilage and microorganism contamination during storage is a major concern. Some of the pathogenic fungi species are mainly responsible for the spoilage of foodstuffs as well as damage to vegetables and fresh fruits, especially when there are high temperatures and high moisture content in a humid environment.

In addition, production of the ROS in the lipid peroxidation of the lipid components in food can lead to deterioration of cells. These processes may be a serious risk for the consumers using these food products. Synthetic preservatives have been used to prevent these processes. However, degradation of synthetic preservatives over a long period of time and also their carcinogenicity and other toxicities can lead to some health and environmental problems. The need to avoid these health and environmental problems has provided considerable interest in developing natural compounds from botanical sources to replace synthetic compounds to be use as preservatives in order to delay the destruction of foods, vegetables, and fresh fruits in storage.

One of the natural agents known suitable for these purposes is the essential oils of the plants. Studies have shown that the essential oils of the plants and their major constituents may have antioxidant activity and/or may have the ability to control food contamination and can be used as natural agents for food preservation. Generally, most of the volatile oils extracted from plants are recognized as safe compounds.

Regarding the chemical composition and properties of dill, its essential oil can be a suitable natural agent to be considered as a food preservative and food additive in order to replace synthetic preservatives in the food industry.

Dill Oil as an Antimicrobial for Food Preservation

The essential oil of dill has been successfully used in China to reduce the rate of decay on cherry tomatoes. In this study, the essential oil obtained from the *A. graveolens* seeds has been employed to evaluate its in vitro and in vivo antifungal

activity (Tian et al., 2011). They used poisoned food technique against several fungi. The results obtained from both in vivo and in vitro experiments were promising. Significant antifungal activity for the essential oil of dill suggested that it is possible to improve food storage by avoiding food spoilage and improve the food safety Table 2. However, there is the need for further investigation to standardize the safety limit of this oil, but it is clear that using this oil as an antifungal agent increases food safety. In addition, it is also economical in comparison to the use of comparative commercial compounds (Tian et al., 2011).

Saviuc et al. examined the antifungal activity of the essential oil of dill. The antimicrobial activity of this volatile oil has been proven on biofilm-grown cells. Essential oil stability on microbial biofilm-related infections has been investigated. Nanoparticles prepared from modified Massart were tested. Results of the study showed strong antibiofilm activity when the nanosystem was used for the *A. graveolens* volatile oil stabilization (Saviuc et al., 2011).

Evaluation of antifungal activity of volatile oil obtained from *A. graveolens* has been investigated in several research studies. Tian et al. investigated the antifungal activity of this oil and the mechanism of action on *Aspergillus flavus*. Results indicated that the volatile oil extracted from dill affects the mitochondria and *A. flavus* plasma membrane. This activity of the oil resulted from its ability to change the plasma membrane's permeability. This result helps in understanding the way the volatile oil of dill acts as a natural antifungal agent (Tian et al., 2012).

TABLE 2 Minimum Inhibitory Concentration (MIC) of Dill (*Anethum graveolens* L.) Volatile Oils

Pathogens		MIC	Ref
Proteus vulgaris		2000	Osman et al. (2009)
Salmonella enterica		500	Osman et al. (2009)
Escherichia coli		2000	Osman et al. (2009)
E. coli	ATCC 35218	4	Orhan et al. (2012)
	Isolated strain ESBL+	64	Orhan et al. (2012)
Pseudomonas aeruginosa	ATCC 10145	2	Orhan et al. (2012)
	Isolated strain	32	Orhan et al. (2012)
Proteus mirabilis	ATCC 7002	4	Orhan et al. (2012)
	Isolated strain ESBL+	64	Orhan et al. (2012)
Klebsiella pneumoniae	RSKK 574	8	Orhan et al. (2012)
	Isolated strain ESBL+	128	Orhan et al. (2012)
Acinetobacter baumannii	RSKK 02026	8	Orhan et al. (2012)
	Isolated strain	64	Orhan et al. (2012)
Staphylococcus aureus	ATCC 25923	1	Orhan et al. (2012)
	Isolated strain MRSA	>128	Orhan et al. (2012)
Enterococcus faecalis	ATCC 29212	1	Orhan et al. (2012)
	Isolated strain	128	Orhan et al. (2012)
Bacillus subtilis	ATCC 6633	1	Orhan et al. (2012)
	Isolated strain	4	Orhan et al. (2012)
Candida albicans		16	Orhan et al. (2012)
Candida parapsilosis		8	Orhan et al. (2012)
E. coli		8	Badar et al. (2008)
B. subtilis		9	Badar et al. (2008)
S. aureus		8	Badar et al. (2008)
Salmonella typhi		7	Badar et al. (2008)

Badar et al. examined the composition and antimicrobial effect of the volatile oil extracted by steam distillation from seeds of dill. In this study, the activity of the oil was tested on *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, and *Salmonella typhi* using disk method (Badar et al., 2008). Results indicated that the volatile oil of dill seeds has antimicrobial effect when compared to standard compounds like penicillin G, chloramphenicol, oxytetracycline, and gentamicin (Badar et al., 2008).

In Egypt, the antimicrobial activities of the essential oil of dill and also the mixture of the dill essential oil with the essential oils obtained from other plants have been studied using whole plate diffusion method. They compared their results with an antibiotic (Velosef, a brand name of cephradine). Depending on the oil's chemical composition, they showed different levels of antimicrobial effects. This activity of the oil can lead to the application of this oil by itself or in mixtures with other oils in food industry in order to reduce food contamination. It is important to mention that the estimated cost for treating food-borne diseases caused by food contamination is about 500 million dollars per year (Osman et al., 2009), so prevention of food contamination would be a major benefit to society.

The antimicrobial and antifungal activity of the essential oil extracted from *A. graveolens* was studied using different procedures. The results showed the effectiveness of this volatile oil against four different Gram-positive bacteria and three different fungi. The antimicrobial and antifungal activity of the *A. graveolens* essential oil suggested the possibility of using this plant oil as a source of natural compounds that could extend packaged foodstuffs' shelf life again several Gram-positive, Gram-negative bacteria and fungi (Kaur and Arora, 2010; Lopez et al., 2005; Zeng et al., 2011).

It has been suggested that the aromatic plants as well as their extracts having antimicrobial activity can affect the growth of mycotoxicogenic fungals. A common microorganism that spoils cheese is *Penicillium verrucosum*. Studies have shown that in order to prevent the growth of some fungal spoilers, the essential oil of certain aromatic plants including dill essential oil can be used as natural covers on cheese. Therefore, it is possible to control the growth of this microorganism in cheese by using a natural cheese cover of particular aromatic plants. Moro et al. evaluated the effect of *A. graveolens* volatile oil on *P. verrucosum*. They concluded that it is possible to avoid growth of mycotoxicogenic fungals and inhibit the cheese spoilage by using dill as a natural cheese cover. Such findings can help the food industry replace harmful commercial synthetic compounds with harmless natural compounds from botanical sources (Moro et al., 2013).

Another effective application of the volatile oil of *A. graveolens* is using it to protect packages of wheat flour against the two species *Ephestia kuehniella* and *Tribolium castaneum* and against contact toxicity of their larvae. This work may lead to the extensive usage of the oil as a natural compound to potentially protect flour packages (Mikhaiel, 2011).

Dill Oil as Antioxidant

Since free radicals in food production can result in cell deterioration, it is important to use antioxidants in the food industry to prevent damage to tissues. Because synthetic antioxidants may cause environmental as well as health problems, they need to be replaced by natural compounds like essential oils. Studies have indicated that the *A. graveolens* leaf and seed essential oils possess good antioxidant activity and commercially have good potential to be used in food processing industries to replace synthetic antioxidants. Using this natural compound may provide the ability of controlling food contamination. Dadashpour et al. investigated the antioxidant properties of *A. graveolens* volatile oil. In their study, they measured various methods of antioxidant evaluation of ferrous-ion chelating assay, β-carotene bleaching activity, tyrosinase inhibition, superoxide anion radical scavenging, and nitric oxide radical scavenging. Results indicated that the *A. graveolens* essential oil has good antioxidant activity and suggest that it has a good potential to be used in food and cosmetic industries (Dadashpour et al., 2011).

Prakash et al. examined the antifungal and antioxidant activity of essential oils extracted from leaves and seeds of *A. graveolens*. They used these volatile oils' effect against antioxidant, fungi, and production of aflatoxin efficacy on stored groundnut samples of fungal contamination. The oils showed good antioxidant activity when two different methods of thin-layer chromatographic assay and spectrophotometric assay for DPPH free radical scavenging were used. Showing these high activities, they suggested that the essential oil extracted from dill seeds is a good candidate for use in the food industry (Prakash et al., 2012).

During the postharvest processing of foodstuffs, mold and mycotoxin contamination is one of the problems in food industry. This is especially important in countries with tropical and subtropical climates. Harmful effects of these compounds in plants and in animals system have been reported extensively. Due to the thermostable nature of aflatoxins, they can be found in foods even after being cooked. In addition, they have a role in the generation of ROS. These activities may affect the quality and the quantity of the food items. This can be an attractive subject for food industries to increase the shelf life of their food products if they apply encapsulation technology for the *A. graveolens* essential oil in order to obtain the most effective concatenation of this oil in the desired time during postharvest processing (Wagacha and Muthomi, 2008).

SUMMARY POINTS

- Dill (*Anethum graveolens*) is an important aromatic plant.
- Dill is used in food industry.
- Dill is an ancient medicinal plant.
- Dill essential oil and its extracts are used as flavoring agent.
- Dill essential oil has various pharmacological activities.
- Dill essential oil has antimicrobial activity.
- Dill essential oil has antioxidant activity.

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Chapter 46

Eucalyptus (*Eucalyptus citriodora* Hook., Myrtaceae) Oils

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Abbreviation

EC *Eucalyptus citriodora*

INTRODUCTION

The medicinal applications and uses of plants may vary in different human communities and cultures. The therapeutic effects of the plants are essentially functions and reflections of the secondary metabolites naturally embedded in the various organs. These metabolites include alkaloids, saponins, flavonoids, tannins, anthracene derivatives, coumarins, and essential or volatile oils. The latter confer the "essence" or the aromatic odor some plants exude when crushed or indented with fingers. The nature and chemical compositions of the volatile oils are different and they are naturally found in many plant families like poaceae, lamiaceae (also called labiatae), zingiberaceae, rutaceae, asteraceae (also called compositae), and myrtaceae to mention a few. *Syzigium aromaticum*, *Callistemon, Vitex*, and *Eucalyptus* species are some of the plants in the Myrtaceae family that have been observed to contain volatile or essential oils with medicinal applications in aromatherapy. This review focuses on *Eucalyptus* species, particularly *Eucalyptus citriodora* (EC) due to the high variations in the nature of the essential oils they produce and also due to wide applications of such oils in cosmetic and pharmaceutical areas. The review also highlights the probable application of the oil of *E. citriodora* in food sciences in view of remarkable reports of its outstanding antimicrobial and insect repellent properties. It is hoped that these applications can be harnessed to improve planting, field harvest, post-harvest, storage and processing, and consumption of agricultural products.

BOTANICAL DESCRIPTION OF *EUCALYPTUS*

Eucalyptus species are believed to have originated in Australia and Tasmania from where they got introduced to various parts of the world. The genus consists of many species (14–550 according to some authors). Generally, the young leaves are mostly opposite, cordate at the base, and stalkless, while the adult leaves are mostly alternate, stalked, and usually narrowly lanceolate. The calyx and corolla are modified to form a cap that falls off in one piece when the filaments carrying numerous stamens expand. The ovary is inferior and the fruit is usually a woody capsule (Keay, 1989).

The above characteristics are largely found in almost all the members of the genus. However, there are other physical parameters that are used to differentiate one species from another. For instance, *Eucalyptus camaldulensis* Dhnh, *Eucalyptus tereticorms* Hook, and *E. citriodora* Hook may be up to 12–20 m high with glabrous, thin, and flexible stems. They can be differentiated from one another by critical evaluation of the macroscopy of their juvenile, intermediate, and adult leaves. As for *E. camaldulensis*, the juvenile leaves are ovate to elliptic in shape, 13–25 cm long and 4–8 cm wide, while the intermediate leaves are widely to narrowly lanceolate, 10–30 cm long and 2–6 cm wide. The adult leaves are more or less narrowly lanceolate, 8–30 cm long and 1–2 cm wide. The petiole is channeled below and up to 1.5–2 cm long. However, the juvenile leaves of EC are ovate to broadly lanceolate, 14–21 cm long and 4–8 cm wide, while the intermediate ones are more or less narrowly lanceolate, 13–39 cm long and 2–5 cm wide. The adult or matured leaves are narrowly lanceolate 8–16 cm long and 0.5–2 cm wide. The petioles are flattened measuring 1.3–2 cm in length (Arbonnier, 2004). Figure 1 shows a stand of EC cultivated and maintained in the University of Benin, Benin City, Nigeria.

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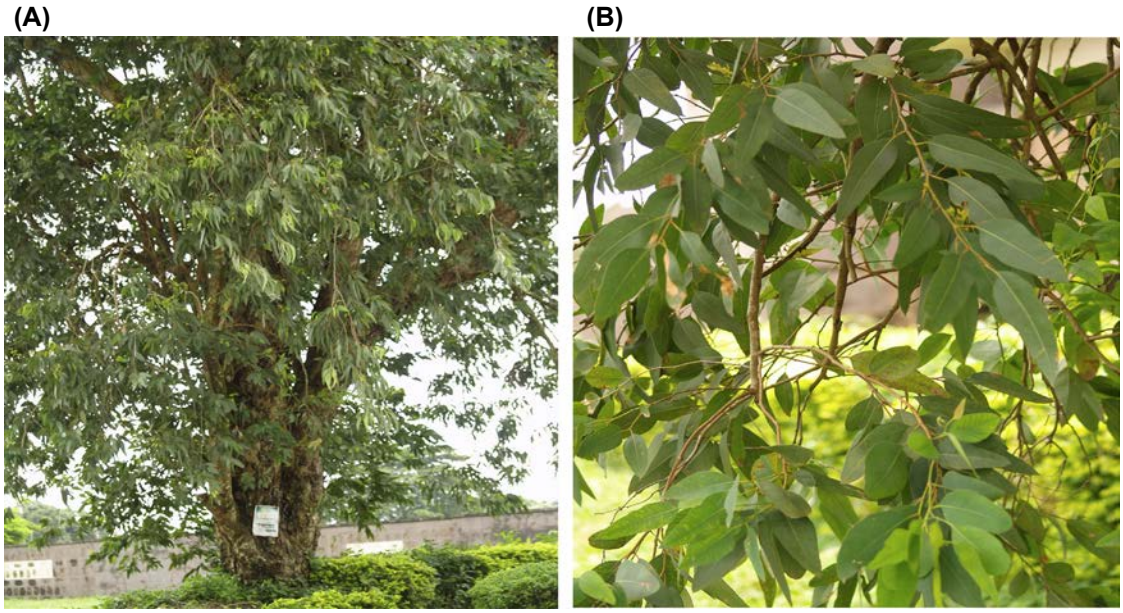

FIGURE 1 The picture of *Eucalyptus citriodora* cultivated at the University of Benin, Ugbowo Campus, Benin City, Nigeria. (A) The whole plant growing on the field. (B) Part of the twigs carrying the leaves.

Like other *Eucalyptus* species, EC can be planted on any type of soil including temporarily flooded soils. It flowers at the beginning of the dry season and can blossom for a long part of the year depending on the age of the offshoot and the humidity (Arbonnier, 2004).

Propagation has been noted to favor use of seeds over vegetative method. The flowers are bisexual and can be self or cross-fertilized (Eldridge, 1976).

USAGE AND APPLICATIONS

The fresh leaves of EC are well known to contain and yield faint yellow or yellowish-brown lemon-scented aromatic essential oil with fresh citrus taste. Knowledge of the uses and applications of the volatile oil of EC seems to vary from one culture to another. Generally, these uses can be categorized as medicinal, perfumes, and industrial. The first category has to do with the use of the plant or its volatile oil products in the treatment of ailments or in the alleviation of disease conditions, some of which have been reported in scientific literature.

In Nigerian traditional medicine, particularly in the Ondo, Edo-Delta axis of the Niger Delta, *Eucalyptus globules*, which is closely related to EC, is used to treat a range of internal and external infections. It is used as a nasal decongestant and to relieve asthma (Gill, 1992). Arbonnie (2004) noted that the leaf oil of EC (like that of *E. camaldulensis*) is used as an expectorant to treat coughs, colds, and rhinitis. It is also useful for asthmatic patients, and the oil can be used as antiseptic. The gum is useful in treating diarrhea.

The volatile oil of EC has been reported to successfully inhibit the growth of some of both Gram-negative and Gram-positive bacteria like *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, as well as *Candida albicans* (Akin-Osanaiye et al., 2007; Elaissi et al., 2012; Mohammed et al., 2012). The activities were reported to be higher than that of *E. camaldulensis*. With these remarkable reports on the antimicrobial potency of the volatile oil against some dermal and enteric pathogenic organisms, the oil may find applications in the preparation of topical and oral antimicrobial agents with appropriate adjuvants to mitigate or completely remove the probable side effects on the skin and gastric mucosa.

In the area of analgesic and anti-inflammatory effects, the volatile oil of EC was reported to produce higher and significant analgesic and anti-inflammatory effects in mice and rats, respectively, more than the oils of *E. globules*, *Eucalyptus tereticornis*, and *Cympopogon citratus* (Silva et al., 2003; Gbenou et al., 2013). The volatile oil may be acting through the inhibition of the synthesis of lipoxygenase L-1, one of the enzymes involved in the production of mediators of inflammation with an IC_{50} of 72 µg/mL (Sahouo et al., 2003).

TABLE 1 Relative Percentage Composition of Leaf Essential Oils of Three *Eucalyptus* Species

Constituents	RI	*Eucalyptus staigeriana*	*Eucalyptus citriodora*	*Eucalyptus globulus*
α-Pinene	928	3.27	1.1	4.15
o-Cymene	1021	1.76	–	2.93
(+) Limonene	1025	28.82	–	8.16
1,8-Cineole	1029	5.39	0.8	83.89
A-Terpinolene	1081	9.4	–	–
(–) Isopulegol	1145	–	7.3	–
β-Citronellal	1149	0.8	71.77	–
Isopulegol	1155	–	4.3	–
β-Citronellol	1223	–	2.9	–
Z-Citral	1235	10.77	–	–
Trans-geraniol	1247	4.2	–	–
E-Citral	1265	14.16	–	–
Methyl geranate	1317	3.66	–	–
Geraniol acetate	1374	3.86	–	–
Total		86.09	88.17	99.13

Obtained from Maciel et al. (2010).

Inclusion of the volatile in topical anti-inflammatory drugs may be suggested. However, the preparation of such drugs can be achieved with the use of appropriate adjuvants in order to mitigate irritating effects that the absolute volatile may have on the skin.

From a chemical composition viewpoint, the volatile oil of EC has been reported to contain citronellal as the major constituent whose concentration varies from one season to another (Maciel et al., 2010; Gbenou et al., 2013; Bossou et al., 2013). The presence and concentration of the compound in the plant can assist in proper identification of the essential oil of *E. citriodora* in relation to other species (Table 1). The perfumery and medicinal uses of the oil may be due to the presence of this compound. For example, the anti-inflammatory effects have been attributed to the presence of citronellal, which significantly inhibited the carrageenan-induced rat edema and leukocyte migration at a dose of 200 mg/kg (Melo et al., 2011). Almost a decade earlier, Ramezani et al. (2002) reported the potency of citronellal in reducing drastically the mycelia growth of rice pathogens *Rhizoctonia solani* and *Helminthosporium oryzae*. The inhibitory effects were noted to be far higher than that of the volatile oil. A recent report by Manzoor et al. (2013) has indicated the presence of terpinen-4-ol in the essential oil of EC. The compound has been found to have insecticidal effects and has been reported to induce apoptosis in lung cancer cells both in vitro and in vivo (Zhi-qing et al., 2008; Wu et al., 2012).

APPLICATION IN FOOD SCIENCE

Vegetable materials often get deteriorated through infestation with fungi and bacteria. Some of these organisms attack the seeds or vegetative propagation materials before and after germination, at harvest and storage stages. Consequently, such microbial attacks threaten and compromise the physicochemical and nutritional integrity of the materials thereby reducing their economic value and sometimes completely rendering them unfit for human or animal consumption.

Since microbial contamination and deterioration cut across different stages ranging from planting, harvest, storage, to processing procedures, coupled with the fact that different species of microorganisms may be involved in each of the stages, it is highly essential to approach their control at each stage with specific methods using appropriate natural or synthetic pesticides.

In order to achieve the goal, it will be economically beneficial to use chemicals or natural products that have the capacity to prevent the proliferation of the target microorganisms and other associated pests without necessarily threatening the well-being of beneficial and harmless ones.

Apart from being absolutely safe to humans and nontarget organisms, such pesticides should be biodegradable, with wide-spectrum and prolonged bioactivity. In practice, there are many pesticides of synthetic origin, most of which are specifically used to inhibit categories of microorganisms. Some of them are extremely toxic and therefore require highly trained personnel for effective application. In addition to all these, some of them are highly expensive and out of reach to peasants. Considering all these facts, it becomes appropriate to advocate use of plant-based secondary metabolites, especially the volatile oils like that of EC, particularly in areas populated by low-income earners.

Effects of *Eucalyptus citriodora* Oil on Plant Pathogens

Apart from keeping the moisture content of seeds and vegetable products below 6% and adding aromatic fragrance to the environment, the volatile oil of EC can be employed to combat the various stages of pathogenic microbial infestation.

Before planting, the volatile oil of the plant can be used on the soil against pathogenic fungi and bacteria. Lee et al. (2007) showed that the oil effectively inhibited the growth of soil-borne pathogenic fungi like *Fusarium oxysporum*, *Pythium ultimum*, and *R. solani* Kuhn. *Fusarium oxysporum* and *P. ultimum* have been reported to be important pathogens in agriculture crops on which they cause serious diseases that result in reduced yield of many crops (Alhussaen, 2012). *Fusarium oxysporum* has been implicated in tomato (*Lycopersicon esculentum*) wilt and fruit and root rot in pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*) (Ignjatov et al., 2012; Amadi et al., 2012). *Rhizoctonia solani* has been implicated in the damping-off of sugar beets (*Beta vulgaris*) and carrots (*Daucus carota*) (Sadeghi et al., 2006; Grisham and Anderson, 1983). The volatile oil has also been reported to be effective in controlling soil pathogenic nematodes like Chitwood (*Meloidogyne incognita*) (Pandey et al., 2000). *Meloidogyne arenaria*, *Meloidogyne hapla*, *M. incognita*, and *Meloidogyne jaranica* have been associated with root-rot disease of spinach (*Spinacia oleracea*). They are the most common and damaging nematodes of spinach in several countries (Potter and Olthof, 1993).

Histoplasma capsulatum has been known to be the cause of histoplasmosis—a respiratory disease characterized by flu-like symptoms, including fever, cough, and headache in immunocompetent or resistant individuals. It has been noted by researchers that the infection may be worse in immunocompromised individuals. The fungus is found in the soil where it exists in mycelia form (Mihu and Nosanchuk, 2012). Luqman et al. (2008) demonstrated the remarkable fungicidal activities of EC against this dangerous fungus at a concentration of 2.5 mg/mL.

Finding an appropriate method of applying the volatile oil may seem challenging since the oil is not water soluble. However, this can be overcome by use of volatile organic solvents that can easily evaporate off without having any undesirable effect on the soil and its content.

Herbicidal Effects

Growth and subsequent yields of agricultural crops are also usually threatened by the presence of the weeds that in not a small way compete for space, nutrients, and light with desired plants. Mechanical removal of such weeds may be laborious and time-consuming. In addition, application of synthetic herbicides may be environmentally risky as some of them may form toxic residues on the soil thereby interrupting the ecosystem. In addition to these, they may be toxic to mammals and nontarget weeds or organisms, coupled with their costs and the need for proper training of the application personnel. The use of natural products like the volatile oil may therefore be appropriately useful. In this regard, the volatile oil of EC and its constituents have been reported to have remarkable growth inhibitory effects.

In a farmland dominated by weeds like *Cassia occidentalis* and *Echinochloa crus-galli* (grassy weed), their growth can be controlled with the oil and its major constituents citronellal and citronellol (Batish et al., 2006). The researchers further established that the oil significantly reduced the germination rate of *C. occidentalis* while the germination and root length of *E. crus-galli* were more inhibited by the citronellal and citronellol. These effects may be extended to other weeds and even desirable food plants like rice *Oryza sativa*.

Earlier, Kohli et al. (1988) reported the germination inhibitory activity of EC oil on *Panthenium hysterophorus* using fumigation method. The effects were also significantly shown on matured plants as they experienced pronounced retarded and stunted growth. It is important to state that the application of the volatile oil may be challenged by the problem of species specificity.

Storage

The wide-spectrum antimicrobial activities of the volatile oil of EC and its major constituents against bacteria and fungi can be utilized in the post-harvest handling, storage, and processing of food materials. The infection of stored food and agricultural products by fungi and bacteria can effectively be controlled by fumigating such an environment. Among the fungi that attack stored farm products are *Aspergillus* species, which produce toxins that are harmful to both man and livestock. Javed et al. (2012) noted the growth inhibitory potential of the volatile oil of EC against four strains of fungi, including *Aspergillus flavus* Link ex Gray, *Aspergillus fumigatus* Fres., *Aspergillus nidulans* Eidam ex Win, and *Aspergillus terreus* Thom.

Probable Applications of the *Eucalyptus citriodora* Oil in Food Processing

The destructive activities of microorganisms are not limited to plants. Other food products such as dairy foods obtained from cattle, meats obtained from poultry birds, cattle, pork, and aquaculture products like fish are sometimes prone to microbial organisms. The ecto- or endoparasitic infections suffered by live animals usually reduce the quality of meats and other products that are produced. Furthermore, packaged meats and grains are also not safe from the activities of spoilage and pathogenic microorganisms. The color, taste, and subsequently commercial, useful lipid content and nutritive value become threatened and compromised by the proliferation of microorganisms such as *Salmonella*, *E. coli*, *Aspergillus species*, *S. aureus*, *Bacillus cereus*, *Campylobacter*, and *Clostridium perfringens* (Singh et al., 2003; López-Malo et al., 2005; Rydlo et al., 2006). *Listeria monocytogenes*, *Aeromonas* species, and some of these pathogenic organisms have also been implicated in the spoilage and intoxication of fish (Novotny et al., 2004). As stated before, the volatile oil of EC has been demonstrated to effectively inhibit the growth of most of these microorganisms.

With the global campaign supporting the application of natural constituents from plants in the preservation of human consumables, it is imperative to explore the uses of plant-derived volatile oils to reduce microbiological degradation and spoilage occasioned by the proliferation of pathogenic microorganisms on semi-processed or raw food materials. This attempt may replace the already known and established methods such as heat treatment, salting, acidification, and drying that are currently used in the food industry (Davidson and Taylor, 2007).

Lucera et al. (2012) noted that the use of volatile oils from plants is gradually gaining a wide interest in the food industry for their potential as decontaminating agents, as they are "generally recognized as safe" (GRAS).

Incorporation of the volatile oil and its constituents into candies and soft drinks may add flavor and prolong the shelf life of such products by ensuring stability of the components through elimination of microbial growth. Consumption (by chewing) of such candies and chewing gums can reduce microbial load of the oral cavity. As some oral diseases have been associated with infestation by some oral bacteria (Ahonkhai et al., 2009), consumption of candies and chewing gums prepared with appropriate quantities of the volatile oil of EC may eventually reduce the proliferation of such organisms in the oral cavity and may also deodorize the mouth against halitosis, coupled with strengthening the gums. As the oral cavity serves as a major gateway to the internal organs, the influx of microorganisms into inner parts of the body can be effectively controlled with the consumption of EC oil-made products.

The swallowing of the saliva of the chewing or sweet candies may also be medicinally beneficial by reducing diarrhea and dysentery through the combination of the antimicrobial activities (stated earlier) and antispasmodic effects of the oil of EC and its constituents. This is because the volatile oil has been established to relax the rat ileum contracted by acetylcholine (Ayinde and Owolabi, 2012) probably by blocking the ileal muscarinic receptors (Figure 2).

However, an abuse of the ileal relaxant property may also induce constipation. In addition to this, the probable side effects of the oil and its components on blood pressure, blood sugar, the central nervous system, and uterus may limit the applications in the production of consumable materials.

In conclusion, the volatile oil of EC has many uses and applications in traditional medicines and pharmaceutical research results have validated some of them. These uses can be applied to improve the practice in agriculture and food sciences with a view to enhancing the final products for the safety of the end users.

SUMMARY POINTS

- Essential or volatile oils constitute a major group of secondary metabolites that are naturally found in some plants.
- Apart from macroscopical variations that exist in the plants, *Eucalyptus* species generally contain the essential oils whose compositions vary from one plant to another.
- The essential oil of *Eucalyptus citriodora* has been found useful as an antiseptic in the treatment of many microbial infections and as analgesic and anti-inflammatory agent.

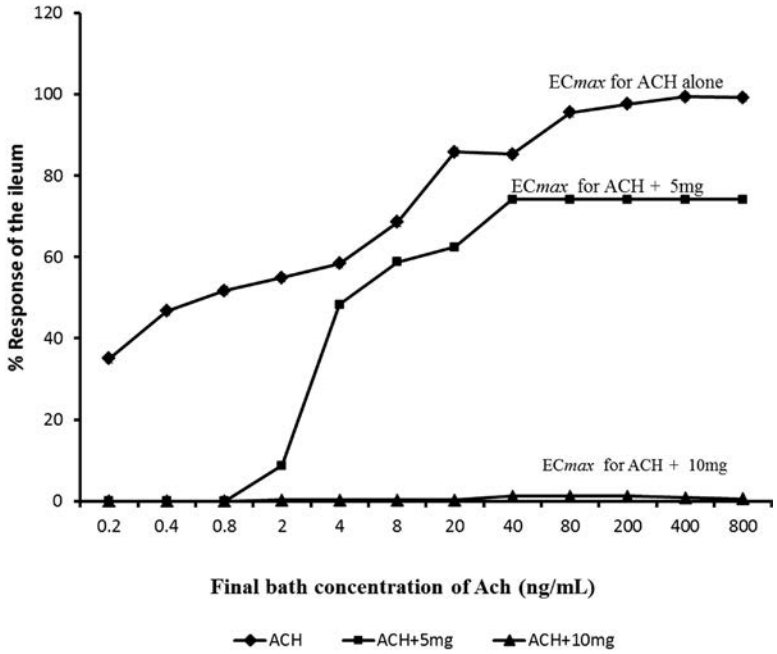

FIGURE 2 The relaxant effect of the volatile oil *Eucalyptus citridora* on rat ileum. *Obtained from Ayinde and Owolabi (2012).*

- The major component of the volatile oil of *E. citriodora* is citronellal, which may be responsible for the various uses of the oil.
- Microbial attack, deterioration, and spoilage cut across different stages of food production and each requires fast, cheap, safe, and effective control.
- The oil of *E. citriodora* can be found useful in treating soil against destructive fungi and bacteria. It can also be used in controlling weed growth.
- The oil can be used in controlling microbial deterioration and spoilage caused by fungi on stored and agricultural products.
- Wide-spectrum antimicrobial activities of the essential oil of EC can be explored in the control of various bacteria that attack and reduce the dietary and commercial value of dairy foods.
- The aromatic fragrance of the oil can be utilized to flavor candies and soft drinks.

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Chapter 47

Fenugreek (*Trigonella foenum-graecum* L.) Oils

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List of Abbreviations

FGHF Fenugreek hexane extract
FGMF Fenugreek methanol extract
GRAS Generally recognized as safe
MDR Multidrug resistant
MIC Minimum inhibitory concentration
ZDI Zone diameter of inhibition
SNP Silver nanoparticles synthesized with the help of *T. foenum-graecum* seed extract
TFSE *T. foenum-graecum* seed extract

INTRODUCTION

Fenugreek (Greek hay), also called methi in Bengali (*Trigonella foenum-graecum*; diploid with $2n = 16$)—an aromatic leguminous plant—is an important spice and has been commonly used as a traditional food and medicine; the dried seeds of the plant have extensive application in foods and beverages as a flavoring additive. The plant is native to many Asian, Middle Eastern, and European countries, and India being the leading producer of fenugreek is recognized as the major consumer for its culinary usage and medicinal application. In Ayurveda, both fenugreek seeds and leaves are used to prepare extracts or powders for medicinal use. The physicochemical characteristics of fenugreek seed oil such as acid value (4.75; mg KOH/g of oil), saponification value (195; mg KOH/g of oil), ester value of 190.25, free fatty acids content (2.38; Oleic acid/100 g oil), and the refractive index of 1.464 indicate fenugreek seed oil as an edible oil, if its characteristic pungent odor is minimized (Sulieman et al., 2008). The fenugreek protein fraction is found to be lysine-rich and comparable in quality to that of soybean protein, and hence seeds are used as food and offer a superlative nutritional profile.

BOTANICAL ASPECTS

Fenugreek, also called Greek hay, belongs to the subfamily Papilionaceae of the family Leguminosae (bean family, Fabaceae). Fenugreek is cultivated all over the world as a semiarid crop. The yields can be significantly augmented in quantity and quality through suitable management of cultivation, irrigation, harvesting, and pest management. Fenugreek is a self-pollinating annual leguminous bean, and seeds sown in well-prepared soil sprout in 3 days; the plants mature in about 4 months and attain a height of 30–60 cm. In this plant, flowering starts after 30–40 days of sawing. The plant has compound pinnate, trifoliate leaves, white to yellow axillary flowers, and 10–15-cm-long thin pointed hoop-like beaked pods (Meghwal and Goswami, 2012) each carrying 10–20 seeds (Figure 1). *Trigonella foenum-graecum* seeds are small (5 mm long), hard, yellowish brown and angular, and are flattened with a characteristic oblong rhomboidal outline (Moradi kor and Moradi, 2013; Mullaicharam et al., 2013). The seed holds a central hard yellow embryo bounded by a large corneous white and semi-transparent endosperm (Betty, 2008).

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FIGURE 1 Water-soaked methi, *Trigonella foenum-graecum*, seeds (processed by the second author and photographed by the first author).

USAGE AND APPLICATIONS

Trigonella foenum-graecum seeds and green leaves, which are used in food as well as in medicinal application, is an old practice of human history. The fresh green leaves of *T. foenum-graecum* consumed as vegetables, dried seeds are used as spice in food preparation, and both leaves and seeds are used in flavoring foods in many countries including India; the seed constitutes an essential part of Bengali five-spice mixture *panch foron* that makes the food tasty and provide attractive flavor during preparation. The *T. foenum-graecum* seed can be cooked or sprouted and can even be eaten raw; the whole seeds are taken as an antacid and against dysentery and stomach disturbances.

The *T. foenum-graecum* cold water extract, known as fenugreek tea, has been traditionally used against respiratory infections (bronchitis and pneumonia), and since it nourishes the body during illness, the herb has also been used to reduce fever, when taken with lemon and honey. *Trigonella foenum-graecum* has been shown to possess hypoglycemic, antihypertensive, and hypolipidemic activities (Micallef and Garg, 2009). Fenugreek also has a long history of use in the treatment of reproductive disorders, in inducing labor, treating hormonal disorders, increasing milk supply, and reducing menstrual pain. The plant has been known to have health potential with the ability to maintain blood glucose and cholesterol levels, and hence in the prevention and treatment of diabetes and heart disorders. *Trigonella foenum-graecum* has also been reported to exhibit pharmacological properties including antiviral, antimicrobial, hypotensive, antioxidant, anti-inflammatory, and antitumor activity (Mullaicharam et al., 2013). Al-Oqail et al. (2013) demonstrated a decrease in the cell viability of cancerous cells exposed to seed oil of fenugreek.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Trigonella foenum-graecum oil possesses a pungent odor with bitter taste and is used as an insect repellent agent in order to protect food grains. The seeds, as spice, are essential ingredients in food preparation, and because of the presence of characteristic pungent aromatic compounds the seeds add flavor and color to the foods, especially curries and pickles, making them attractive and tasty. Fenugreek oil is used in flavoring canned food and syrups, such as artificial maple syrup, which is flavored with fenugreek seed extract since *T. foenum-graecum* odor imitates the maple syrup smell. Due to the presence of galactomannan gum in fenugreek seed, its extracts are used as a thickening agent in foods and as a food emulsifier. The fenugreek essential oils and extracts, as natural agents, are found to be very important in preserving foods due to their excellent antioxidant, antibacterial, and antifungal activities, and also in preparing foods as spices discussed below, including their chemistry and nutritional requirements.

TABLE 1 Percentage of Carbohydrate, Protein, and Fat in Fenugreek Leaves and Seeds

Plant Parts	Carbohydrate	Protein	Fat	References
Seed	62.48	28.55	4	Sulieman et al. (2008)
Seed	45–60	20–30	5–10	Mehrafarin et al. (2010)
Seed	42.3	9.5	7.5	Hemavathy and Prabhakar (1989)
Seed	42.3	25.4	7.9	Meghwal and Goswami (2012)
Leaf	6.6	4.4	0.9–1	Meghwal and Goswami (2012), Snehlata and Payal (2012)
Seed	–	20–30	6.53	Sheikhlar (2013)
Seed	44	24.7	7.6	Abdel Aal et al. (1985), Shankaracharya and Nalarjan (1973)
Seed	58	23–26	6–7	Lu et al. (2008)

Nutritional Value

The edible part of *T. foenum-graecum* leaves contains moisture (86.1%), carbohydrate (6%), protein (4.4%), minerals (1.5%), fiber (1.1%), and fat (0.9%), while the contents of carbohydrate, protein, fat, fiber, and moisture in *T. foenum-graecum* seeds are 44.1, 26.2, 5.8, 7.2, and 13.7%, respectively (fenugreek oil: www.kazimaperfumers.com). *Trigonella foenum-graecum* seed is good source of protein, fat, and sugar; requirements of food types that can be obtained from *T. foenum-graecum* are represented in Table 1.

Fenugreek is a natural source of minerals (Ca, P, Fe, Zn, and Mn) and vitamins such as A, B1, C, and nicotinic acid (Moradi kor and Moradi, 2013). It has been reported that fresh 100 g fenugreek leaves contain ascorbic acid of 220.97 mg and β-carotene of 19 mg (Meghwal and Goswami, 2012), while Srinivasan (2006) reported vitamin C, β-carotene, thiamine, riboflavin, nicotinic acid, and folic acid contents as 52 mg, 2.3 mg, 40 µg, 310 µg, 800 µg, 0 (zero) µm in leaves, and 43 mg, 96 µg, 340 µg, 290 µg, 1.1 mg, 84 µg in seeds, respectively. According to the report of Shakuntala et al. (2011), germinated endosperm, ungerminated endosperm, germinated sprouts, germinated seed coat, and ungerminated seed coat contain 39.25, 48.20, 36.12, 10.51, and 7.35% of protein, respectively, and 11.44, 12.26, 6.65, 1.75, and 1.22% of fat, respectively.

Antispoilage Activity

Food-borne illness is an important public health problem, and outbreak of food-borne diseases is not uncommon (Sasidharan et al., 2011). Microorganisms, including different fungi and bacteria, cause spoilage of foods altering their color, texture, and odor, making them unfit for human consumption, such as souring of milk, fungal growth on bread, jam, and jelly, and rotting of fruits and vegetables. This type of spoilage is known as microbial spoilage of food. Food spoilage is also caused due to oxidation causing the destruction of vital biochemical compounds or the plant and animal cells or both. When oils and spices, including *T. foenum-graecum*, are added with salt and sugar to foods, they prevent growth of microorganisms in foods. *Trigonella foenum-graecum* has antibacterial, antifungal, and antioxidant properties and thus can effectively prevent the growth of microbial pathogens and help in food preservation. Thus, foodstuffs wrapped in the fenugreek-coated paper have their freshness preserved for long time because no pathogens such as bacteria and fungi can grow on their surface, and hence shelf life of the foods is protected. Hegazy (2011) reported that fenugreek seed flour when mixed into beef burger patties, instead of soybean flour, had effective antimicrobial properties and high antioxidant activity that help in preserving the food.

Antibacterial Activity

Trigonella foenum-graecum oil possesses broad-spectrum antibacterial property against bacteria involved in food spoilage as well as food poisoning, and bacteria related to food-borne diseases of humans.

The antibacterial activity of *T. foenum-graecum* seed oil (100%) has been determined in terms of zone diameter of inhibition (ZDI), and has been recorded as 10, 20, 15 mm for *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*, respectively (Sulieman et al., 2008). It has been reported that the essential oil of *T. foenum-graecum* showed strong inhibition against *S. aureus* and *Pseudomonas aeruginosa*, and the inhibition was found to be concentration

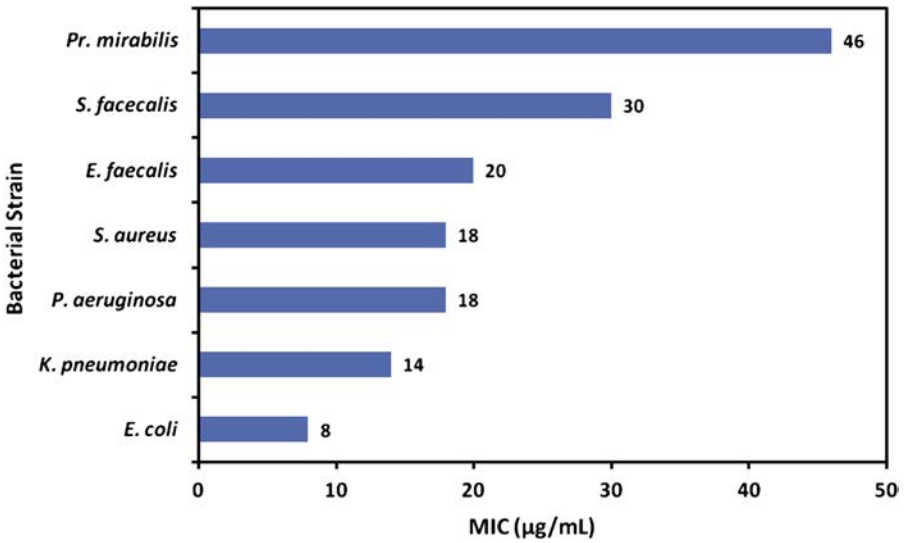

FIGURE 2 MIC of methanolic *Trigonella foenum-graecum* seed extract against clinical bacterial isolates (Dubey et al., 2010: text data converted). MIC: minimum inhibitory concentration.

dependent; the highest activity was noted at 100% (ZDI 24 and 22 mm for *S. aureus* and *P. aeruginosa*, respectively), and lowest activity was noted at 12.5% (ZDI 12 and 10 mm for *S. aureus* and *P. aeruginosa*, respectively) (Wagh et al., 2007). The five bacterial isolates *Proteus*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *Klebsiella pneumoniae* were sensitive to essential oil of *T. foenum-graecum* (at 75% dilution), based upon the ZDI 9.3, 13, 11.67, 12.33, and 10.5 mm, respectively, for the isolates (Mehani and Segni, 2012).

Dubey et al. (2010) determined ZDI of methanolic *T. foenum-graecum* seed extract for *E. coli* (16 mm), *P. aeruginosa* (16 mm), *Enterobacter faecalis* (12 mm), *K. pneumonia* (19 mm), *Streptococcus* facecalis (11 mm), *S. aureus* (14 mm), and *Proteus mirabilis* (10 mm); the minimum inhibitory concentrations (MICs) for the isolates ranged between 8 and 46 µg/mL (Figure 2). The MIC values of methanol *T. foenum-graecum* extract have been recorded as 64 µg/mL against *Shigella dysenteriae*, *Salmonella typhi*, and *E. coli*, and 32 µg/mL against *Pseudomonas* spp., while the MICs of acetone extract were 64 and 32 µg/mL against *Pseudomonas* spp. and *S. dysenteriae*, and 16 µg/mL against *E. coli* (Dash et al., 2011). The MIC of ethanolic *T. foenum-graecum* seed extract has been reported as 400 mg/mL for *E. coli*, *S. typhi*, *Vibrio cholerae*, *S. aureus*, and *Bacillus subtilis* (Khanra et al., 2010). Pooloth (2013) demonstrated the antimicrobial activity of silver nanoparticles synthesized with the help of *T. foenum-graecum* seed extract and the *T. foenum-graecum* seed aqueous extract against multidrug resistant gram positive (*S. aureus*, Str. pneumonia) and gram negative (*P. aeruginosa*, *Pr. vulgaris*) and found the nanoparticles thus synthesized as highly toxic to the test bacterial strains (Figure 3). Combinations like aqueous extract of cumin and fenugreek showed synergistic activity against *Pr. vulgaris* and additive effects against *S. aureus*, *Bacillus cereus*, and *Aspergillus niger* (Das et al., 2012). The bacterial isolates mentioned herein are associated with food-borne diseases, food poisoning in humans, and spoilage of food.

Antifungal Activity

The importance of fenugreek seed and its oil as an antimicrobial agent to be used as a food preservative or in medical industries is known. The fenugreek seed oil completely inhibited the growth of *A. niger*, which can potentially cause black mold on fruits (Sulieman et al., 2008). Das et al. (2012) showed additive effect of aqueous extract of fenugreek in combination with cumin extract against *A. niger*. The *T. foenum-graecum* seed essential oil was found active against two potential human pathogens—*A. niger* and *A. fumigatus*—having ZDI 22 mm and 24 mm, respectively (at 100% concentration), while the activity was reduced at 12.5% showing ZDI 8 mm and 10 mm, respectively (Wagh et al., 2007). The post-harvest green mold from *Penicillium digitatum* infection is a universal disease that leads to the spoilage of almost all kinds of mature citrus fruits. Kanan and Al-Najar (2008) reported that the ZDI of ethanolic *T. foenum-graecum* seed extract (97 µg/mL) against *P. digitatum* isolates from spoiled citrus orange (*Citrus sinensis* L.), and lemon (*Citrus limon* L.) fruits ranged 38–41 mm, and the percent inhibition ranged 8.5–58.23 at concentrations 50–520 µg/mL, with IC_{50} value of 142 µg/mL (Figure 4). Kanan and Al-Najar (2009) reported that the extract of *T. foenum-graecum* seed (520 µg/mL) gave inhibition values 25.79–31.38% against four isolates of *Penicillium italicum* causing a universal post-harvest disease of citrus fruits (Prusky et al., 2004).

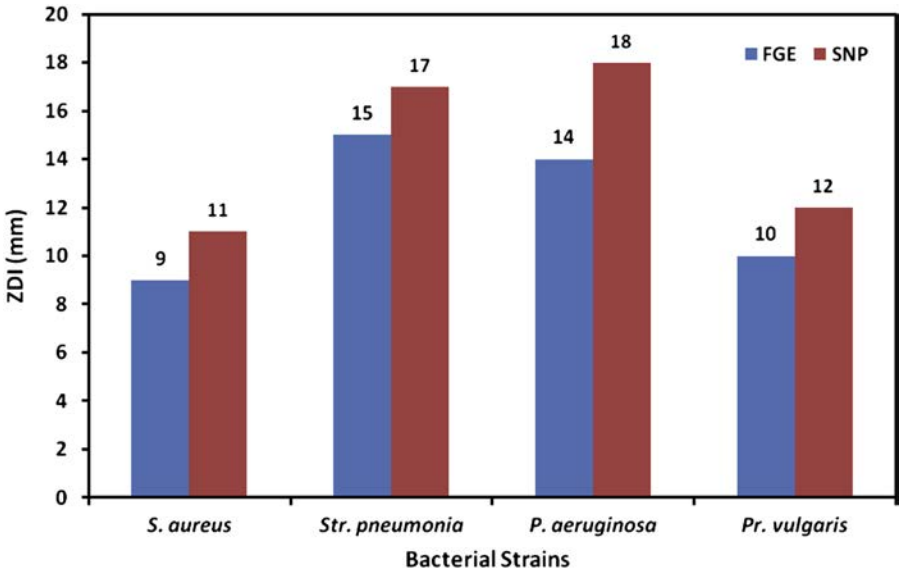

FIGURE 3 ZDI for bacterial isolates due to the action of fenugreek extract (FGE) and silver nanoparticles (SNP) (Data table converted from Pooloth, 2013). ZDI: zone diameter of inhibition; FGE: fenugreek extract; SNP: silver nanoparticles synthesized with the help of *T. foenum-graecum* seed extract.

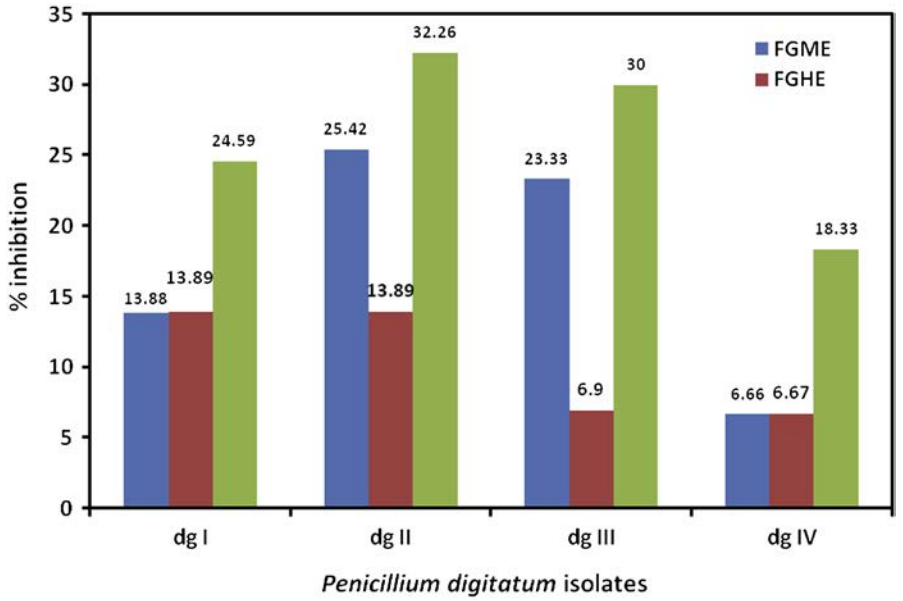

FIGURE 4 Antifungal activity of fenugreek seed extract (Kanan and Al-Najar, 2008; text data converted). FGHF: fenugreek hexane extract; FGMF: fenugreek methanol extract.

Antioxidant Activity

Fenugreek can act as potent source of antioxidant, and such activity is due to the presence of polyphenols and flavonoids. The phenolic and flavonoid levels in the leaf extracts of *T. foenum-graecum* have been reported as 414 µmol/mL and 0.7 µg/mL, respectively (Joglekar et al., 2012). Premanath et al. (2011) determined the level of polyphenols (4.9 mg/g) in the ethanol extract of leaves with flavonoid content of 0.47 mg/g and a total antioxidant activity of 47 µM Fe (II)/g. *Trigonella foenum-graecum* that is used as food and in food preparation removes ions like copper, iron, and nickel and helps preserve foods because in their free state those ions increase oxidation of foods. The total phenolic content was determined as 1.35–6.85 mg/g of the fenugreek extract, which was highest in ethanol extract (6.85 mg/g) and lowest in hexane extract (1.35 mg/g); the flavonoid content of ethanol fenugreek seed extract was 653 and 208 µg/g in hexane extract (Bukhari et al., 2008).

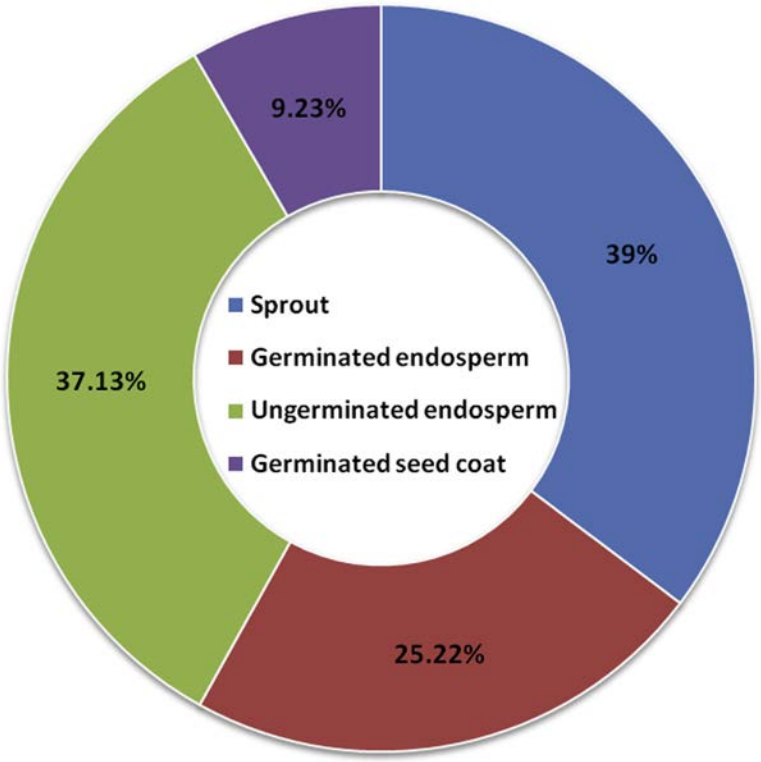

FIGURE 5 Polyphenolic contents in fenugreek seeds. (Shakuntala et al., 2011; text data converted).

The polyphenol content in fenugreek seeds, as has been reported by Shakuntala et al. (2011), is depicted in Figure 5, and the antioxidant activity (at 200 ppm extract concentrations) of seed coat, sprouts, and endosperm of germinated seed was determined as 79.87, 49.05, and 13.42%, respectively; the ungerminated seed coat and endosperm had 90.94 and 10.13% antioxidant activity.

Fenugreek Chemistry

The biological and pharmacological actions of fenugreek are attributed to the variety of its constituents including steroids, polyphenolic substances, volatile constituents, amino acids, etc. (Mehrafarin et al., 2010). The *T. foenum-graecum* water extracts contain flavonoids, saponins, volatile oils, and phenolics; while methanol extracts contain flavonoids, saponins, alkaloids, phenolics, and volatile oils (Sheikhlar et al., 2013). Moradikor and Moradi (2013) documented that fenugreek seed contains alkaloids (trigonelline: 0.2–0.38%, choline: 0.5%), gentianine and carpaine, flavonoids (apigenin, luteolin, orientin, quercetin, vitexin, and isovitexin), free amino acids, (4-hydroxyisoleucine: 0.09%, arginine: histidine and lysine), saponins (0.6–1.7%), glycosides steroidal sapogenins (diosgenin, yamogenin, tigogenin, neotigogenin). Pande et al. (2011) reported that *T. foenum-graecum* had antifungal (against *A. niger*) and antibacterial (against *S. aureus*) activity that might be due to the constituents presented in Figure 6. Hamden et al. (2011) demonstrated identification of 13 compounds representing 97.2% of the total fenugreek essential oil (Figure 7). The galactomannans (fenugreek gum derived from the seed endosperm) are water-soluble polysaccharides, which are used as thickening, water holding, and emulsifying agents in food products (Brummer et al., 2003). Volatile constituents contribute to the aroma and flavor of fenugreek: anethol, an aromatic compound, occurs in fenugreek and produces a licorice-like aroma, and sotolone (3-hydroxy-4,5-dimethyl-2(5H)-furanone) has been recognized as the main constituent contributing to the flavor of fenugreek in foods.

SAFETY AND TOXICITY

Fenugreek has gained "generally recognized as safe" status by the US Food and Drug Administration. Although it has conventionally been considered safe and well tolerated, toxic effects associated with its use are not uncommon, and some such cases include transient diarrhea, flatulence, mild hepatitis, and dizziness. It has also been considered as allergenic (Faeste et al., 2009), thus caution is warranted in persons allergic to fenugreek. Fenugreek showed antifertility activity

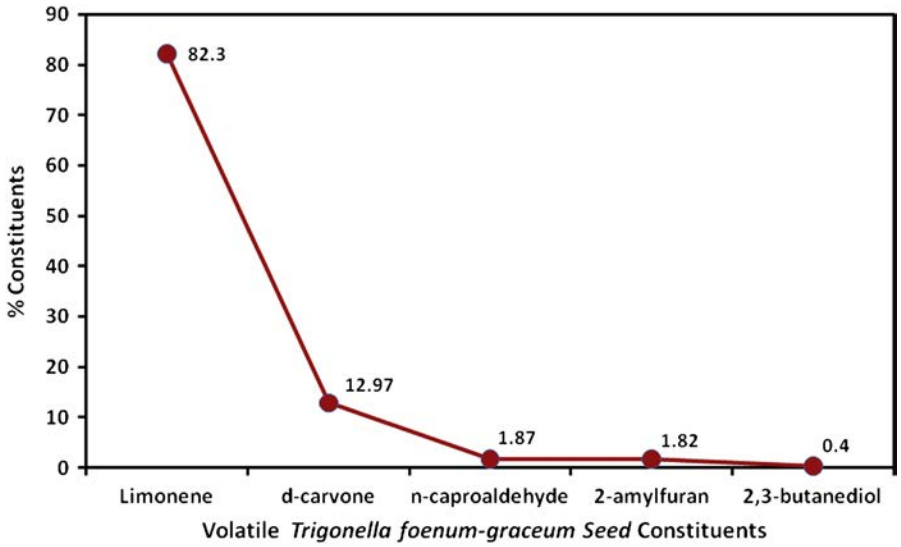

FIGURE 6 Fenugreek volatile seed contents showing antimicrobial activity (Pande et al., 2011).

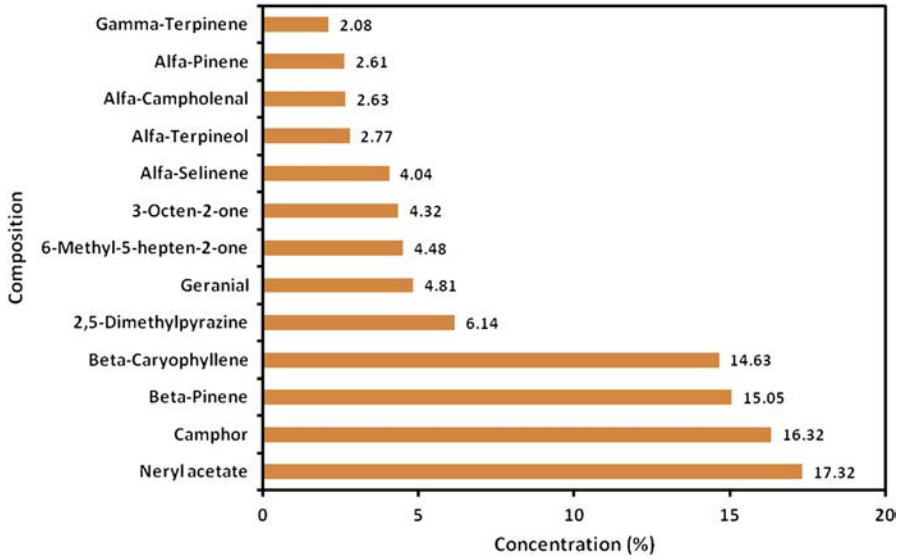

FIGURE 7 Chemical composition of fenugreek essential oil. (Hamden et al., 2011: data table partially converted to figure; http://www.lipidworld.com/content/10/1/226).

in experimental rabbits (Kassem et al., 2006). Since *T. foenum-graecum* possesses glucose-lowering activity, its use by hypoglycemic individuals may lead to serious health problems if consumed without proper monitoring of blood glucose levels. Turner (2011, 2012) reported an outbreak of *E. coli* infection, which was spread by contaminated *T. foenum-graecum* sprouts, that swept across northern Germany infecting thousands and killing 53; *E. coli* strain O104 produces proteins that help it stick to food and to the human gut.

SUMMARY POINTS

- Methi (*T. foenum-graecum*) is a popular kitchen herb due to its unique aroma and benefits to human health.
- Methi seeds, which are bitter and aromatic, are important in having restorative and nutritive properties; its germinated endosperm is rich in protein and seed coat is rich in dietary fiber and unsaturated fatty acids.
- Its seeds are used as a spice and the leaves are consumed as a green vegetable.
- Methi endosperm contains high amounts of saponin and protein, while the husk contains high amounts of polyphenols and total dietary fiber.

- The essential oils and plant extracts are considered as potential alternatives to synthetic antimicrobials, and as a lead compound for nonantibiotic drugs.
- Its extracts and oils provide a promising alternative to chemical preservatives, used in food products, because of the antimicrobial and antioxidative activities.
- Sotolone (3-Hydrocy-4,5-dimethyl-2(5H)-furanone) present in fenugreek essential oil is the main reason for the characteristic strong odor of the plant that provides the sweet smell of curry/foods.
- When plant oils and extracts are to be used in order to preserve foods, addressing the issues of safety and toxicity is required.

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Chapter 48

Frankincense (*Boswellia*) Oils

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List of Abbreviations

BHT Butylated hydroxytoluene
DPPH 2,2-Diphenyl-1-picrylhydrazyl
MIC Minimum inhibitory concentration

INTRODUCTION

The name *frankincense* is derived from the French term *franc encens* and means the true or "free lighting" incense. Frankincense comprises the resin produced by a group of trees belonging to the genus *Boswellia* (Burseraceae) (Vuuren et al., 2010). Frankincense (Olibanum, oleogum) is known in Arabic as *Luban* and it is reported that frankincense resin played an important role in the civilizations of the Arabian Peninsula and North Africa as an incense material (El-Nagerabi et al., 2013).

Although tremendous improvements in food production techniques have been developed in the past decade, food safety still remains a crucial issue of public health and safety. Although synthetic preservatives have been used in foodstuffs for many years, they do, however, have significant drawbacks, viz., increasing costs, handling hazards, concerns about residues in food, and a real threat to the general human environment. Therefore, natural preservatives comprising effective and nontoxic compounds are consequently needed to replace synthetic preservatives (Skrinjar and Nemet, 2009). It has been reported that 300 essential oils out of about 3000 plant species are commercially available in the flavors and fragrances market. In addition, a few essential oils are commercially available as food preservatives in the form of sodium citrate and sodium chloride solutions, respectively (Hamedo and Abdelmigid, 2009). This chapter gives an overview of the antimicrobial (antibacterial and antifungal) and antioxidant activity of a selection of essential oils derived from frankincense of different *Boswellia* species.

BOTANICAL ASPECTS

Botanically, the frankincense tree belongs to the family of the Burseracea and its genus is denoted as *Boswellia*. Moreover, the word *Burseracea* means that these plants produce balms and resins in special tissue canals. The Burseraceae consist of approximately 700 species from 18 genera. There are three tribes, viz., Canarieae, Protieae, and Bursereae in the family. The genus of *Boswellia* (named after Johann Boswell) is widespread in the dry areas of the Horn of Africa (Somalia, Sudan, Ethiopia, and Eritrea), the Arabian Peninsula (Oman and Yemen), and in India (Basar, 2005; Paul, 2012).

The name *Boswellia carterii* Birdw. was adopted from the Somalian tree and the name *Boswellia sacra* Flückiger from the South-Arabian (Oman, Yemen) plant. However, most of the authors suggest that *B. sacra* Flückiger and *B. carterii* Birdw. are in fact two different species. On the other hand, a few reports considered *B. sacra* Flückiger and *B. carterii* Birdw. to actually be the same species. Recently, however, it was confirmed on the basis of aroma and color, physical and chemical analyses, botanical description, and geographical distribution that *B. sacra* Flückiger and *B. carterii* Birdw. are in fact not synonymous but rather two distinct and separate *Boswellia* species (Al-Harrasi et al., 2013a). The other members of the genus *Boswellia* include *Boswellia serrata* Roxb. ex Colebr. (India), *Boswellia ovalifoliolata* Bal. & Henry (India), *Boswellia pirottae* Chiov. (Ethopia), *Boswellia dalzielii* Hutch. (West Africa), *Boswellia frereana* Birdw. (Somalia), *Boswellia neglecta* S. Moore (Kenya), *Boswellia papyrifera* (Del.) Hochst. (Ethopia), *Boswellia rivae* Engl. (Ethopia), *Boswellia hildebrandtii* Engl. (Somalia), *Boswellia ogadensis* Vollesen (Ethopia), *Boswellia popoviana* Hepper (Yemen),

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Boswellia nana Hepper (Yemen), *Boswellia bullata* Thul. & Gifri (Yemen), *Boswellia dioscorides* Thul. & Gifri (Yemen), *Boswellia elongata* Balf. f. (Yemen), *Boswellia ameero* Balf. f. (Yemen), *Boswellia odorata* Hutch. (Tropical Africa), *Boswellia dalzielli* Hutch. (Tropical Africa), and *Boswellia socotrana* Balf. f. (Yemen) (Basar, 2005; Paul, 2012).

The *Boswellia* plants are deciduous trees that can reach a height of up to 5 m and even more but mostly are dependent on the particular species and the growing area to determine their final height. The *Boswellia* plants have bald stems and branches with a peeling bark and appear bush or tree like. Branches of *Boswellia* plants have compound leaves and an odd number of leaflets. Generally, the frankincense tree is wounded in March or April and the resin is harvested through the summer and autumn. It has been reported that a *Boswellia* tree can produce this exudate in high quality only for three consecutive years, after which the quality of the resin decreases (Basar, 2005; Paul, 2012).

USAGE AND APPLICATIONS

Boswellia plants have been used in traditional medicines in India, Africa, and China. Frankincense was for centuries commonly employed for medicinal purposes, viz., Pliny the Elder in the first century employed frankincense as an antidote for hemlock poisoning. Similarly, in the tenth century, the Iranian physician Avicenna used frankincense for ailments such as tumors, vomiting, dysentery, fevers, and for memory support. For example, even today, the frankincense of *B. sacra* is employed for dental infections. Moreover, frankincense of *B. sacra* is furthermore used for treatment of the digestive system and for stomachaches. On the other hand, frankincense of *B. sacra* is used for the relief of muscle pain, joint pain (from arthritis), and for the treatment of various fevers, asthma, colds, and cough (Al-Harrasi et al., 2013a). The burnt incense is employed to reduce general pain and as an expectorant. Moreover, the burnt form of the incense is used in the treatment of colds and flu. Similarly, *B. dalziellii* Hutch. is employed to treat madness, boils, tetanus, cough, catarrh, internal bleeding, skin diseases, fever, rheumatism, and gastrointestinal symptoms as well as for treatment of snake bite (Adelakun et al., 2001). Traditionally, the frankincense of *B. carterii* has been employed for the treatment of rheumatic and inflammatory diseases, viz., ulcerative colitis and Crohn's disease (Mothana et al., 2011). *B. carterii* Birdw. has been used in traditional Chinese medicine for relieving pain caused by leprosy, activating blood circulation, gonorrhea, cancer, and carbuncles, and as an astringent (Zhao et al., 2003). It has also been reported that *B. ameero* Balf. f. and *B. elongata* Balf. f. are used for the treatment of the common cold, bronchitis, asthma, and rheumatism (Mothana and Lindequist, 2005). Furthermore, in Iranian folk medicine, *B. papyrifera* is used for analgesia, anti-inflammation, antitumor, antirheumatism, and for improving intelligence (Farshchi et al., 2010).

In Unani medicine, *B. serrata* Roxb. is used as a heart and memory tonic, carminative, antipyretic, antiseptic, and expectorant. Similarly, in Unani medicine, *B. serrata* is used for the treatment of ringworm infection, healing wet wounds and ulcers, inflammation of the breast, contusions of the ear, red and chronic pterygium, eye cancer, night blindness, palpitations, chronic cough, asthma, phlegmatic cough, pneumonia, vomiting, digestion problems, hemoptysis, hemorrhage, stomachache, diseases of the eyes, diarrhea, dribbling of urine, phlegmatic fevers, and septic ulcers (Sultana et al., 2013). In ethnomedicine, *B. serrata* Roxb. is used as an analgesic, antiallergic, antialzheimeran, antiarthritic, antiasthmatic, anticancer, anticomplementary, antiedemic, anti-inflammatory, antileukemic, antileukotriene, antipyretic, antirheumatic, astringent, carminative, central nervous system depressant, collyrium, COX-2-inhibitor, demulcent, depurative, diaphoretic, diuretic, emmenagogue, expectorant, heptatonic, hypoglycemic, 5-lipoxygenase inhibitor, pectoral, propecic, sedative, stomachic, tonic, antidysenteric, antiseptic, sedative, and analgesic stimulant. Similarly in ethnomedicine, *B. serrata* is also used for the treatment of pitta, asthma, dysentery, ulcers, hemorrhoids, skin diseases, urinary disorders, gout, piles, rheumatism, cutaneous and nervous diseases, chronic diarrhea and dysentery, cystic breast, chronic breast, tumors and ulcers, and gonorrhea (Sultana et al., 2013).

Usage and Applications in Food Science

Antimicrobial Activity

Frankincense has been carefully classified according to its color, size, and smell. Thus, the frankincense of *B. sacra* has been classified into six grades according to its degree of excellence. The color, size, and hardness of the resin lumps differ significantly from one grade to another as shown in Figure 1. The geographical location of the trees might have contributed to this variation because frankincense trees producing Sha'bi grade grow in coastal areas that are more affected by monsoon rains than the Hougari-producing areas. As expected, the moisture content of the Sha'bi oleo-gum resin was significantly greater than that of the Hougari.

It has been reported and is well known that different synthetic compounds have been employed for controlling fungal invasion of agricultural crops and stored food commodities. Furthermore, it is interesting to note that aflatoxins are the

FIGURE 1 Six grades of frankincense of *Boswellia sacra* available in Dhofar from left to right (up): Royal Hougari White, Super Hougari Green, Hougari Regular, (down) Hougari Yellow, Shabi, Shazri; photographed by the authors.

most toxic natural products produced by the genus *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nominus*, and *Aspergillus pseudotamarii* (El-Nagerabi et al., 2013).

In order to study and understand the detoxification properties of *B. sacra* frankincense essential oil, El-Nagerabi et al. (2013) recently evaluated its in vitro antifungal activity on the mycelial growth and aflatoxin production by two *Aspergillus* species, viz., *A. flavus* (Strain SQU21) and *A. parasiticus* (Strain CBS921.7). The reported results demonstrated that the essential oil of *B. sacra* frankincense significantly ($p<0.05$) reduced aflatoxin secretion for *A. flavus* (45.8–83.7%) and *A. parasiticus* (41.3–83.5%). Furthermore, *B. sacra* oil significantly inhibited ($p<0.05$) the mycelial dry weights of the *two Aspergillus* species. These studies suggested that the essential oil of *B. sacra* frankincense can be used as a natural plant additive in food and feed products based on their toxicity studies. In another study, essential oils of *B. carterii* frankincense at 2% (v/v) demonstrated the strongest mycelium inhibition against the pathogenic fungi *A. flavus* (11.1%), *Fusarium moniliform* (61.1%), *Pyrincularia grisea* (33.3%), *Rhizoctonia solani* (44.4%), *Fusarium proliferatum* (16.7%), *Bipolaris oryzae* (33.3%), and *Alternaria brassicicola* (71.3%) (Udomsilp et al., 2009) (Table 1). Pathogenic fungi such as *Pyricularia oryzae*, *B. oryzae*, *F. moniliforme*, *F. proliferatum*, *A. brassicicola*, *A. flavus*, and *R. solani* caused fungal rice diseases (Udomsilp et al., 2009). Prakash et al. (2014) in their research, demonstrated that *B. carterii* oil strongly inhibited both the growth and aflatoxin production of the food-borne toxigenic strain of *A. flavus* by an encouragingly 1.75 mL/mL and 1.25 mL/mL, respectively. Moreover, the oil of *B. carterii* elicited a broad fungitoxic potential toward 12 different food-borne molds. The antifungal potential of *B. carterii* oil was noted in terms of its ability to reduce the ergosterol content of the plasma membrane of *A. flavus*. Of note is the fact that when used as a fumigant in food systems when in storage containers, the *B. carterii* oil displayed a 65.3% protection against the fungal deterioration of *Piper nigrum*.

Baratta et al. (1998) evaluated the antibacterial potential of essential oils of the frankincense of *Boswellia thurifera* against 25 different genera of bacteria. The results indicated that this frankincense was active against *Beneckea natriegens*, *Enterobacter aerogenes*, *Leuconostoc cremoris, Citrobacter freundii*, *Serratia marcescens*, and *Micrococcus luteus*. Moreover, essential oils of this frankincense displayed significant activity toward growth of food poisoning organisms such as *Staphylococcus aureus*, *Salmonella pullorum*, and *Yersinia enterocolitica*. Similarly, frankincense essential oils of *B. thurifera* displayed promising activity against food spoilage organisms such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Lactobacillus plantarum*

TABLE 1 Antimicrobial Activity of Frankincense of Different *Boswellia* Species

	Boswellia Species										
Test Organisms	**1**[a]	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Aspergillus flavus	A	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Aspergillus parasiticus	A	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Fusarium moniliform	NT[c]	A[b]	NT	NT	NT	NT	NT	NT	NT	NT	NT
Fusarium proliferratum	NT	A	NT	NT	NT	NT	NT	NT	NT	NT	NT
Pyrincularia grisea	NT	A	NT	NT	NT	NT	NT	NT	NT	NT	NT
Bipolaris oryzae	NT	A	NT	NT	NT	NT	NT	NT	NT	NT	NT
Rhizoctonia solani	NT	A	NT	NT	NT	NT	NT	NT	NT	NT	NT
Alternaria brassicicola	NT	A	NT	NT	NT	NT	NT	NT	NT	NT	NT
Beneckea natriegens	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Enterobacter aerogenes	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Leuconostoc cremoris	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Citrobacter freundii	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Serratia marcescens	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Micrococcus luteus	NA[d]	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Staphylococcus aureus	A	A	A	A	A	A	NT	NA	A	A	A
Salmonella pullorum	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Yersinia enterocolitica	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Bacillus subtilis	NA	NT	A	A	A	A	NT	NT	NT	NT	NT
Pseudomonas aeruginosa	A	A	A	A	A	A	NT	A	A	NT	NT
Lactobacillus plantarum	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Alcaligenes faecalis	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Escherichia coli	A	A	A	A	A	A	A	A	A	A	A
Streptococcus faecalis	NT	NT	A	NT	NT	NT	NT	NT	NT	NT	NT
Aspergillus niger	NT	NT	A	NT	NT	NT	NT	NT	A	NT	NT
Acinetobacter baumanii	NT	A	NT	NT	NT	NT	NT	NT	NT	NT	NT
Aeromonas sobria	NT	A	NT	NT	NT	NT	NT	NT	NT	NT	NT

Candida albicans	A	A	NT	NA	NA	NA	A	A	A	A	A
Enterococcus faecalis	A	A	NT	NT	NT	NT	NT	NT	A	NT	NT
C. tropicalis	NT	A	NT	NT	NT	NT	A	A	A	NT	NT
Klebsiella pneumoniae	NA	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Shigella dysenteria	A	NT	NT	NT	NT	NT	NT	NT	A	NT	NT
Salmonella enterica	A	NT	NT	NT	NT	NT	NT	NT	A	NT	NT
Bacillus cereus	A	A	NT	NT	NT	NT	NT	NT	A	A	A
Listeria innocua	A	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Staphylococcus camorum	A	NT	NT	NT	NT	NT	NT	NT	A	NT	NT
Proteus mirabilis	A	NT	NT	NT	NT	NT	NT	NT	A	NT	NT
Proteus vulgaris	A	A	NT	NT	NT	NT	NT	NT	NT	A	A

[a]Boswellia sacra *(1)*, B. carterii *(2)*, B. thurifera *(3)*, Boswellia dioscorides *(4)*, Boswellia elongate *(5)*, Boswellia socotrana *(6)*, Boswellia rivae *(7)*, Boswellia serrata *(8)*, Boswellia papyrifera *(9)*, Boswellia neglecta *(10)*, Boswellia frereana *(11)*.
[b]*A: active.*
[c]*NT, not tested.*
[d]*NA, not active.*

as well as organisms of fecal origin such as *Alcaligenes faecalis*, *Escherichia coli*, and *Streptococcus faecalis*. Frankincense essential oils of *B. thurifera* demonstrated significant activity against the common spoilage fungus *Aspergillus niger* at a concentration of 1 µL/mL broth. In another study, Hammer et al. (1999) reported that essential oils of frankincense of *B. carterii* showed antimicrobial activity against *Aeromonas sobria*, *Acinetobacter baumanii*, *Enterococcus faecalis*, *Candida albicans*, *S. aureus,* and *E. coli*. However, no activity was observed against Gram-negative bacteria (Table 1).

Mothana et al. (2011) reported the antimicrobial potential of essential oils of *B. dioscorides* Thulin & Gifri, *B. elongata* Balf. f., and *B. socotrana* Balf. f. toward two Gram-positive and two Gram-negative bacteria as well as toward one fungal strain. The results showed that the essential oils of *B. dioscorides*, *B. elongata*, and *B. socotrana* demonstrated a higher activity toward Gram-positive strains than Gram-negative strains. It is noteworthy that the essential oil of *B. socotrana* exhibited strong activity toward *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* but lower activities with minimum inhibitory concentration (MIC) values (1.87 mg/mL) (Table 2) were observed toward *S. aureus* and *B. subtilis*. However, essential oils of *B. dioscorides*, *B. elongata*, and *B. socotrana* did not show any activity against *C. albicans*. It has been reported that rather the oxygenated monoterpenes, viz., borneol, camphor, α-terpineol, and linalool were responsible for the antimicrobial potential of several volatile oils (Mothana et al., 2011). Furthermore, it has been suggested that the significant antibacterial potential of *B. socotrana* may in fact be due to the presence of the relatively higher percentage of oxygenated monoterpenes such as α-fenchol, borneol, camphor, and terpinen-4-ol.

Camarda et al. (2007) reported that the *B. carterii* essential oil displayed significant encouraging activity toward the methicillin-resistant *S. aureus* (MIC 3.52 µg/mL) as well as toward *P. aeruginosa* (MIC 6.60 µg/mL). On the other hand, *B. rivae* essential oil displayed the lowest MIC value toward *E. coli* (3.53 µg/mL) (Table 2). However, *B. serrata* resin oil was the least active toward all of the tested bacterial strains. *Boswellia papyrifera*, *B. carterii*, *B. serrata*, and *B. rivae* essential oils were also investigated against two human pathogenic fungal strains, viz., *C. albicans* and *Candida tropicalis*, and the results showed that *B. rivae* resin oil displayed strong activity with lower MIC value toward *C. albicans* (2.65 µg/mL). On the other hand, *B. carterii* and *B. papyrifera* resin oils demonstrated good activity toward *C. albicans* and *C. tropicalis*. However, *B. serrata* resin oil showed weak activity toward *C. albicans* and *C. tropicalis*. Although Mothana et al. (2011) reported that the volatile oils of *B. dioscorides*, *B. elongata*, and *B. socotrana* did not show any activity against *C. albicans*, the results of Camarda et al. (2007), on the other hand, showed that *B. carterii*, *B. papyrifera*, and *B. rivae* resin oils indeed demonstrated activity toward *C. albicans* and *C. tropicalis*. This could in part be explained by the fact that previous studies demonstrated that limonene inhibits the growth of several fungal and Gram-positive bacterial strains (Camarda et al., 2007) and thus the absence of limonene in the Mothana et al. (Mothana et al., 2011) investigation of volatile oils of *B. dioscorides*, *B. elongata*, and *B. socotrana* resins explains the lack of antifungal activity in the latter case accounting for the discrepancy.

Al-Saidi et al. (2012) evaluated the antibacterial potential of the essential oils of four grades of *B. sacra* Flueck, locally known as Najdi, Hoojri, Shaabi, and Shathari. It was interesting to note that all four of the oils of *B. sacra* showed significant antimicrobial potential toward both Gram-positive and Gram-negative bacteria. The clinical isolates of *M. luteus*, *B. subtilis*, *Klebsiella pneumoniae, S. aureus*, and *E. aerogenes* were sensitive to all four oils of *B. sacra*. On the other hand *P. aeruginosa*, *E. coli*, and *Proteus vulgaris* were resistant to the Shathari, Najdi, and Hoojri oils, respectively. Interestingly, *S. aureus* and *E. coli* demonstrated inhibition zones with Hoojri, Najdi, Shathari, and Shaabi (Table 3). On the other hand, no activity was observed for the Najdi and Hoojri essential oils toward *P. aeruginosa*, and most interestingly, all tested organisms were sensitive to the Shaabi oil. From these results it was evident that these essential oils were slightly more active toward Gram-positive than Gram-negative bacteria (Al-Saidi et al., 2012). In another study, *B. sacra* oils showed strong activity toward *E. faecalis*, *Shigella dysenteria*, *Salmonella enterica*, *Bacillus cereus*, and *Listeria innocua* (Abdoul-latif et al., 2012) (Table 3). Similarly *B. papyrifera* oils showed strong activity toward *S. enterica*, *B. cereus*, *E. faecalis*, *S. dysenteria*, and *Staphylococcus camorum*. It was interesting to note that essential oils of *B. papyrifera* and *B. sacra* showed a higher antimicrobial activity than the reference drug tetracycline (Abdoul-latif et al., 2012). Interestingly, oils of *B. papyrifera* and *B. sacra* demonstrated stronger antimicrobial activity than the reference drug ticarcycline against *E. faecalis*, *E. coli*, *S. dysenteria*, *S. camorum*, *P. aeruginosa*, and *Proteus mirabilis*. The essential oil of *B. sacra* and *B. papyrifera* displayed strong fungicidal activity against *C. albicans* and *A. niger*. In a separate study, frankincense (*Boswellia* sp.) essential oil showed moderate antibacterial activity toward *Proteus* sp., *S. aureus*, and *E. coli*, while the same frankincense oil was not active toward *K. pneumoniae*, *Brucella* sp., and *P. aeruginosa* (Shareef, 2011).

Vuuren et al. (2010) reported the antimicrobial potential of a total of 16 oil fractions: *B. carterii* (nine fractions), *B. neglecta* (one fraction), *B. sacra* (two fractions), *B. thurifera* (one fraction), and *B. frereana* (three fractions). The measured antimicrobial potential of these 16 fractions toward *E. coli* varied between 4.0 and 12.8 mg/mL, while activity toward *P. vulgaris* showed the greatest variability between fractions where MIC values ranged between 2.0 and 12.0 mg/mL (Table 2). Fractions of *B. thurifera* demonstrated the highest sensitivity (2.0 mg/mL) against Gram-negative pathogens while their antimicrobial potential toward *C. albicans* was moderate to poor with MIC values ranging between 5.3 and 12.0 mg/mL.

TABLE 2 Antimicrobial Activity (MIC-Values) of the *Boswellia* sp. Essential Oils

Plant Species	Tested Organisms						
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*C. tropicalis*	References
Boswellia socotrana	1.87[a]	1.87	7.5	7.5	NA	NA	Mothana et al. (2011)
B. carterii	3.52[b]	–	54.6	6.60	6.16	6.16	Camarda et al. (2007)
Boswellia rivae	13.25[b]	–	3.53	45.76	2.65	27.4	Camarda et al. (2007)
Boswellia papyrifera	22.6	–	26.9	26.9	6.09	6.09	Camarda et al. (2007)
B. carterii	16.0–6.0[a]	–	12.0–4.0	–	12.0–5.3	–	Vuuren et al. (2010)
Boswellia neglecta	6.0	–	6.0	–	6.6	–	Vuuren et al. (2010)
Boswellia sacra	6.0–4.0	–	6.0–4.0	–	8.0	–	Vuuren et al. (2010)
B. thurifera	10.0		6.0		6.0	–	Vuuren et al. (2010)
Boswellia frereana	12.0–4.0		6.0–4.0		12.0–6.0	–	Vuuren et al. (2010)

[a]*MIC values are given as mg/mL.*
[b]*MIC values are given as µg/mL.*

TABLE 3 Antimicrobial Activity (Inhibition Zone Diameter) of the *Boswellia* sp. Essential Oils

Tested Organism	Inhibition Zone Diameter (mm)					
	Hoojri (*Boswellia sacra*)[a]	Shaabi (*B. sacra*)[a]	Najdi (*B. sacra*)[a]	Shathari (*B. sacra*)[a]	*B. sacra*[b]	*Boswellia papyrifera*[b]
Bacillus subtilis	15.0	16.0	14.7	15.5	–	–
Micrococcus luteus	11.3	20.0	15.7	20.0	–	–
Staphylococcus aureus	14.0	24.3	13.6	21.2	–	–
Pseudomonas aeruginosa	23.3	29.7	25.7	NI[c]	–	–
Klebsiella pneumoniae	8.0	12.7	7.0	8.7	–	–
Enterobacter aerogens	23.0	11.0	19.7	9.3	–	–
Escherichia coli	11.6	26.3	NI	8.7	–	–
Proteus vulgaris	NI	24.3	15.7	22.5	–	–
P. aeruginosa	NI	10.0	NI	7.0	–	–
Enterococcus faecalis	–	–	–	–	37.0	39.0
Shigella dysenteria	–	–	–	–	37.0	31.0
Salmonella enterica	–	–	–	–	35.0	40.0
Bacillus cereus	–	–	–	–	34.0	39.0
Listeria innocua	–	–	–	–	34.0	28.0
Staphylococcus camorum	–	–	–	–	27.0	30.0

[a] *Al-Saidi et al. (2012)*.
[b] *Abdoul-latif et al. (2012)*.
[c] *NI: No inhibition zone.*

The essential oils from *Boswellia* species comprise different terpenes and thus the antimicrobial potential of these frankincense oils may be in part due to the presence of these terpenes. Generally, essential oils usually present themselves in the form of complex mixtures, and thus the antimicrobial potential of this mixture could possibly be due to the major monoterpenoids. Moreover, roughly 300 volatiles have been reported to be present in *Boswellia* oils and a broad diversity of these volatile compounds have been discovered in a wide range of different *Boswellia* species (Mertens et al., 2009). For example, a report from our laboratory on *B. sacra* resin oil showed that limonene and *E*-β-ocimene were the major components. However, in another study on the *B. sacra* resin oil, it was reported that α-pinene was one of the major components in four grades of *B. sacra*. Furthermore, myrcene, α-thujene,duva-3,9,13-triene-1a-ol-5,8-oxide-1-acetate, and octylacetate were individually reported as the major components for *B. serrata* and *B. carter* (Mertens et al., 2009). On the other hand, α-pinene, limonene, and octanol were reported separately as the major components for *B. rivae* while *trans*-verbenol and terpinen-4-ol were the major constituents for *B. pirottae*. It is interesting to note that only α-pinene was reported as the major constituent for *B. neglecta* and *B. frereana* and octylacetate for *B. papyrifera* (Mertens et al., 2009).

The antimicrobial activity demonstrated by the essential oil of the various *Boswellia* species may be therefore partly due to the presence of major constituents like *E*-β-ocimene, limonene, α-pinene, myrcene, α-thujene, duva-3,9,13-triene-1a-ol-5,8-oxide-1-acetate, octylacetate, octanol, *trans*-verbenol, and terpinen-4-ol. Furthermore, individually α-pinene (Yasodamma et al., 2013), limonene (Espina et al., 2013), and myrcene (Yasodamma et al., 2013) showed significant antimicrobial activity in their own right. Moreover, the authors believe that some volatile compounds that present themselves in rather low concentrations may contribute to the antimicrobial potential of the frankincense essential oil, more than likely involving some type of synergism with these other active compounds.

Antioxidant Activity

Baratta et al. (1998) evaluated the antioxidant potential of the essential oils of frankincense of *B. thurifera*, which demonstrated significant antioxidant activity, comparable with α-tocopherol and butylated hydroxytoluene. Mothana et al. (2011)

reported that oils of *B. dioscorides*, *B. elongata*, and *B. socotrana* showed at a concentration of 1 mg/mL a weak radical scavenging potential (22%, 21%, and 28%, respectively). The weak antioxidant activity of these oils may be due to their relatively low concentration of the active phenolic compounds, viz., thymol and carvacrol, in the oils of *B. dioscorides*, *B. elongata*, and *B. socotrana*. The antioxidant activity of *B. socotrana* Balf. f, *B. ameero* Balf. f, and *B. elongata* Balf. f were additionally evaluated, and the results showed that they were indeed active with IC_{50} values of 121.4, 211.2, and 175.2 µg/mL, respectively (Ali et al., 2008). The scavenging potential of *B. socotrana* volatile oils has significantly higher activity than those of *B. elongata* and *B. ameero*. It was postulated that the higher activity of *B. socotrana* essential oils was due to the relatively higher concentration of oxygenated monoterpenes (Ali et al., 2008). Recently, the authors tested the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and superoxide anion scavenging activity of essential oils of Royal lower, Hougari regular, Shabi frankincense, and Royal upper of *B. sacra* (Al-Harrasi et al., 2013b). Interestingly, Royal upper, Hougari regular, Shabi frankincense, and Royal lower showed good superoxide anion scavenging activity with more than 50% inhibition. On the other hand, Shabi frankincense showed moderate DPPH free radical scavenging activity while Royal upper, Royal lower, and Hougari regular frankincense showed weak DPPH free radical scavenging activity. The antioxidant activity of Royal upper, Hougari regular, Shabi frankincense, and Royal lower is not surprising since these frankincense grades comprise a wide range of terpenes. These terpenes may act as electron donors and react with free radicals to transform them to more stable products and thereby terminate any radical chain reaction (Al-Harrasi et al., 2013b). Prakash et al. (2014) reported that *B. carterii* oil demonstrated significant antioxidant potential with IC_{50} value and percent inhibition of linoleic acid peroxidation being 0.64 mL/mL and 51.6%, respectively.

SUMMARY

- Frankincense essential oils of the genus *Boswellia*, viz., *B. sacra*, *B. carterii*, *B. thurifera*, *B. dioscorides*, *B. elongate*, *B. socotrana*, *B. rivae*, *B. serrata*, *B. papyrifera*, *B. neglecta*, and *B. frereana* demonstrated significant antimicrobial activity against the growth of food poisoning organisms.
- Oils of *B. thurifera*, B. *dioscorides*, *B. elongate*, *B. socotrana*, *B. ameero*, and *B. sacra* showed the most promising antioxidant activity.
- The antimicrobial and antioxidant activity of frankincense (*Boswellia*) essential oils confirms the potential use of these essential oils in the food industry.
- The antimicrobial and antioxidant activity of frankincense (*Boswellia*) essential oils also confirms the potential use of these essential oils in the preservation of foodstuffs against bacteria and fungi.
- These oils may also be valuable for increasing the shelf life of foodstuffs in general.
- The review clearly illustrates that frankincense (*Boswellia*) essential oils have a significant role to play as a potential for serious consideration by the food industry.

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Chapter 49

Garlic (*Allium sativum* Linn.) Oils

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List of Abbreviations

GO Garlic oil
GOG Garlic, onion and ginger oils
GO-β-CD Garlic oil encapsulated in β-cyclodextrin
TBARS Thiobarbituric acid reactive substance

INTRODUCTION

Garlic (*Allium sativum* Linn.) is an ancient medicinal plant that originated from Central Asia over 6000 years ago. Garlic essential oil (GO) can be obtained by steam distillation of the minced raw or dry material, yielding 0.1–0.2% of essential oil on a fresh basis (Santhosha et al., 2013). Its strong pungent flavor potency is equal to that of 900 mg of fresh garlic/200 g of dehydrated powder. Sulfur compounds, mostly diallyl disulfide, diallyl sulfide, diallyl trisulfide, allylmethyl trisulfide, and diallyl tetrasulfide emit strong odor and are kept in GO as their active constituents (Figure 1) (Casella et al., 2012).

GO is recognized for its antimicrobial property, which has been attracting attention to consider it as a food additive (Janz et al., 2007). GO is generally recognized as a safe natural product by the US Food and Drug Administration (CFSAN/FDA, 2013), and its listed volatile compounds are the main reason for its bioactive properties. The medicinal, antimicrobial, and sometimes preservative mechanisms of GO constituents are attributed to the inactivation of key metabolic enzymes of food deteriorative and pathogen microorganisms or enzymes involved in deteriorative reactions (Chung et al., 2007). These attributes had the potential to stop rancidity, browning, and reduce microbial growth in several food systems (Richardson, 2013). However, its volatility and reactivity hinders its handling as a liquid and its strong odor can represent a problem affecting the sensorial acceptability of the treated produce (Ayala-Zavala et al., 2008b). In these terms, research proposed the encapsulation process to optimize the functionality of GO applied as food additive (Ayala-Zavala et al., 2008a). This chapter provides a description and analysis of the bioactive properties and effects of GO applied in food systems.

BOTANICAL ASPECTS

Garlic is a member of the Liliaceae family, which is a common food spice used in many parts of the world (Hacıseferoğulları et al., 2005). This product is a heavily consumed crop around the world due to its characteristic flavor and stable postharvest life (Benkeblia, 2004). The *Allium* genus has over 500 members, each different in taste, color, or appearance but similar in bioactive content and properties (Hacıseferoğulları et al., 2005). Among the botanical characteristics of the plant, it presents long, narrow, and flat leaves. The edible bulbs are arranged in groups known as "cloves," entrapped by a white skin.

After harvest, garlic must be cured by drying the outer leaf sheaths and neck tissues of the clove. This process is more efficient in warm temperatures, low relative humidity, and airflow conditions. In addition, this process is essential to maximize the postharvest life and quality of the product. The harvest of garlic occurs at different stages of development for special markets, but most of the product is collected when the bulbs are well developed; this condition is visualized when the tops of the plant have fallen and are dry. The concept of quality for garlic is perceived as firm, clean, white (or other colors typical of the variety), and well-cured cloves. Sulfur compounds are the bioactive compounds of garlic products (Benkeblia, 2004; Benkeblia and Lanzotti, 2007). The powerful and unusual flavors of many of these plants and their possible medical applications have attracted the attention of plant physiologists and chemists.

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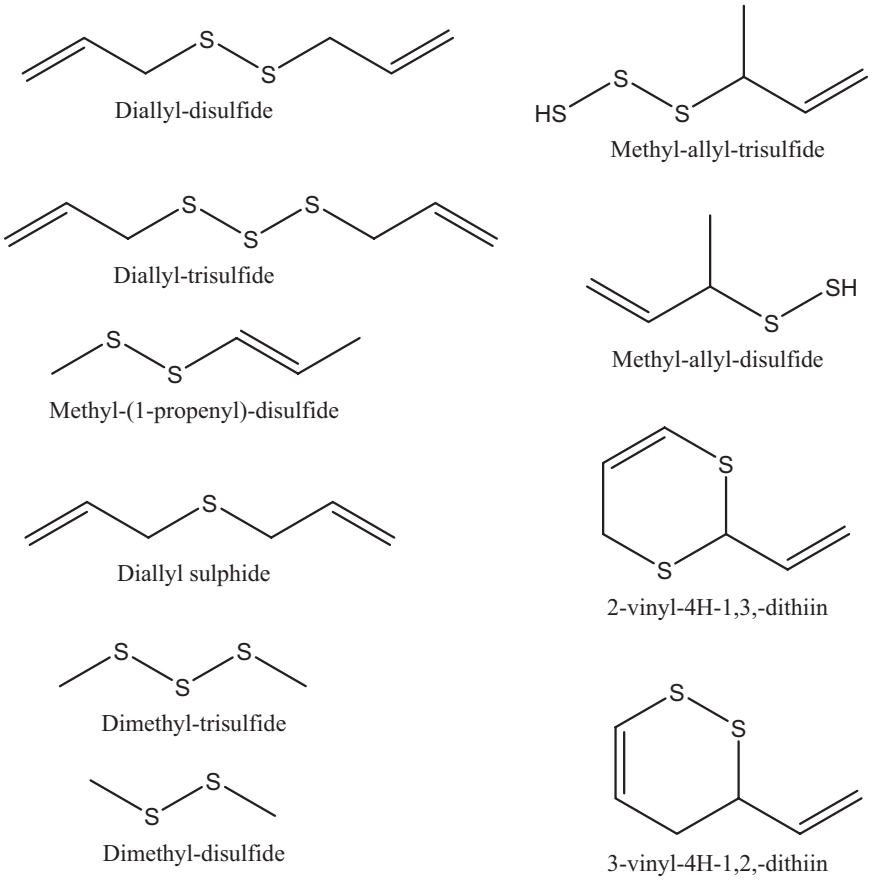

FIGURE 1 Principal components of garlic essential oil.

USAGE AND APPLICATIONS

Garlic is one of the most extensively researched products for investigating the beneficial health effects of plant products. Potential health benefits of garlic have been reported since ancient times (Tsai et al., 2012). Among the first reported uses of garlic is that it was consumed by the working class, of ancient Egypt, who helped build the pyramids (Rivlin, 2001). In addition, it is reported by the Ebers papyrus, an ancient Egyptian compilation of medical records of that time, that garlic was prescribed for the treatment of abnormal growths. This source also prescribed garlic's consumption for circulatory ailments, general malaise, and as an antiparasitic (Rivlin, 2001).

In vitro and animal model studies have addressed the protective effect of garlic against cardiovascular disease and cancer (Tsai et al., 2012). Among the mentioned health benefits of garlic are the antioxidant defense, low blood lipids, inhibiting of blood aggregation, inducing apoptosis of cancer cell lines, inhibiting metastasis, and modulating drug metabolism. Clinically, garlic has been evaluated for lowering blood pressure, cholesterol, and glucose concentration, as well as for the prevention of arteriosclerosis and cancer (Tsai et al., 2012). Epidemiologically, garlic consumption inversely correlates with the risk of oral, stomach, esophageal, colon, and prostate cancers (Tsai et al., 2012). In addition, the biological activities of garlic include antibacterial, antithrombotic, antioxidant, immunomodulatory, and antidiabetic actions and modulation of drug metabolism (Tsai et al., 2012). The medicinal, antimicrobial, and sometimes preservative mechanisms of garlic can be found in GO constituents and are attributed to the inactivation of key metabolic enzymes of food deteriorative and pathogen microorganisms or enzymes involved in deteriorative reactions (Chung et al., 2007).

USAGE AND APPLICATIONS IN FOOD SCIENCE

GO properties have attracted the attention of the food industry, mainly as flavoring, however, several alternative uses as an antimicrobial and antioxidant agent are being proposed to use GO as a preservative agent (Table 1). Organic compounds containing sulfur are commonly found in GO; they have demonstrated antimicrobial activity against a wide variety of microorganisms.

TABLE 1 Antimicrobial and Sensorial Effects of the Addition of Garlic Preparations in Food Matrices

Treatment	Antimicrobial Effect	Antioxidant Effect	Sensorial Effect	References
Stewed pork treated with GO and chitosan	Reduced 6.3 log CFU/g aerobic mesophilic	Reduced the content in TBARS	Acceptable flavor notes	Cao et al., 2013; Lenon et al., 2010
Chicken sausages added with GO, garlic powder and fresh garlic	Reduced 4.4 log CFU/g of aerobic mesophilic bacteria	ND	Acceptable at low concentrations	Sallam et al., 2004
In vitro GO encapsulated in β-CD	Inhibited *Staphylococcus aureus*, *Salmonella enteritidis*, *Aspergillus niger*, *Penicillium cyclopium*, *Fusarium oxysporum* growth	ND	ND	Harris et al., 2001
Fresh-cut tomato treated with GO	Reduced 4 log CFU/g of aerobic mesophilic, 2 log CFU/g of aerobic coliform bacteria	ND	ND	Ayala-Zavala et al., 2008b
Fresh-cut tomato treated with encapsulated GO	ND	ND	Like moderately after 14 days	Ayala-Zavala and González-Aguilar, 2010
Wheat flour added with garlic and garlic capsules	ND	ND	Non-encapsulated garlic affected dough strength	Miller et al., 1997
Belly lean and loin treated with garlic powder	Reduced 7 log CFU/g aerobic mesophilic bacteria	Reduced formation of peroxide an TBARS	Reduced off-flavor compounds	Park et al., 2008
Irradiated cooked ground beef added with fresh garlic	ND	Reduced formation of peroxide an TBARS	The garlic-added beef had the same overall acceptance as controls	Yang et al., 2011
Lamb fed with garlic	ND	ND	The diet increased the acceptability of the obtained meat	Strickland et al., 2011

ND, no detected.

Organosulfur compounds from garlic such as diallyl mono-, di-, and trisulfide have been reported to have antimicrobial activity against fungi and bacteria (Chung et al., 2007). For example, diallyl sulfide and dimethyl trisulfide are volatile constituents of garlic oil that have shown antimicrobial activity against *Enterobacter aerogenes*, *Escherichia coli*, *Salmonella* sp., *Listeria monocytogenes*, and *Yersinia enterocolitica*, are perceived as sulfur onion, garlic, and/or cabbage-like odorants (Table 2) (Benkeblia, 2004).

To conceive a sensorially acceptable addition of GO to food systems, a common relation of flavor profiles normally used in the global seasoning and flavoring mixtures could be helpful to support a given combination of flavors and odors (Rozin and Rozin, 1981). The aroma profile of a given food could be merged with GO using its aroma notes as connectors. Notes aroma of food are different among them, however, in dishes prepared notes of herbs and spices are pleasing to the consumer. In this context, aroma notes of a given food and GO could be combined. The idea of adding an extra flavor to the GO-treated product could be perceived within the philosophy of seasoning (Rozin and Rozin, 1981). The traditional art of seasoning food consists generally of the addition of fresh herbs and the pungent odors of herbs and spices, like garlic, could be considered for this purpose. Among the different EOs studied, much research has been focused on the oil extracted from garlic, which possesses the aroma and odor notes of its source plant (Ayala-Zavala et al., 2008a). In some traditional Chinese, Egyptian, Mediterranean, and Mexican dishes, garlic flavor provides seasoning in salad dressing, vinaigrettes and sauces, pork, beef, shrimp, among other foods, providing a special taste and smell dishes (Sherman and Billing, 1999).

Mixtures of GO with onion and ginger oils (GOG) incorporated in different proportions within chitosan have been shown to affect in different ways the sensorial acceptability of stewed pork (Cao et al., 2013). Chitosan (0.5%) + GOG (5%) treatment had a positive effect, increasing sensorial acceptability, while the high concentration of composite solution had

TABLE 2 Antibacterial Effect in vitro of Garlic Essential oil

Bacteria	MIC (mg/mL)	Zone Diameter (mm)	Reference
Staphylococcus aureus	ND	9.3[a]	Chung et al., 2007
Salmonella enteritis	ND	13.1[a]	
S. aureus	>1	ND	Arora and Kaur, 1999
Campylobacter jejuni	>1	ND	
S. enteritidis	>1	ND	
Listeria monocytogenes	>1	ND	
Escherichia coli	>1	ND	
S. aureus	>1	ND	
E. coli	ND	4[a]	Smith-Palmer et al., 1998
S. aureus	ND	4[a]	
L. monocytogenes	ND	4[a]	
S. enteritidis	ND	4[a]	
C. jejuni	ND	4[a]	

ND, no detected.
[a]*25 μl/L.*

an adverse effect on odors and overall acceptance. Similarly, stewed-pork with GO had the same acceptability stewed-pork control as to the sensory quality. In this context, the production of pork with garlic-flavored notes using GO was evaluated (Leong et al., 2010). In a similar way, the addition of garlic extracts in different presentations, including GO, on the acceptability of chicken sausages showed that garlic powder followed by GO at a concentration of 0.6 g/kg can serve as a flavoring agent (Sallam et al., 2004). However, higher concentrations were not well accepted. As reviewed, the sensorial effect of GO addition must consider carefully the selection of the appropriate concentration used.

An important characteristic of GO volatile compounds is their reactivity and volatility, given by their short carbon chains and sulfur atoms (Chung et al., 2007). Reactivity of GO compounds is related to its antimicrobial activity; however, this attribute can make it difficult to control the reaction target. The sulfur atoms of the GO volatiles can interact with other electronegative atoms present in proteins, forming sulfur bridges and affecting the functionality of biomolecules (Vermeiren et al., 2002). It means that in a food system, reaction of GO volatiles with the food matrix and package could deplete the volatiles' power when microbial growth occurs (Vermeiren et al., 2002).

Another limitation to be overcome is the poor aqueous solubility that restricts the application of GO in industry. The formation of garlic oil encapsulated in β-cyclodextrin inclusion complex has been demonstrated by different studies. Microemulsions of GO have been developed to solve this issue (Zheng et al., 2013). Microemulsions were prepared with ethoxylated hydrogenated castor (Cremophor RH40) as surfactant, *n*-butanol (or ethanol) as cosurfactant, oleic acid-containing garlic oil as oil phase, and ultrapure water as water phase. The prepared microemulsion solution exhibits remarkable antibacterial activity against *Staphylococcus aureus*. GO exhibited a marked inhibitory activity on *S. aureus* and *Salmonella enteritidis*, and three fungi, *Aspergillus niger*, *Penicillium cyclopium*, and *Fusarium oxysporum* (Harris et al., 2001). A previous study reported that natural volatile compounds including tea tree oil and GO, among other treatments, inhibit the growth of aerobic mesophilic microorganisms of fresh-cut tomato as compared to control samples (Ayala-Zavala et al., 2008b). In fact, fresh-cut tomatoes treated with tea tree oil showed remarkable low aerobic plate counts throughout storage at 5 °C. Growth of total coliform bacteria was inhibited to a higher extent in fresh-cut tomatoes treated with GO followed by tea tree oil, among other treatments, when compared to control fresh-cut tomatoes (Ayala-Zavala et al., 2008b). However, the effect of these treatments on the sensorial acceptability of the treated produce was not evaluated.

Odor acceptability expressed as liking/disliking of fresh-cut tomato treated with free and encapsulated GO was evaluated at days 7 and 14 of storage at 5 °C, respectively (Ayala-Zavala and González-Aguilar, 2010). The highest reduction in acceptability was observed in fresh-cut tomatoes with 100 and 200 μg of free GO per 100 g of fresh-cut tomatoes. Only the control and tomatoes treated with the 50 μg/100 g of free GO remained acceptable at day 7 of storage. Although the

lowest free GO concentration used presented the highest acceptability, it did not achieve a significant reduction of microorganisms. Conversely, fresh-cut tomatoes treated with the most effective antimicrobial concentrations of free GO (200 and 100 μg/100 g) were not acceptable for panelists. In fact, panelists did not find differences of odor acceptability among controls and GO capsule-treated produce, on day 14 of storage (Ayala-Zavala and González-Aguilar, 2010). It is important to point out that acceptability on day 14 did not reach the line above disliking. The major comments from panelists were that the treated produce presented a slight garlic odor, and some proposed that it could be used without problem in a salad mixture. Thus, GO capsule treatment with 1 g/100 g of fresh-cut tomato was accepted by panelists and additionally offered good antimicrobial effect.

Other premises that could help to sustain the use of GO as food additive are the uses of other forms of garlic applied in other food systems. The addition of garlic powder to wheat flour dough caused breakdown during mixing, due to disulfide bridges among garlic compounds and dough proteins (Miller et al., 1997). Garlic in the bread formula weakened the dough and resulted in bread with undesirable crumb grain characteristics and low volume. However, encapsulated garlic increased dough strength and did not affect loaf volume or crumb grain. Another study presented the addition of garlic powder to increase redness (1) and yellowness (2) in both the belly lean and loin (Park et al., 2008). In addition, free fatty acid values in both pork belly and loin cuts were reduced with the addition of this ingredient, as compared to the control. Significant differences in peroxide values were observed in sodium ascorbate and garlic injected belly as compared to the control. Thiobarbituric acid reactive substance values (TBARS) in the pork belly with garlic powder were significantly lower than in the belly without these ingredients or with sodium ascorbate. Total plate counts were lower in both the belly and loin containing garlic powder, as compared to the control. With similar results, the addition of garlic (0.1%) changed the lipid oxidation, volatiles, and sensory characteristics of irradiated cooked ground beef at 7 days storage (Yang et al., 2011). TBARS content was lower for treatments with garlic that was proposed to be an option to mask the odor and flavor produced by the irradiation of meat flavor and aroma providing acceptable garlic scores compared to controls. Moreover, positive results of the sensorial acceptability of meat from lambs fed with garlic during 10 weeks at different concentrations (0, 1.8, and 3.6%) were observed (Strickland et al., 2011). Their results suggest that the inclusion of garlic into the animals' feed did not have a negative impact on the flavor of the lamb and, at the high rate (3.6%), made the meat was more acceptable to the panelists.

As reviewed, the appropriate or compatible use of GO with the food matrices would involve its application to add positive sensory characteristics and improve the safety and/or quality of the treated food. As observed, GO has been proven to be an effective antimicrobial; however, its aromatic volatile constituents can be absorbed and its sensory characteristics added to the treated food product. The analyzed information indicated that the potential uses of GO in the food industry are beyond its flavoring properties. As can be observed in these examples, several foods are consumed with aroma notes also found in GOs. Therefore, other application forms in other food matrices could be implemented and performed with similar purposes, increasing its use as natural additives and/or preservatives. Their potent antimicrobial properties, their efficacy to inhibit deteriorative enzymes, and their efficacy to reduce lipid oxidation should be contemplated; however, the strong impact of their flavor must be considered. Several studies indicated the benefits of using encapsulation systems for the application of this oil in food systems, which could be helpful to improve its uses in the food industry.

SUMMARY POINTS

- GO is a mixture of reactive sulfured compounds with strong odor-flavor, antimicrobial, and antioxidant properties.
- GO supplemented in animal diets can be used to improve the quality of food products, in terms of oxidation and sensorial acceptability.
- Encapsulation can be an option to control reactivity of GO and obtained better results in adding value to food systems.
- GO addition can reduce the impact of flavor artifacts produced by other food preservation techniques.
- GO uses can improve the sensorial acceptability of certain food products depending on the dose, application form, and treated food.

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Chapter 50

Ginger (*Zingiber officinale* Rosc.) Oils

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List of Abbreviation

BHA Butylated hydroxyanisole
BHT Butylated hydroxytoluene
PG Popyl gallate
TBHQ Tertbutylhydroquinone
H_2O_2 Hydrogen peroxide
MIC Minimum inhibitory concentration

INTRODUCTION

Ginger (Figure 1), the root of the plant *Zingiber officinale* Rosc. that belongs to the family Zingiberaceae, is globally one of the most commonly used spices and medicinal agents. The plant is known as *Sringavera* in the ancient Indian language of Sanskrit, and it is speculated that this term may have given way to *Zingiberi* in Greek and then to the Latin term *Zingiber* (Vasala, 2004). The names of ginger in various languages appear in Table 1.

Historical evidence indicates that ginger was originally indigenous to Southeast Asia (today's northeast India), and during medieval times, it was exported from India to other parts of the world. Today ginger also grows in many other parts of the world (Govindarajan, 1982a,b; Warrier, 1989), including Nigeria, Sierra Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica, Nepal, Haiti, Mexico, and Hawaii. India and China are the leading providers of ginger to the world market (Govindarajan, 1982a,b; Warrier, 1989).

Ginger has been cultivated for thousands of years as a spice. It is an important cash crop in India and is grown primarily in the states of Kerala, Karnataka, and northeast India (Govindarajan, 1982a,b; Vasala, 2004). Among the Indian varieties, the Cochin and Calicut ginger have a lemon-like bye note and are very popular (Govindarajan, 1982a,b; Vasala, 2004). When compared to the Indian varieties, Chinese ginger is low in pungency and is principally exported preserved in sugar syrup or as sugar candy (Govindarajan, 1982a,b; Vasala, 2004). The yield and oil characteristics and content vary with cultivars and environmental factors. Many local varieties are grown around the world. More than 400 accessions of ginger are maintained at the Indian Institute for Spice Research in Calicut, Kerala, India (Vasala, 2004). The Indian Institute for Spice Research selected the following Indian cultivars for their high yield and high oil content (Vasala, 2004). With respect to the African varieties, the Jamaican ginger is highly popular basically due to its delicate aroma and fine-textured powder, while the Nigerian and Sierra Leone dried ginger possess camphoraceous and coarser odor and are rich in both aroma and pungency factors (Govindarajan, 1982a,b; Vasala, 2004).

CLASSIFICATION

Zingiber officinale Rosc.
Kingdom: Plantae-Plants
Subkingdom: Tracheobionta–Vascular plants
Superdivision: Spermatophyta–Seed plants
Division: Magnoliophyta–Flowering plants

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FIGURE 1 Photograph of ginger.

TABLE 1 The Different Vernacular Names of Ginger in India and Other Parts of the World

Language	Names
Scientific name	*Zingiber officinale* Rosc.
English	Ginger
Indian Languages	
Assamese	Ada
Bengali	Ada
Gujarati	Adu
Hindi	Adrak
Kannada	Shunti
Konkani	Shunti
Malayalam	Inji
Manipuri	Shing
Marathi	Aale
Mizo	Thingpuidum
Oriya	Ada
Pali	Singivera
Punjabi	Adaraka
Sanskrit	Adaraka
Tamil	Inji
Telugu	Allam
Urdu	Adrak

TABLE 1 The Different Vernacular Names of Ginger in India and Other Parts of the World—cont'd

Language	Names
Other Languages	
Arabic	Zanjbeel
Burmese	Sif
Dutch	Gember
Filipino	Luya
French	Gingembre
German	Ingwer
Italian	Zenzero
Javanese	Jahe
Khmer	Khnyyi
Malay	Halia
Nepali	Sano Adwa
Russian	Imbir
Sinhala	Inguru
Spanish	Jengibre
Swahili	Tangawizi
Thai	K˘lng
Tibetan	Sga skya
Vietnamese	Gừng

Class: Liliopsida–Monocotyledons
Subclass: Zingiberidae
Order: Zingiberales
Family: Zingiberaceae - Ginger family
Genus: Zingiber P. Mill.–Ginger
Species: *Zingiber officinale* Rosc. – Garden ginger

BOTANICAL ASPECTS

Ginger is said to be Indian in origin and is known as *Adrak* in the Hindi language (Govindarajan, 1982a,b; Vasala, 2004). It is a tropical plant that needs plenty of heat and humidity for good growth. It is a perennial rhizomatous plant with pale yellowish, thick-lobed rhizomes having tuberous joints. On cultivation, the plant may grow as high as 90 cm with a stalk resembling a green reed arising directly from the root and with a flowering stalk on the top (Schauenberg and Paris, 1977). The leaves are simple, distichously narrow, alternate, lanceolate, and oblong with a sheathing base. The flowering stalk or inflorescence arises directly from the root and ends in a solitary, pedunculated oblong scallop spike. The flowers are calyx superior, gamosepalous and open on one side. Even though the ginger flowers and bruised stem have the characteristic aromatic fragrance, the rhizome is the most useful part of the plant (Schauenberg and Paris, 1977). A well-grown plant may reach a height of about 90 cm in good soil and climatic conditions (Schauenberg and Paris, 1977).

Globally, ginger is cultivated in India, China, the West Indies, Jamaica, and Africa. India is the leading producer, and the plant is cultivated in most of India's states, especially Kerala, Meghalaya, Arunachal Pradesh, Mizoram,

Sikkim, Nagaland, and Orissa. Several cultivars of ginger are grown in India and are named after the localities, such as Maran, Ernad, Himachal, and Nadia. But, international cultivars like Rio-de-Janeiro also have become famous for their higher yield (Srinivasan et al., 2009). Some of the important Indian cultivars promoted by the Indian Institute for Spice Research include Rejatha, Mahima, IISR- Varada, Suprabha, Suruchi, Suravi, and Himagiri (Srinivasan et al., 2009).

Ginger needs a warm, humid climate for good yield and is cultivated successfully at an altitude of 1500 m above sea level. It grows well in a climate with moderate rainfall with well-drained soil like sandy loam that is rich in humus. It is recommended not to grow ginger in the same soil year after year, but to use crop rotation. It is best to plant ginger in the pre-monsoon period, after burning the surface soil to produce higher yield and reduce disease incidence.

The seed rhizome, a portion of rhizome, is used for propagation, measuring 2.5–5.0 cm length and weighing 20–25 g, each having one or two buds. It is manured with cattle or compost manure in split doses. The crop is harvested after 8 months of planting when the leaves turn yellow and start drying up gradually. The rhizomes are lifted carefully after digging soil and are separated from the dried leaves and roots. The rhizome is washed to clean away the soil and the outer skin is removed for domestic use. To prevent damage of oil cells that are just below the outer skin, deep scraping is avoided. The volatile oil is extracted from the rhizome/plant material by water, steam distillation, or application of microwaves and liquid carbon dioxide (Sellar, 2001).

USAGE AND APPLICATIONS

Studies have shown that ginger oil contains a plethora of biologically active compounds and that the aroma and flavor are determined by a number of factors that include the geographic origin, the maturity of the rhizomes at the time of harvest, and the method by which the extracts are prepared (Govindarajan, 1982a,b). Phytochemical studies have shown that ginger rhizomes contain 3–6% fatty oil, 9% protein, 60–70% carbohydrates, 3–8% crude fiber, about 8% ash, 9–12% water, and 2–3% volatile oil (Govindarajan, 1982a,b; Ali et al., 2008). The oil from the rhizomes also contains proteolytic enzyme zingibain, extractable oleoresins, vitamins, and minerals (Govindarajan, 1982a,b; Vasala, 2004). Ginger oil also contains the nonvolatile pungent phytochemicals of ginger consisting of gingerols, shogaols, paradols, and zingerone that contribute to the warm pungent sensation in the mouth; their concentration and ratio varies with the form of the ginger (Govindarajan, 1982a,b).

Fresh ginger is rich in gingerols, a series of chemical homologs differentiated by the length of their unbranched alkyl chains—[3–6]-, [8]-, [10]-, and [12]-gingerols—and having a side chain with 7–10, 12, 14, or 16 carbon atoms. Of all the gingerols, the compound 6-gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] is the most abundant (Govindarajan, 1982a,b). Studies with fresh Indian varieties of ginger have shown that the quantity of [6]-gingerol was about 104–965 μg/g (Govindarajan, 1982a,b). Due to the presence of a β-hydroxy keto group, these gingerols are highly labile and easily undergo dehydration to form the corresponding shogaols (Govindarajan, 1982a,b). Shogaols may be further converted to paradols by hydrogenation and are similar to gingerols (Govindarajan, 1982a,b).

The distinctive organoleptic properties of ginger are reported to be due to the steam volatile oil and their concentration is highly dependent on the growing conditions, temperature, harvesting, and processing of the ginger rhizome (Govindarajan, 1982a,b). Reports indicate that the volatile oil of ginger contains over 70 constituents and that the chief constituent sesquiterpene hydrocarbons, which are responsible for the aroma, seem to remain almost constant (Govindarajan, 1982a; Vasala, 2004). The sesquiterpene hydrocarbon α zingiberene predominates and accounts for 20–30% of the oil obtained from dry ginger (Connell and Sutherland, 1969; Yoshikawa et al., 1993). Reports also indicate that citral with its two isomers, geranial and neral, is especially high in the Brazilian-grown cultivars "Capira" (6.6–7.0% citral) and "Gigante" (14.3–20.7% citral), while it is only 1.9–4.3% in some Chinese oils. Australian oils also have high citral content, up to 27%, averaging 19% and imparting a lemony aroma to the final product. The details of the ideal characteristics in accordance with the Food Chemical Codex Standards for Ginger oil are stipulated in Table 2.

The composition of fresh ginger oil shows that it contains more oxygenated compounds (29%) compared to dry ginger oil (14%) (Sasidharan and Menon, 2010). The higher content of geranial and other oxygenated compounds makes fresh ginger oil more potent than dry ginger oil (Sasidharan and Menon, 2010). The content of hydrocarbon compounds is higher in dry ginger oil compared to fresh ginger oil (Sasidharan and Menon, 2010). Additionally, zingiberol is also another predominant aromatic component of the rhizome (Govindarajan, 1982a; Vasala, 2004). The other important constituents of ginger oil are the mono and sesquiterpenes; camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terphenol, terpenes, borneol, geraniol, limonene, β-elemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol, and α-farmesene (Govindarajan, 1982a,b). Some of the important phytochemicals of ginger oils are depicted in Figure 2.

Depending on the variety, abundance, availability, and processing units, ginger oil may be produced from fresh or dried rhizomes. Oil yield from dried rhizomes is generally from 1.5% to 3.0%. Ginger oil is extracted by steam distillation

TABLE 2 The Food Chemical Codex Standards for Ginger Oil (Anandaraj et al., 2001)

Standard	Value	ISO Method
Relative density at 20 °C	0.870–0.882	ISO 279-1981
Refractive index	1.488–1.494	ISO 280-1976
Optical rotation	−47° to −28°	ISO 592-1981
Saponification	Not more than 20	

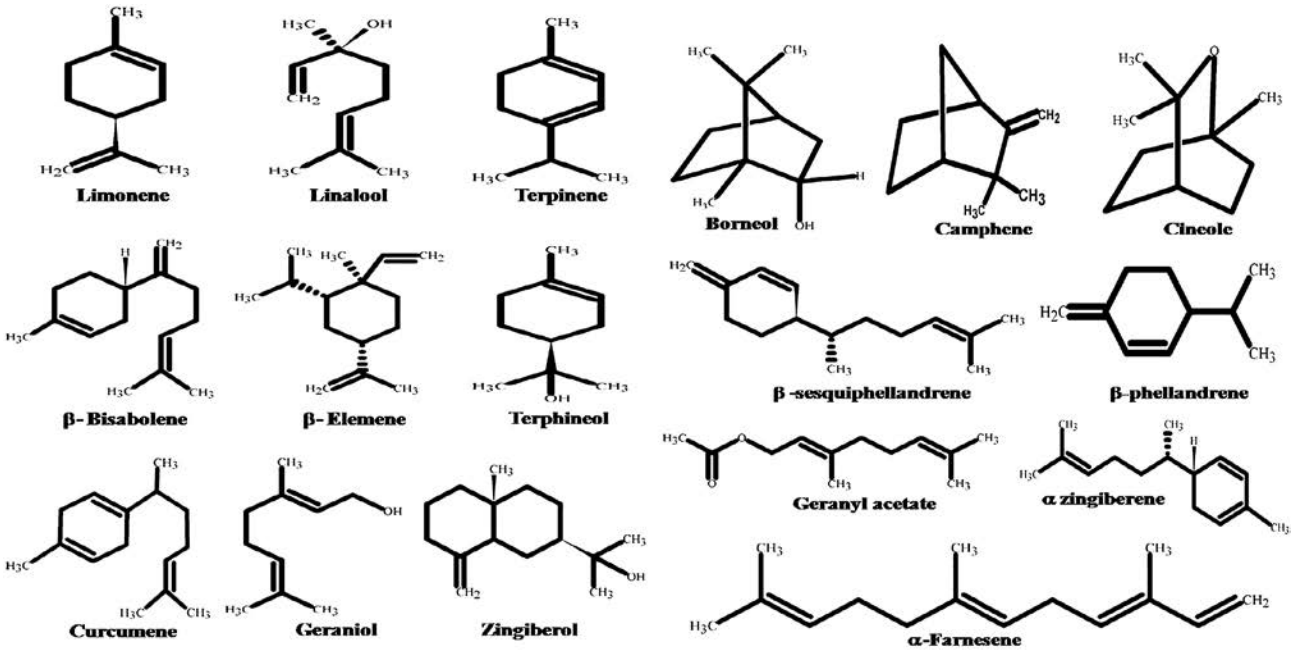

FIGURE 2 Important phytochemicals present in the ginger oil.

whereas dried rhizomes are ground to a coarse powder and loaded into a still. Following this, steam is passed through the powder to entrain the volatile components. Then these oil components are facilitated to condense with cold water as the process separates it from the water. Additionally, cohobation, or redistillation, is also shown to increase oil yield. Results published by the Indian Institute for Spice Research have shown that the Mahima variety of ginger contains 2.4% of oil while the Himagiri contains 1.6% of the essential oil. The details appear in Table 3.

When compared to fresh ginger, the oil from dried rhizomes has fewer of the low boiling point volatile compounds as most of them evaporate during the drying process. Additionally, the unpeeled rhizomes have been shown to yield more oil than peeled rhizomes. Chemical analysis has shown that the concentration of citral is lower in the oil from dried plant material than in the raw ginger. The Cochin variety of ginger has shown a yield of 1.5–2.2% of an oil rich in citral. The other low boiling point monoterpenes like α-pinene, cineole, borneol, geraniol, geranial, and neral are less abundant and present in various proportions in dried varieties.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Antimicrobial Properties

Ginger oil has been reported to possess antimicrobial effects, and studies by Natta et al. (2008) have shown that the essential oil of ginger extracted by hydrodistillation possesses high antibacterial effects on food pathogens (*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*), with a minimum concentration of 6.25 μg/mL to inhibit *B. cereus* and *L. monocytogenes* (Natta et al., 2008). Subsequent studies have shown that the oil extracted from the leaf and

TABLE 3 Characteristics of Improved Cultivars from the Indian Institute for Spice Research, Cochin, India (Anandaraj et al., 2001)

Ginger	Fresh Yield (Ton/hector)	Essential Oil (%)
Rejatha	23.2	1.7
Mahima	22.4	2.4
IISR- Varada	22.6	1.8
Suprabha	16.6	1.9
Suruchi	11.6	2
Suravi	17.5	2.1
Himagiri	13.5	1.6
Rio-de-Janeiro	17.6	2.3

rhizome was moderately active against the Gram-positive bacteria *Bacillus licheniformis*, *Bacillus spizizenii*, and *S. aureus*, and the Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas stutzeri* (Sivosathy et al., 2011).

Studies with the Gram-positive bacteria, *Bacillus subtilis* (NCIM 2162), *S. aureus* (NCIM 2602), *Micrococcus luteus* (NCIM 2704), and Gram-negative bacteria, *E. coli* (NCIM 2576), *Pseudomonas aeruginosa* (NCIM 2200), *Proteus vulgaris* (NCIM 2813), *K. pneumoniae* (NCIM 2957) have also shown ginger oil to be effective (Sayyad and Chaudhari, 2010). The results indicate that the antibacterial effects were as follows: *B. subtilis* > *S. aureus* > *E. coli* = *P. vulgaris* > *P. aeruginosa* > *M. luteus* > *K. pneumoniae* (Sayyad and Chaudhari, 2010). Ginger oil has also been shown to possess antibacterial effects on the growth of psychotropic food-borne bacteria (Fabio et al., 2003).

On a comparative note, recent studies by Sasidharan and Menon (2010) have shown that the fresh ginger oil was more effective than the dry ginger in inducing the antimicrobial effects on *Aspergillus niger*, *Candida*, and *P. aeruginosa*, weaker toward *Saccharomyces cerevisiae*, and inactive against *B. subtilis*, *Penicillium* spp., and *Trichoderma* spp.; while the dry ginger oil was more active toward *P. aeruginosa*, on par with standard toward *Candida*, weaker than standard against *B. subtilis*, *A. niger*, *Penicillium* spp., and *S. cerevisiae*. Fresh ginger oil had a minimum inhibitory concentration (MIC) value of <1 μg/mL against *A. niger* and *Candida albicans*, and dry ginger oil had an MIC value of less than 1 μg/mL against *P. aeruginosa*, *Penicillium* spp. and *C. albicans*. Fresh ginger is more abundant in oxygenated compounds, and the observed variance in the antimicrobial effects is attributed to this factor (Sasidharan and Menon, 2010).

Nanasombat and Lohasupthawee (2005) studied the antibacterial effects of the fresh ginger oil in 25 standardized bacterial strains (20 serotypes of *Salmonella* and 5 species of other enterobacteria) commonly involved in the spoilage of food and associated with food-borne illness. The observed antibacterial effects were as follows: *Serratia marcescens* > *Klebsiella pneumoniae* = *S. Typhimurium* DT104 (8748A-1) > *E. coli* (DMST 4212) > *S. Anatum* (DMST 7108) = *S. Choleraesuis* ssp. *Choleraesuis* (DMST 8014) = *S. Derby* (DMST 8535) = *S. Enteritidis* (DMST 10,633) = *S. Hadar* = *S. Newport* (DMST 7101) = *S. Newport* (DMST 7101) = *S. Orion* (SAP 08,991/02) = *S. Rissen* (DMST 7097) = *S. Senftenberg* (DMST 7113) = *S. Typhimurium* (DMST 0562, non-DT104 strain) = *Citrobacter freundii* (DMST 1959) > *S. Virchow* (DMST 10,635) = *Salmonella Agona* (DMST 10,338). The essential oil extract of ginger showed significant reduction in total vial count, *Staphylococcus*, *E. coli*, and *Salmonella* counts at dilution of 1:150 and 1:250 than the dilution of 1:500. The dilution at 1:150 is the best dilution for the effective reduction of bacterial counts for both Gram-positive cocci (*Staphylococcus*) and Enterobacteriaceae (*E. coli* and *Salmonella*), which are the main contaminants seen in poultry meat (Sudharshan et al., 2010).

Ginger Oil Prevents Oxidative Damage of Food

The observation that certain synthetic compounds like the synthetic antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), popyl gallate (PG), and tertbutylhydroquinone (TBHQ) can retard/inhibit the process of lipid peroxidation and thereby increase the shelf life of food paved the way for using these compounds to prevent/inhibit rancidity. However, the use of these synthetic antioxidants needs to be highly monitored as at higher concentrations they can

impart ill effects—therefore, they are strictly regulated (Hettiarachchy et al., 1996). Additionally, many consumers are increasingly avoiding foods prepared with preservatives of chemical origin due to their possible ill effects. In lieu of these observations, a need for alternative antioxidant compounds was determined and studies have shown that many aromatic spices and some phytochemicals are beneficial. Scientific studies carried out in accordance with the principles of free radical biology assays have shown that ginger oil was effective in inhibiting hydrogen peroxide-induced oxidative damage (Lu et al., 2003). This observation validates its use in inhibiting the free radical damage, preventing rancidity, and increasing the shelf life of food.

CONCLUSIONS

This chapter has summarized the validity of using ginger oil in the food industry, which has been extensively reported. Ginger's antimicrobial properties and ability to prevent free radical-induced damage and rancidity help increase the shelf life of food. Widespread application of ginger in food preservation will provide food with desirable organoleptic properties, increase the shelf life, and prevent wastage. Detailed studies are required to understand more effective means of combining ginger with other food preservation methods as this will make precious food available for the needy.

SUMMARY

- *Zingiber officinale*, commonly known as ginger, is one of the most commonly used spices worldwide with medicinal value. It has been cultivated for thousands of years.
- Marinating or usage of ginger in food preparation helps prevent food spoilage.
- Ginger rhizomes contain a plethora of biologically active compounds like zingiberol, the mono and sesquiterpenes, camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, β -elemene, linalool, α-zingiberene, β sesquiphellandrene, β bisabolene, zingiberenol, and β –farmesene.
- Ginger oil has antimicrobial activity against Gram-positive, Gram-negative microbes as well as fungi like *A. niger* and *Candida*, which are responsible for food spoilage.
- Ginger oil has been found to retard/inhibit the process of lipid peroxidation and thereby increase the shelf life of food, which paved the way for using it to prevent rancidity.
- Ginger oil possesses free radical scavenging, antioxidant, and antiperoxidative effects.

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Chapter 51

Grape Seed (*Vitis vinifera*) Oils

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INTRODUCTION

Humans have relied on plants as medicine for centuries and their use for treatment of ailments and diseases is practiced in many parts of the world despite the widespread practise of modern medicine and the use of synthetic drugs worldwide. Many natural products are used as adjuvants/prophylactics or for therapeutic purposes. Grapes have attracted much interest due to their frequent use as fruit or in the processed form as wine. Extracts from skin/whole blue grapes, grape juice, and wine were reported to possess important abilities such as scavenging free radicals, reducing activity, chelating prooxidant metals, inhibiting lipid peroxidation, and quenching singlet oxygen. Usually grape seed oil is not regarded as an essential oil. However, several volatile compounds were identified in grape seed oil, such as ethyl octanoate, ethyl acetate, ethanol, acetic acid, ethyl hexanoate, and 3-methylbutanol (Bail et al., 2008), which may overlap with the requisite characteristic "volatility and the plant origin are the characteristic properties of essential oils" (Tyagi, 2013). Grape seed oil has been considered as an essential oil and investigated for antibacterial activity by Si et al. (2006). Phenolic compounds from grapes and wine, also found in oil, exhibited numerous activities including antioxidant and antimicrobial activities (Bekhit et al., 2008; Cheng et al., 2012) and there is growing evidence for immunomodulation properties of grape polyphenols (Mitjans et al., 2004) which can be beneficial in designing novel healthy food products and drinks. This chapter covers information on the biological activities of grape seed oil.

BOTANICAL ASPECTS

Grapes are ellipsoid berries that appear in clusters on deciduous woody vines of the genus *Vitis*. Grape cultivation started in the Near East about 60–80 centuries ago and then spread to Europe, North Africa, and finally North America. Yeast occurs on grape peels and subsequent fermentation results in the production of alcoholic drinks such as wine. Several native *Vitis* species grew wildly and consumed by the natives in North America but were not considered to have the appropriate quality for wine making by European colonists. *Vitis vinifera* cultivars were imported for that purpose. Grapes come in a variety of colors including crimson, black, dark blue, green, and yellow. It is believed that mutations in the genes regulation the synthesis of anthocyanin cause the formation of green/white grapes.

Most grapes are from cultivars of *V. vinifera* native to Central Asia and the Mediterranean. Some grapes and wine originate from American and Asian species such as *Vitis labrusca*. Several *Vitis* species, such as *Vitis riparia* which grows wildly in North America and *Vitis rotundifolia* which is native of the South Eastern United States, are used for making wine and jam. For the Asian region, *Vitis amurensis* is considered the most important Asian species. Commercially cultivated grapes are either table grapes or wine grapes, depending on whether they are to be eaten raw (table grapes) or utilized for wine production (wine grapes) depending on their sensory profile, especially the sugar content and acidity of the fruits. Several differences are found between *V. vinifera* table grapes and wine grapes due to selective breeding techniques employed in their production. Table grapes are larger and thinner-skinned than wine grapes and often seedless. They are vegetatively propagated by cuttings. Wine grapes are often seeded and their thick skin contributes to the aromatic flavor in wine. Wine grapes have higher sugar content than table grapes. The world vineyard area was increased by 4.0% over the period from 2008 to 2011 (from about 17.8 to 18.5 million acres) (http://www.wineinstitute.org/resources/statistics). The highest reported increase in vineyard areas were: China (54.0%), Réunion (50.0%), Uzbekistan (32.1%), Greece (27.9%), India (27.9%), Iraq (26.3%), Cuba (26.7%), Colombia (25.3%), Azerbaijan (24.2), Afghanistan (21.5%), and New Zealand (21.3%). Most of the grown grapes are destined for wine and grape juice production. During the wine making process, several waste streams are generated with an estimated 20% of fresh grapes used end up as residues, and wine making generates about five to nine million tonnes of wastes annually (Lafka et al., 2007). This waste stream has offer great potential for the production of grape seed oil as well as other valuable by-products.

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USAGE AND APPLICATIONS

Grapes have been used as food and eaten raw or producing a variety of products (grape seed extract, grape seed oil, grape juice, wine, brandy, vinegar, raisins, jam, and jelly). Grape seed oil is used for cooking purposes.

MOLLUSCIDAL AND INSECTICIDAL ACTIVITIES

Grape seed alcoholic extracts brought about mortality in the snail (*Biomphalaria alexandrina, Lymnea cailliaudi*) but did not exert snail ovicidal or larvicidal effects on *Culex pipiens* (Taher et al., 2012).

ADSORPTION OF MYCOTOXINS

Grape pomace (pulp and skin) could speedily and concurrently remove in vitro a number of mycotoxins from liquid media, with the biosorbent efficacy in the order: aflatoxin B1 > zearalenone, ochratoxin A, and fumonisin B1 >> deoxynivalenol (Avantaggiato et al., 2014).

COSMETIC APPLICATIONS

A mixture of grape seed oil, linseed oil, retinyl palmitate, tocopheryl acetate, and coenzyme Q_{10} encapsulated in nanoparticles produced a significant wrinkle-reducing effect after topical application for three weeks (Felippi et al., 2012). A patent by Spiers and Cleaves (1999) claims correction of face scar and discoloration as well as relieving chronic dry skin symptoms by the use of grape seed oil. Grape seed oil has an abundance of the essential fatty acid linoleic acid, vitamins, and minerals that are beneficial to the skin. Grape oil has a wound-healing potential as revealed by a study using an excision wound rat model (Shivananda Nayak et al., 2011). Wine lees extracts have antiinfluenza virus activity (Figure 1) and may be useful in sanitation applications.

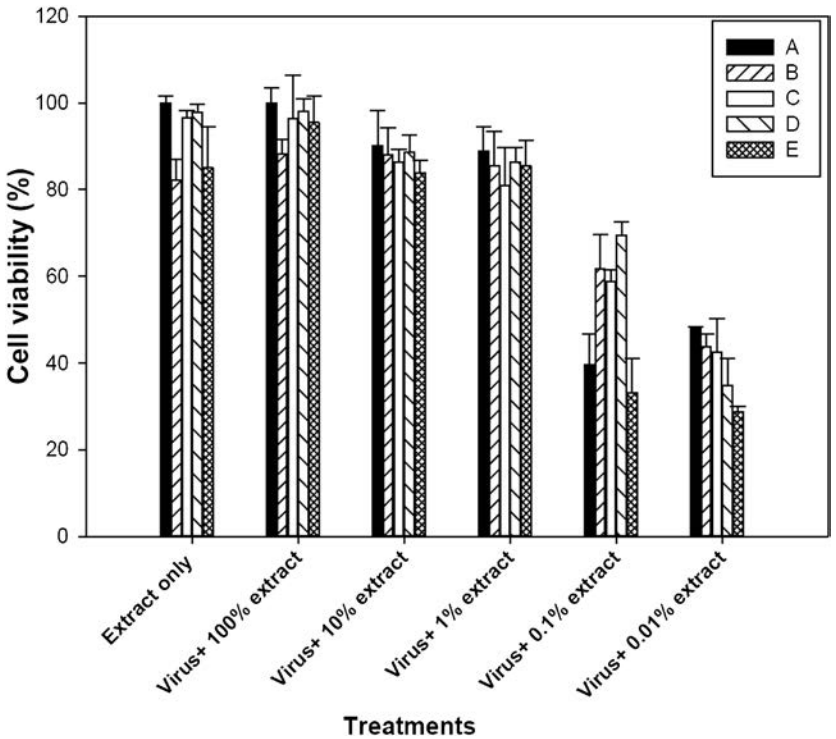

FIGURE 1 Effect of wine lees extracts (100, 10, 1, 0.1, and 0.01 mg/mL) on inhibition of influenza virus in MDCK cells. Percentage cell viability infected with influenza virus was 33.2 ± 7.3%. A, South Island Shiraz; B, South Island Pinot Noir after fermentation; C, North Island Pinot Noir after fermentation; D, North Island Pinot Noir after aging, and E, South Pinot after aging. *Data are from Bekhit et al. (2008) and are expressed as mean ± SD (n = 3). Reproduced by kind permission from Elsevier, permit No. 3340080721049.*

USAGE AND APPLICATIONS IN FOOD SCIENCE

Chemical Composition

Tangolar et al. (2009) investigated fatty acid profiles and mineral content of grape seed oil of seven grape cultivars (Alicante Bouschet, Alphonse Lavallée, Horoz karasi, Muscat of Hamburg, Narince, Razaki, and Oküzgözü) and two rootstocks (Cosmo two and Salt creek). It was found that the oil content varied with the cultivar, ranging from 10.45% (Razaki) to 16.73% (Salt creek). In all genotypes the major fatty acids comprised linoleic acid, oleic acid, and palmitic acid. The saturated fatty acid content was below those of monounsaturated and polyunsaturated fatty acids. All varieties had an appreciable quantity of macroelements and microelements. Thus, grape seed extract is valuable as a dietary supplement (Tangolar et al., 2009).

Grapes at the initial stage of berry development gave a meager oil yield and the oil was characterized by elevated sterol content and a dissimilar fatty acid composition compared with grapes at later stages. However, oil from grapes collected prior to and following veraison did not differ substantially with regard to oil yield, fatty acid composition, and total sterol content and composition.

Analysis of the chemical composition of virgin oils derived from grape seeds of different varieties disclosed that the two most abundant fatty acids were linoleic acid and oleic acid and that β-sitosterol was the predominant phytosterol. The volatile compounds comprised 1-butyl-3-methylacetate, *trans*-2-hexenal, E-2-pentenal, hexanal, and heptanal which may account for the fruity flavor. Tocopherols and α and γ isomers of the tocotrienols which are cardioprotective were also present. The deep green color was attributed to chlorophylls and other pigments (Navas, 2009).

The predominant fatty acids in the Pkatsiteli grape seed oil were palmitic, oleic, and stearic acids. De Marchi et al. (2012) reported that trilinolein was the most abundant triglyceride in seed oil samples from 32 hybrid grape varieties, followed by dilinoleoyl-oleoylglycerol, and dilinoleoyl-palmitoylglycerol.

The chemical compositions of grape seed oil from three varieties of *V. vinifera* (Cabernet Sauvignon, Merlot, and Moscato Giallo), and two varieties of *V. labrusca* (Bordô and Isabel), harvested in 2005 and 2006, were compared after extraction using supercritical fluid system. Bordô, 2005 and 2006, and Merlot, and Moscato Giallo, 2006 had the highest concentrations of palmitic, stearic, and linoleic acids. The highest oleic acid and α-tocopherol concentrations were found in Bordô 2005 and 2006. A comparison of the fatty acid profiles of grape seed oils from different countries is presented in Table 1. Grape seed oil is rich in procyanidins and other valuable vitamins and minerals. Procyanidins did not show much variation among the varieties. Bordô and Cabernet Sauvignon had higher total phenolic concentration than the other studied varieties. Cheng et al. (2012) reported the composition of various phenolic compounds in New Zealand grapes (Tables 2 and 3). Catechins and procyanidins are the major phenolics found in the grape seeds.

In grape seed oil, hexacosanol is the most abundant policosanol, followed by octacosanol, tetracosanol, and triacontanol. The grape seed oil concentration varied from 7.3 to 22.4% among different grape varieties. Linoleic acid was the predominant constituent, followed by oleic acid, palmitic acid, and stearic acid. α-tocopherol was present at higher concentrations than γ-tocopherol and δ-tocopherol. β-tocopherol was not detected in grape seed oil. The levels of α-tocopherol, γ-tocopherol, and δ-tocopherol in Turkish grape seed varieties are in the range of 10.5–18.25 and 20.51–34.01; 0.94–1.86 and 2.30–5.58; and 0.14–0.34 and 0.09–0.20 mg/kg seed for hot extracted and cold extracted oils, respectively. It is interesting to note that while

TABLE 1 Effects of Origin and Grape Variety (Red vs White) on the Fatty Acids Profile in Grape Seed oil. The Results are Presented as Percentage Fatty Acid of the Extracted Oils

		Fatty Acid (%)					
Origin	**Variety**	**14:0**	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**
American	White	0.04–0.07	6.8–8.1	2.7–3.5	15.9–21.5	66.9–73.9	Traces
	Red	0.04–0.06	5.8–7.1	2.5–3.1	13.7–20.3	69.7–77.8	Traces
Turkish	Various	ND	6.5–9.0	4.0–5.4	17.8–25.5	60.1–70.1	0.0–0.9
India	Unknown	8.1	3.5	–	19.5	66.7	1.7
Spain	White	ND	8.12	5.6	19.59	66.16	0.37
New Zealand	Red	0.01	6.76	3.71	12.38	76.23	0.45

TABLE 2 Phenolics Profile of Extracts (mg/g Extract) Obtained from Pinot Noir Wine Residues Using 50% (v/v) Extraction Solvents of Acetone/Water (Ace/W), Ethanol/Water (Eth/W), and Methanol/Water (Meth/W) as Quantified by Liquid Chromatography–Mass Spectrometry Analysis

Groups	Phenolic Compounds[a]		Pinot Noir Seed			Pinot Noir Skin			Pinot Noir Pomace		
			Ace/W	Eth/W	Meth/W	Ace/W	Eth/W	Meth/W	Ace/W	Eth/W	Meth/W
Nonflavonoids	Gallic acid		0.37	0.39	0.52	0.18	0.16	0.17	0.33	0.29	0.28
Flavonoids	Flavanols	Catechin	13.41	14.31	15.18	1.08	0.61	0.64	10.01	8.97	11.81
		Epicatechin	8.79	9.65	10.23	0.72	0.34	0.35	6.74	5.70	5.00
		Epicatechin gallate	0.16	0.14	0.16	0.03	0.01	–	0.13	0.09	0.08
		Procyanidin A[b]	6.02	5.67	7.80	0.52	0.30	0.29	4.39	3.93	3.81
		Procyanidin B[b]	5.01	5.45	6.07	0.49	0.28	0.27	3.98	3.30	2.88
	Anthocyanins	Delphinidin-3-glucoside[c]	–	–	–	0.49	0.38	5.31	0.18	0.20	0.10
		Cyanidin 3-glucoside[c]	–	–	–	–	0.06	0.07	–	–	0.02
		Petunidin-3-glucoside[c]	–	–	–	1.19	0.98	1.17	0.53	0.53	0.34
		Peonidin-3-glucoside[c]	–	–	–	0.95	0.66	0.78	0.46	0.43	0.33
		Malvidin-3-glucoside[c]	–	–	–	10.64	8.36	9.95	5.94	5.42	5.18
		Vitisin[c]	–	–	–	0.14	0.04	0.07	0.07	0.05	0.04
		Total anthocyanin	–	–	–	13.41	10.48	17.35	7.18	6.63	6.01
	Flavonols	Quercetin	0.30	0.27	0.20	1.923	1.54	0.66	0.65	0.68	0.33
		Quercetin methyl-glucoside	0.24	0.25	0.22	2.135	1.77	1.25	0.82	0.82	0.32

[a]*Values (mg/g of extracts) are the means of duplicate samples.*
[b]*Data expressed as mg of catechin equivalent per g extracts.*
[c]*Data expressed as mg of malvidin equivalent per g extracts.*
Data from Cheng et al. (2012). Reproduced With Kind Permission From Elsevier, Permit No. 3340080285928.

TABLE 3 Phenolics Profile of Extracts (mg/g Extract) Obtained from Pinot Meunir Wine Residues Using 50% (v/v) Extraction Solvents of Acetone/Water (Ace/W), Ethanol/Water (Eth/W), and Methanol/Water (Meth/W) as Quantified by Liquid Chromatography–Mass Spectrometry Analysis

Groups	Phenolic Compounds[a]		Pinot Menuir Seed			Pinot Menuir Skin			Pinot Menuir Pomace		
			Ace/W	Eth/W	Meth/W	Ace/W	Eth/W	Meth/W	Ace/W	Eth/W	Meth/W
Nonflavonoids	Gallic acid		0.63	0.58	0.63	0.01	0.003	0.01	0.12	0.08	0.08
Flavonoids	Flavanols	Catechin	8.49	17.38	11.85	0.16	0.12	0.13	4.35	3.61	3.50
		Epicatechin	18.32	15.21	15.05	0.11	0.08	0.09	3.12	2.13	2.13
		Epicatechin gallate	0.44	0.22	0.25	0.01	0.04	0.01	0.04	0.03	0.03
		Procyanidin A[b]	11.52	9.44	11.37	0.10	0.09	0.09	0.83	0.77	0.69
		Procyanidin B[b]	7.47	7.01	7.28	0.08	0.08	0.08	0.75	0.58	0.47
	Anthocyanins	Delphinidin-3-glucoside[c]	–	–	–	0.07	0.06	0.06	0.06	0.06	0.05
		Cyanidin 3-glucoside[c]	–	–	–	0.05	0.04	0.03	0.04	0.03	0.04
		Petunidin-3-glucoside[c]	–	–	–	0.22	0.19	0.18	0.16	0.13	0.14
		Peonidin-3-glucoside[c]	–	-	–	1.13	0.75	0.75	0.76	0.57	0.62
		Malvidin-3-glucoside[c]	–	–	–	3.78	2.34	2.46	2.44	1.74	1.90
		Vitisin[c]	–	–	–	0.03	–	0.01	0.01	–	0.01
		Total anthocyanin	–	–	–	5.28	3.38	3.49	3.47	2.55	2.75
	Flavonols	Quercetin	–	–	0.14	0.15	0.15	0.14	0.16	0.18	0.15
		Quercetin methyl-glucoside	0.18	0.17	0.15	0.50	0.44	0.44	0.45	0.42	0.39

[a]*Values (mg/g of extracts) are the means of duplicate samples.*
[b]*Data expressed as mg of catechin equivalent per g extracts.*
[c]*Data expressed as mg of malvidin equivalent per g extracts.*
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higher tocopherols can be extracted in cold extracted grape seed oil compared with hot extracted oil, the case was the opposite with grape pomace and bagasse. The use of ethanol to remove tannin from grape seed reduced the tocopherols content in the oil (Oomah et al., 1998).

Virgin grape seed oil is characterized by a pleasant and fruity taste and aroma, whereas the refined oil normally has a neutral taste and aroma (Matthaus, 2008). Upon storage, degradation products accumulate and several aroma-active volatiles (e.g., acetic acid, ethyl acetate, and ethanol) are formed and give rise to off-notes. The degradation rate of the oil is accelerated by the presence of any sediment or fine materials transferred from the seed during pressing (Matthaus, 2008).

Use as Replacement in Food Products

The consequences of reducing pork lipid levels from 30% to 20% and partly replacing the pork fat with a mixture of grape seed oil (0%, 5%, 10%, and 15%) and 2% rice bran fiber were examined based on chemical composition, cooking characteristics, physicochemical characteristics, textural characteristics, and viscosity of reduced-fat meat batters. The ash content, moisture content, uncooked pH value, cooked pH value, yellowness, cohesiveness, chewiness, gumminess, and sarcoplasmic protein solubility were increased in the decreased-fat meat batters made with grape seed oil and rice bran fiber compared with control samples. The decreased-fat meat batters with escalating grape seed oil concentrations were characterized by lower cooking loss, emulsion stability, and apparent viscosity. The changes were all desirable. Similarly, decreasing pork fat levels in frankfurters from 30% to 10% and partly replacing the pork fat with grape seed oil ensued in higher moisture and ash content; lower caloric values, cholesterol level, *trans*-fat level, and fat content; increased pH and cooking yield; and comparable sensory properties compared with control frankfurters containing pork fat (Choi et al., 2010).

Antimicrobial Activity

The total phenolic content in grape seed oil ranged between 59 μg/g and 115.5 μg/g oil, depending on the grape variety (Bail et al., 2008) and was higher in virgin grape oils. The phenolic compounds in grape oil will probably exhibit similar activities to those found in grape seed extract. Chitosan films containing grape seed extract (10 g/kg) used to wrap mortadella sausages demonstrated inhibitory effects on lipid oxidation and growth of aerobic mesophiles and *Listeria monocytogenes* (Moradi et al., 2011).

In grapes infected with *Botrytis cinerea*, ethylene emission increased while *trans*-resveratrol decreased. A brief (5 s) immersion of the grapes in *trans*-resveratrol solution curtailed the concentration and rate of ethylene given off. The treatment also doubled the shelf-life of grapes at room temperature and preserved the postharvest quality for 10 days (Montero et al., 2003).

Refrigeration of eggs may not be feasible in some developing countries. Coating of eggs with grape seed oil, sunflower oil, soybean oil, olive oil, corn oil, or canola oil to facilitate storage at 25 °C for 5 weeks has been found to be efficacious in preserving the eggs and preventing microbial contamination for prolonging shelf-life (Ryu et al., 2011).

Use as Carrier for Essential Oils

Owing to its excellent physicochemical properties, grape seed oil is commonly used as a carrier for other essential oils (Thompson et al., 2013).

Antioxidant Activity and Inhibitory Effects on Generation of Heterocyclic Aromatic Amines during Frying of Beef Patties

Natale et al. (2014) examined the suppressive action of liposome-encapsulated grape seed extract on the generation of heterocyclic aromatic amines during frying of beef patties. 2-amino-1-methyl-6-phenylimidazo[4,5b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), Norharman, and Harman were detected following marinade application and frying. There was a decline in PhIP level after marinating with grape seed extract (0.1%) and grape seed extract-containing liposomes (1% and 5%). MeIQx level declined but no alterations in β-carboline levelswere discernible. Liposomal encapsulation of grape seed extract failed, compared with grape seed extract, to produce further inhibition of the generation of MeIQx and PhIP. The data appeared to be contradictory to earlier findings that liposomal encapsulation, and also a water-in-oil marinade with grape seed extract being encapsulated in water droplets suspended in an oil phase, augmented the efficacy of polyphenols to suppress radical reactions and lipid oxidation in model systems. Natale et al. (2014) attributed the discrepancy in the observations to mechanisms affected by the structural and physical features of the

encapsulation system employed and its interaction with the application matrix involving diffusion and partitioning behavior and interaction behavior (Natale et al., 2014).

SUMMARY POINTS

- Owing to its excellent physicochemical properties, grape seed oil is commonly used as a carrier for other essential oils.
- Linoleic acid is the predominant constituent in grape seed oil, followed by oleic acid, palmitic acid, and stearic acid. α-tocopherol is present at higher concentrations than β-tocopherol, γ-tocopherol, and δ-tocopherol.
- Grape seed extract has molluscidal, insecticidal, and antimicrobial activities.
- Decreasing pork fat levels in frankfurters and partly replacing the pork fat with grape seed oil ensued in higher moisture and ash content; lowered caloric values, cholesterol levels, *trans*-fat level, and fat content; increased pH and cooking yield; and had comparable sensory properties
- Grape seed oil has some antimicrobial activity which can be made use of in the coating of eggs with grape seed oil for prolonging shelf-life.

DISCLAIMER

This article was written in a personal capacity (E.F.F.) and does not represent the opinions of the United States Food and Drug Administration, the United States Department of Health and Human Services, or the United States Federal Government.

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Chapter 52

Grapefruit (*Citrus paradisii*) Oils

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INTRODUCTION

Grapefruit essential oil, although less renowned than some of the other essential oils, is regarded as a potent oil with several food and therapeutic applications. It is produced by cold-pressing the peel (rind) for extracting the oil. The oil has been found to possess **a**ntioxidant, antiseptic, disinfectant (due to antiviral and antimicrobial activities), diuretic, and stimulant properties. Grapefruit essential oil can be inhaled for its energizing and soothing aroma, applied to young or sensitive skin, used in cooking, or consumed as a dietary supplement. However, it is worth noting that grapefruit oil inhibits cytochrome P-4503A4 and thus interferes with the metabolism of some therapeutic drugs if taken orally.

BOTANICAL ASPECTS

Grapefruits occur as clusters like grapes on the subtropical evergreen tree. The fruit is approximately spherical in shape with a diameter of four to five inches. It has a yellow- or orange-colored peel and pink or red pulp. It may or may not have seeds depending on the variety. It is juicy like oranges and its taste ranges from acidic and sour to sweet due to grapefruit mercaptan, a sulfur containing terpene. The foliage is long, slender, and dark green. The flowers are white with four petals. In the 1830s, the species name *Citrus paradisii* was first given to grapefruit. The species name *paradisii* refers to its wonderful flavor. Earlier it was mixed with and not distinguished from the pomelo. When its name is written as *Citrus*×*paradisii,* it refers to its hybrid origin from the pomelo or shaddock (*Citrus maxima*) and the sweet orange (*Citrus sinensis*).

USAGE AND APPLICATIONS

As Gutta-perch Solvent

Grapefruit oil and tangerine oil were better in performance as gutta-perch solvents than lime oil and lemon oil in root canal retreatment procedures.

Insecticidal Activity and Antifeedant

Grapefruit peel oil exerted toxicity to adults of the housefly *Musca domestica.* Grapefruit oil had high fumigant activity against adult cowpea beetles *Callosobruchus maculatus* (Moravvej and Abbar, 2008).

The nootkatone in grapefruit extract displayed toxicity against fleas with an LC50 (concentration lethal to 50% of fleas) as low as 0.0029% and against unfed nymphs of the ticks *Rhipicephalus sanguineus*, *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* (Flor-Weiler et al., 2011). A lignin-encapsulated formulation of nootkatone has improved toxicity for tick control and diminished volatility and phytotoxicity. Thus it is promising as a safe and environmentally friendly alternative for biocontrol of disease vectors. Joffe et al. (2012) demonstrated the higher efficacy of piperonyl butoxide than natural oils examined including grapefruit oil, bergamot oil, neem oil, dillapiole oil, and parsley oil in synergism with pyrethrum in anti-housefly activity, which brought about 100% mortality in insecticide-susceptible

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World Health Organization (WHO) and insecticide-resistant 381 zb housefly strains. Using WHO houseflies, grapefruit oil resulted in higher mortality compared to unsynergized pyrethrin (50% and 18%, respectively).

Grapefruit oil showed high insect-repellent activity against cockroaches (*Periplaneta americana* and *Periplaneta fuliginosa*), and its activity was higher than that exhibited by lemon, lime, and orange oils (Yoon et al., 2009).

Grapefruit essential oil exhibited various levels of effectiveness in controlling the population of *Callosobruchus maculatus*, *Culex pipiens*, and *Musca domestica* with effects ranging from reduction in egg viability, insecticidal activity against larvae, and negative impact on the growth of pupae and adults and reduction in fecundity of survived adult insects. Grapefruit oil components (limonene, α-pinene, and β-myrcene) were the most effective repellents against *Sitophilus oryzae*.

Furthermore, grapefruit oil was a more effective repellent against the German cockroach *Blattella germanica*, the American cockroach *Periplaneta americana*, and the smoky brown cockroach *P. fuliginosa* compared with essential oils from caraway seed, clary sage, clove leaf, coriander, eucalyptus, lemon citrus, lime citrus, marjoram, myrrh, orange citrus, petitgrain citrus, pine needle, rosemary, spearmint, strawberry, and ylang-ylang.

The LC_{50} and LC_{90} values obtained for second instars of the yellow fever vector *Aedes aegypti* after exposure to grapefruit peel essential oil for 24 h were 180.460 and 334.629 ppm, respectively, and corresponding values for fourth instars were 210.937 and 349.489 ppm, respectively (Ivoke et al., 2013).

Anti-elastase Activity

In aroma massage, grapefruit essential oil, diluted with vegetable oil, is rubbed onto the skin; it penetrates the skin and acts on the dermis. Grapefruit essential oil has been shown to inhibit porcine and human elastases, enzymes that catalyze the breakdown of dermal elastin thereby bringing about cutaneous wrinkling and aging (Mori et al., 2002), and therefore this oil may have potential applications in skin care products.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Composition

Aurapten (7-geranyloxycoumarin) found in the peel, sarcocarp, and juice of grapefruit (Takahashi et al., 2002), and decanal, octanal, and citral in grapefruit essential oil are chemopreventive compounds. Bergamottin (5-geranoxypsoralen) has been reported as a principal constituent of grapefruit oil. The chiral coumarin meranzin and epoxyaurapten and the furocoumarin epoxybergamottin are present in grapefruit essential oils (Dugo et al., 2012).

Polymethoxylated flavones including tangeretin (4′,5,6,7,8-pentamethoxyflavone), nobiletin (3′,4′,5,6,7,8-hexamethoxyflavone), 3,3′,4′,5,6,7,8-heptamethoxyflavone and tetra-O-methylscutellarein (4′,5,6,7-tetramethoxyflavone) were the oxygen heterocyclic compounds identified in grapefruit essential oils (Dugo et al., 2000). Volatile compounds emitted by flushing shoots of Rio Red grapefruit included alpha-pinene, sabinene, beta-pinene, beta-myrcene, (Z)-3-hexen-1-ol acetate D-limonene, (Z)--ocimene, (E)--ocimene, linalool, allo-ocimene, neo-allo-ocimene, alpha-terpineol, neral, geranial, beta-copaene, beta–elemene, alpha-bergamotene, beta-caryophyllene, beta-farnesenee, alpha-caryophyllene, and delta-cadinene (Patt and Sétamou, 2010).

Limonene is the main volatile constituent of cold-pressed essential oil from redblush grapefruit peel. Alpha-pinene, alpha-terpinene, alpha- and beta-sinensal, alpha-cubebene, (E,E)-alpha-farnesene, beta-caryophyllene, decanal, citronellal, octanal, (Z)-carvone, perillene, (E)-carveol, heptyl acetate, nootkatone, perillyl acetate, methyl-N-methylanthranilate, and (Z,E)-farnesol are prominent compounds found in grapefruit oil (Njoroge et al., 2005). A summary of the components of the grapefruit oil is shown in Table 1. The origin of the material can lead to expression of different levels and new levels of compounds in the oil such as with the Kenyan Redblush variety (Table 1). The concentration of nootkatone in the oil is used as criterion for the oil quality. The concentration of nootkatone increases with the increase in storage temperature and time.

The application of wax decreased the levels of nonanal and nootkatone (Table 2). The levels of 3-carene, *trans*-linalool oxide, beta-Pinene, ocimene, octanol, alpha-phellandrene, and *cis-p*-mentha-2,8-dien-1-ol were increased, whereas limonene level decreased, with the increase in storage temperature. Levels of citronellal, linalool, dodecanal, limonene, alpha-humulene, alpha-pinene, neral, and alpha-terpineol decreased, whereas the levels of 3-carene, alpha-phellandrene, ocimene, and *trans*-linalool oxide increased with storage time. Some aldehydes, alpha-pinene, alpha-terpineol, and limonene that are toxic to the Caribbean fruit fly decreased with storage time, which explains the vulnerability of grapefruit to the fly during storage (Sun and Petracek, 1999).

Sodium orthophenylphenate has been employed extensively for over four decades to control diseases in grapefruits after harvest. The compound is metabolized to produce orthophenylphenol as the major metabolite and

TABLE 1 Composition (%) of Components of Grapefruit Oil

Chemical Class	Compound	%
Alcohols	Decanol	0.04
	Linalool	0.1–0.3
	1-Nonanol	0.1–0.8
	Terpinen-4-ol	0.08
	α-Terpineol	0.1–0.2
Aldehydes	Citral	0.05–0.06
	Citronellal	0.14–0.2
	Decanal	0.3–0.6
	Dodecanal	0.1–0.2
	Furfural	0.1
	Geranial	0.2
	Heptanal	0.05–0.06
	Neral	0.03
	Nonanal	0.04–0.1
	Octanal	0.3–0.71
	Perillaldehyde	0.2
	Tetradecanal	0.1
Esters	Decyl acetate	0.15
	1-*p*-Menthen-9-yl acetate	0.2
	Octyl acetate	0.09–0.1
Hydrocarbons	Δ-Cadinene	0.1
	Caryophyllene	0.3
	α-Copaene	0.06
	β-Copaene	0.01
	p-Cymene	0.4
	β-Elemene	0.06
	Limonene	85.5–95
	α-Muurolene	1.4–1.9
	Myrcene	2.1–2.12[a]
	Nonane	0.4
	Ocimene	0.8
	β-Phellandrene	0.1–0.7
	α-Pinene	0.5–1.6
	Sabinene	0.7
	α-Terpinene	0.1
	γ-Terpinene	0.5–0.8
Ketones	Nootkatone	0.3–0.8

Continued

TABLE 1 Composition (%) of Components of Grapefruit Oil—cont'd

Chemical Class	Compound	%
Miscellaneous	*cis*-Linalool oxide	0.03
	trans-Linalool oxide	0.03
Non-volatiles		1.37–9.5
	Heptyl acetate[b]	0.5
	α-Cubebene[b]	0.1
	Linalyl acetate[b]	0.1
	α-Cedrene[b]	0.1
	Sabina ketone[b]	0.2
	Carvone[b]	0.4
	(E,E)-R-farnesene[b]	0.1
	Cumin aldehyde[b]	0.1
	(Z)-carveol[b]	0.3
	(E)-carvone[b]	0.1
	(E)-carveol[b]	0.2

[a]*Calculated with sabinene.*
[b]*Found in Kenyan redblush grapefruit oil (Njoroge et al., 2005).*
Collated from Wilson and Shaw (1978), Shaw (1979), Njoroge et al. (2005).

phenylhydroquinone as a minor metabolite. The maximum whole fruit tolerance for orthophenylphenol in the United States is 10 parts per million (ppm). Grapefruits treated with sodium orthophenylphenate using foamer wash and shipping wax applications contain below the 10 ppm tolerance threshold, and phenylhydroquinone residues were all at or below 0.439 ppm. Increasing the storage time leads to higher phenylhydroquinone residues in whole fruit (Johnson et al., 2001).

A cyclic acetal of marmin (6′,7′-dihydroxy-7-geranyloxycoumarin), two cyclic acetals of 6′,7′-dihydroxybergamottin, and marmin, 7-geranyloxycoumarin, bergamottin, and 6′,7′-dihydroxybergamottin were found in the oil of grapefruit peel. Coumarins (1–3) exhibited negligible inhibitory activity, while the furanocoumarins showed potent in vitro inhibitory activity against intestinal cytochrome P450 3A4, an enzyme involved in the "grapefruit/drug" interactions in humans, with IC_{50} values (concentration resulting in 50% inhibition) of 2.42, 0.13, 0.27, and 1.58 μM, respectively (César et al., 2009).

Voo et al. (2012) deployed laser-capture microdissection and pressure catapulting to isolate epithelial cells and parenchyma cells (as controls with no oil synthesis) from young grapefruit peels. RNA was isolated, and transcript patterns were evaluated based on oligonucleotide microarrays. The biosynthesis of volatile terpenoids and nonvolatile phenylpropanoids in epithelial cells, rather than parenchyma cells, is regulated by expression of genes. The gene expression profiles in epithelial cells paralleled the accumulation of the polymethoxylated flavonoids, prenylated coumarins, and monoterpenes, which account for the majority of the essential oil content (Voo et al., 2012).

Flavoring Agent

The oil has been used as a flavoring agent in food applications as well as in cosmetics and perfumes. There are more than 260 volatile compounds that have been identified in grapefruit oil (Lin and Rouseff, 2001). Using gas chromatography olfactometry technique, Lin and Rouseff (2001) identified 38 aroma-active compounds in cold-pressed grapefruit oil. The most important flavor compound in grapefruit oil is nootkatone, which is produced from the oxidation of valencene (Caputi and Aprea, 2011), but other pathways of production have been proposed (Furusawa et al., 2005). The form of nootkatone (+ or – enantiomeric form) plays an important role in the oil flavor. The (+) form has a strong characteristic aroma of grapefruit while the (–) form has a weak woody/spicy odor (Caputi and Aprea, 2011).

TABLE 2 Percentage Composition of Marsh Grapefruit Peel Oil Influenced by Waxing, and Temperature and Time of Storage. Nootkatone Levels Increased with Storage Time but Were Reduced by Wax Application and Cold Storage

		Day 14				Day 28			
		21.0 °C		4.5 °C		21.0 °C		4.5 °C	
Compound	Index	No Wax	Wax	No Wax	Wax	No Wax	Wax	No Wax	Wax
α-Pinene	938	0.62	0.627	0.63	0.62	0.60	0.614	0.61	0.612
Sabinene	976	0.77	0.723	0.688	0.693	0.850	0.777	0.540	0.677
β-Pinene	980	0.08	0.079	0.067	0.063	0.093	0.084	0.061	0.067
Myrcene	991	1.94	1.912	1.939	1.948	1.932	1.931	1.893	1.911
Octanal	1004	0.67	0.765	0.649	0.734	0.710	0.670	0.660	0.509
α-Phellandrene	1009	0.07	0.057	0.076	0.075	0.190	0.173	0.134	0.096
3-Carene	1023	0.04	0.050	0.053	0.050	0.256	0.272	0.194	0.151
Limonene	1039	92.63	92.25	92.943	92.949	89.083	90.853	92.668	92.467
Ocimene	1051	0.27	0.305	0.223	0.187	0.384	0.238	0.273	0.281
γ-Terpinene	1067	0.06	0.064	0.056	0.037	0.170	0.133	0.108	0.103
Octanol	1074	0.03	0.048	0.019	0.020	0.042	0.020	0.010	0.020
cis-Linalool oxide	1082	0.03	0.043	0.030	0.036	0.028	0.018	0.010	0.054
trans-Linalool oxide	1099	0.07	0.071	0.075	0.070	0.328	0.307	0.213	0.180
Linalool	1107	0.10	0.084	0.076	0.083	0.050	0.054	0.042	0.089
Nonanal	1110	0.11	0.096	0.099	0.109	0.148	0.101	0.125	0.092
cis-p-mentha-2, 8-dien-1-ol	1149	0.07	0.078	0.089	0.095	0.239	0.174	0.051	0.023
Citronellal	1161	0.05	0.049	0.063	0.056	0.038	0.034	0.040	0.054
α-Terpineol	1210	0.05	0.046	0.031	0.036	0.011	0.018	0.009	0.023
Decanal	1216	0.50	0.514	0.480	0.501	0.495	0.514	0.499	0.469
Neral	1258	0.05	0.051	0.046	0.043	0.032	0.032	0.041	0.032
α-Copaene	1418	0.084	0.095	0.084	0.0908	0.0901	0.0900	0.09	0.0900
Dodecanal	1419	0.11	0.097	0.098	0.092	0.069	0.071	0.078	0.084
trans-Caryophyllene	1456	0.17	0.172	0.168	0.171	0.178	0.165	0.158	0.172
α-Humulene	1491	0.03	0.033	0.030	0.027	0.023	0.020	0.024	0.026
γ-Cadinene	1502	0.11	0.106	0.112	0.111	0.111	0.114	0.117	0.123
Nootkatone	1847	0.43	0.282	0.297	0.282	0.636	0.309	0.315	0.322
Total		99.12	99.025	99.204	99.264	96.883	97.8778	99.052	98.816

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Antimicrobial Activity

The grapefruit constituent 4-[[(E)-5-(3,3-dimethyl-2-oxiranyl)- 3-methyl-2-pentenyl]oxy]-7H-furo[3,2-g]chromen-7-one renders methicillin-resistant strains of *Staphylococcus aureus* (MRSA) more susceptible to ethidium bromide and norfloxacin, compounds that these micro-organisms demonstrate resistance to. Citricidal (grapefruit seed extract) was tested alone and in conjunction with other essential oils for antibacterial activity against three strains of *S. aureus*: Oxford *S. aureus* NCTC 6571 (Oxford strain), epidemic MRSA (EMRSA 15), and MRSA (untypable). Citricidal in combination with geranium oil manifested the most potent anti-MRSA activity (Edwards-Jones et al., 2004). Grapefruit oil demonstrated efficacy against several *S. aureus* strains, *Streptococcus* strains, and *Candida* strains including *Candida krusei.* Grapefruit oil and several essential oils (orange, lemon, mandarin, terpeneless lime, D-limonene, terpineol, and geraniol) were examined for their inhibitory effects on the growth of *Salmonella senftenberg, Escherichia coli, S. aureus and Pseudomonas* spp. in milk (Dabbah et al., 1970). The effect of grapefruit oil was the least effective against of *S. senftenberg, E. coli*, and *Pseudomonas* spp. but had similar inhibition effect against *S. aureus* to that found with the other tested essential oils. The sensory analysis indicated that consumers found orange, lemon, and grapefruit oils only were acceptable at 1 mL/L and improved the chalky taste of skim milk.

Five strains of *S. aureus* tested by Adukwu et al. (2012) were susceptible to grapefruit essential oil in the disk diffusion assay although grapefruit essential oil was devoid of antibiofilm activity. Grapefruit essential oil exhibited antibacterial activity against *Propionibacterium acnes*. Alonso et al. (2010) designed a nontoxic procedure for grafting of chitosan-based microcapsules containing grapefruit seed oil extract onto cellulose previously irradiated with ultraviolet light and then functionalized from an aqueous emulsion of the chitosan with the grapefruit oil. The materials are readily obtained with lasting fragrance and increased antimicrobial activity. Complete inhibition of *E. coli* and *Staphylococcus epidermidis* up to 48 h of incubation was achieved.

The oil of grapefruit peels inhibited *S. aureus, Enterococcus faecalis, E. coli, Salmonella typhimurium*, *Serratia marcescens*, and *Proteus vulgaris* except *Pseudomonas aeruginosa*. *Bacillus cereus*, *Mycobacterium smegmatis*, *Listeria monocytogenes*, *Micrococcus luteus*, *Klebsiella pneumoniae*, and the yeast cultures *C. albicans, Kluyveromyces fragilis, Rhodotorula rubra, Debaryomyces hansenii*, and *Hanseniaspora guilliermondii*. *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum*, and *Penicillium verru-cosum*. D-limonene, a major component of the oil, inhibited *E. coli, S. aureus, Salmonella* sp., *Pseudomonas* sp. and the yeasts *Oidium lactis*, *Sachharomyces elipsoideus*, and *Wilia hansenula*. Thus, the antimicrobial activity of the essential oil was probably attributed to D-limonene, flavonoids, and phenolic compounds. The antimicrobial activity depended on the solvent employed, with the methanol solution having the highest activity, followed by ethanol solution and the lowest activity in Tween 80 solution (Okunowo et al., 2013).

Mouthwash solutions currently available consist of synthetic agents (such as chlorhexidine and triclosan) or essential oils (especially Listerine). Many natural extracts, for instance, grapefruit seed extract, can also be employed. They display antiseptic, antioxidant, analgesic, buffering, immunoregulatory, or healing properties. The development of microencapsulation technology may facilitate a slow and steady liberation of active ingredients from mouthwash solutions over several hours (Vervelle et al., 2010). A low pH value augmented the suppressive action of naringenin on growth and adherence of *Salmonella* to intestinal epithelial cells, although the mechanism awaits elucidation (Gyles and Gong, 2012).

The antimicrobial effects of essential oils alone, including grapefruit, cinnamon, *Leptospermum morrisonii*, manuka (*Leptospermum scoparium*), tea tree (*Melaleuca alternifola*), eucalyptus, arnica, and essential oils in conjunction with chlorhexidine digluconate against planktonic and biofilm cultures of *Lactobacillus plantarum* and *Streptococcus mutans*, have been examined. Essential oils from cinnamon, *Leptospermum morrisonii,* tea-tree oils, and manuka, but not grapefruit, were inactive (Filoche et al., 2005).

The quality of blue fish burgers could be improved to ensure microbial acceptability after storage at 4 °C for 4 weeks by employing minute quantities of grapefruit seed extract (100 ppm), thymol (110 ppm), and lemon extract (120 ppm) together with modified atmosphere packaging conditions involving an elevated carbon dioxide. Nevertheless, the sensorial quality reduced the burgers' shelf life to about 22–23 days, although the proposed methodology was also efficacious in curtailing the decline in sensory quality of the product without affecting its nutritional quality (Del Nobile et al., 2009).

1,1-Diphenyl-2-Picryl-Hydrazyl Radical-scavenging Activity

The potencies of essential oils from pink grapefruit, clove buds, coriander, lemon, and their mixtures to inhibit oxidation of hexanal to carboxylic acid were compared. Grapefruit and clove bud essential oils were found to have the lowest and the highest antioxidant activities, respectively (Misharina and Samusenko, 2008).

When six commercially available herb essential oils and their main constituents, including grapefruit, frankincense, lavender, lemon, peppermint, and rosemary, were compared, the most potent 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity was displayed by lavender essential oil and limonene, the major component of the grapefruit essential oil (Yang et al., 2010).

SUMMARY POINTS

- Limonene is the main volatile constituent of grapefruit essential oil. Alpha-pinene, alpha-terpinene, alpha- and beta-sinensal, alpha-cubebene, (E,E)-alpha-farnesene, beta-caryophyllene, decanal, citronellal, octanal, (Z)-carvone, perillene, (E)-carveol, heptyl acetate, nootkatone, perillyl acetate, methyl-N-methylanthranilate, and (Z,E)-farnesol were other prominent compounds in grapefruit oil.
- Grapefruit oil has insecticidal activity against beetles, mosquitoes, fleas, ticks, and houseflies.
- Grapefruit oil inhibits elastase activity and has cosmetic potential due to its antiwrinkling activity.

DISCLAIMER

This article was written in a personal capacity (E.F.F.) and does not represent the opinions of the US Food and Drug Administration, the US Department of Health and Human Services, or the US federal government.

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Chapter 53

Hyssop (*Hyssopus officinalis* L.) Oils

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INTRODUCTION

Hyssop is a perennial herb of Mediterranean origin and well acclimated to the Northern hemisphere. It has been a well-known medical and culinary herb since ancient times. The plant is mentioned many times in the Bible as spiritually purifying. A long list of different valuable therapeutic properties has been reported for hyssop extracts and essential oils (EOs), such as spasmolytic, antiviral, sudorific, emmenagogue, carminative, tonic, diuretic, expectorant, antiseptic, antibacterial, antioxidant, anti-inflammatory, and cardiovascular. Numerous studies have focused on investigation of chemical constituents of *Hyssopus officinalis* L. (both wild and cultivated) EOs and have shown a large compositional variability. Nevertheless, isopinocamphone, pinocamphone, and pinenes are the characteristic constituents. Differences in composition (according to the climatic conditions, geographic origin, variety, phenological growth stage, soil type, cultivation technology, applied extraction technique, etc.) of the oils influence organoleptic, physiological properties, as well as possibilities and fields of oil application.

Recently, the demand for bioherbicides, bioinsecticides, and natural allelochemicals (nontoxic to humans or environment and easily biodegradable) has been increasing rapidly, so hyssop EOs are finding a new range of applications in agriculture. Hyssop oils can be effective in two key areas: (1) improving organoleptic properties (mainly due to its specific odor and bitter taste) and (2) exhibiting antifungal and antimicrobial activities against many food-borne pathogens. For these reasons, the oils could be used more often in food technology and food storage. Usually, plant-derived preservatives in food do not create risks for human health or induce allergic/sensitivity reactions. Hyssop oil (usually in quantity up to 40 ppm) is often applied in various liqueur and aperitif formulations and is also an important ingredient for flavoring soft beverages.

BOTANICAL ASPECTS

Hyssopus officinalis (hyssop) is a species of the genus *Hyssopus* from the family Lamiaceae Lindl. (syn: Labiatae Juss.), tribe *Mentheae*.

Hyssopus officinalis (also known as *Thymus hyssopus*) is native to Mediterranean and Caspian Sea regions, grows naturally in southern Europe, the Middle East, Central Asia, N. Africa, and N. America, while the herb for culinary, decorative, and medicinal purposes is cultivated in many countries worldwide.

The Latin word *hyssopus* most likely originated from the ancient Greek word ὕσσωπος [hyssopos], which relates to the Hebrew word אזוב, Azob (Esov or Esobh)-"a holy herb" (actually, it is an unidentified plant, perhaps oregano species, could be *Origanum hirtum*), used in Jewish rituals of purification. The adjective *officinalis* is from the noun *officina* (the older version "*opificina*"-a place where something is made or a process of manufacturing).

Hyssop is also known by such common names in other languages, as: hysope (Fr.), ysop (Ger.), issopo (Ital.), hisopo (Span.), jufa (Sanskr./Ind.), az-zufa (Arab.), zufa (Pers.).

Hyssop is a perennial herbaceous, semievergreen plant with a pleasant mint/sage odor. The plant is a shrub or subshrub, growing up to 0.2–0.6 m in height. Its stems are straight, woody, and branched at the base. Leaves are lanceolate (2–4 cm long and 2–7 mm wide), stalkless and toothless, dark green in color, covered with thin silky hairs, and bearing essential oil-producing glands. Flower colors could be blue, pink, pale violet, and (more rarely) white or purple. They are fragrant and appear erect, tabular, two-lipped, clustered in upper leaf axils, often forming a spike; four stamens protrude from the flower. Corollas are from 7 to 11 mm long, and its outside is pilose. The flowers are hermaphrodite and are pollinated by insects, mainly by bees. The hyssop taproot is woody and twiggy.

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The blooming period of hyssop is from July to September, and in some regions even to November. Seeds ripen from August to October. The ripe fruit of hyssop is a small, oblong trihedral achene, dark brown in color, up to 2.5 mm in length. Hyssop breeds by generative or vegetative propagation. The plant likes sunny and warm climate, preferring growing locations that are well-drained sandy or loamy soil. The herb grows in gardens, roadsides, and various waste lands as well. Hyssop is a self-fertile plant.

Cultivated plantations of hyssop can be continuously farmed for about 10 years, and crops can be harvested several times: in spring, at the flowering stage, and at the beginning of the fall. The plants are preferably harvested during blooming season in order to collect the flowering tops. Production of hyssop EOs is performed by steam distillation with yields around 0.5% in France, Spain, Italy, Germany, Slovenia, Moldova, Bulgaria, Iran, USA, China, and India, with other countries producing usually less than 5 tons annually.

USAGE AND APPLICATIONS

Today *H. officinalis* is a well-established aromatical and medicinal plant around the world, whose pharmacological properties have been known since ancient times. In the past, it has been used for ritual cleaning of temples and holy places. Hyssop was also used as medicine for cleansing lepers. The use of the plant in spiritual purifying is mentioned many times in the Bible. In the Middle Ages, hyssop tops were strewn on floors to mask bad smells. Due to its fresh, clean, and pleasant aroma, dried leaves are often placed in cupboards and coatrooms. Hyssop is an ornamental plant, and gardens or areas along walkways are often decorated by its bright flowering shrub.

Hyssop has a particularly fine odor, significant in the perfumery industry. Its volatile oils are used as a fragrance component in soaps, perfumes, eau de Cologne, creams, and other cosmetic products, as well as in aromatherapy.

Herb material of *H. officinalis* contains numerous important ingredients, such as EOs, polyphenols, acids (chlorogenic, p-hydroxybenzoic, protocatechuic, ferulic, feruloylquinic, syringic, vanillic, p-coumaric, rosmarinic, caffeic), flavonoids, polysaccharides, tannins, pigments, and resins. Characteristic bioactive compounds identified in hyssop are hyssopin (a glucoside), diosmin and hesperidin (flavonoids), marrubiin (a bitter diterpenoid), and gentisic, ursolic, and oleanolic acids.

The herb possesses numerous valuable therapeutic properties and is used for healing respiratory diseases such as tuberculosis, asthma, coughs, sore throat, respiratory infections, fevers, and the common cold. It is also used in baths to cause sweating.

Preparations of this plant are effective in curing some digestive and intestinal problems, including liver/gall bladder conditions, intestinal pain and gas, loss of appetite, urinary tract infection, menstrual pains and cramps, and formation of kidney stones. Tea prepared from the green tops of the plant not only has an agreeable flavor but also can help digestion of fatty foods and shows anthelmintic activity.

Decoctions or EOs of hyssop could be used on the skin for treating burns, frostbites, bruises, skin irritations, and diminishing the effects of skin aging. *Hyssopus officinalis* oils are active against drug-resistant clinical herpes simplex virus type 1 (HSV-1) and HSV-2 strains, and for treating skin disorders, such as dermatitis and eczema. The plant extracts exhibit antibacterial activity to *Propionibacterium acnes*, as the causative bacteria of acne. Some patented cosmetics and skin-conditioning and antiwrinkle topical formulations contain *H. officinalis* EOs and ethanolic extracts. The plant essential oil is also used externally to treat toothache, earache, and rheumatism. Hyssop volatile oils are also suitable for massage and effective against poor blood and lymph circulation, as a muscle-relaxing treatment.

In addition, crude extracts of dried leaves of *H. officinalis* have shown strong anti-HIV activity and may be useful in the treatment of patients with AIDS.

USAGE AND APPLICATIONS OF HYSSOP IN FOOD SCIENCE

Hyssopus officinalis is a well-known culinary herb with a long history. Pliny the Elder mentioned that ancient Romans made an herbal wine with hyssop, which was called *hyssopites* in the encyclopedic work "Natural History" (*Naturalis Historia*, published around 77–79 BCE). During Medieval times, the herb was used to mask the putrescent smell of meat, while the Benedictine monks were manufacturing liqueurs with this herb.

Nowadays, due to its specific minty aroma, preservative, and tonic properties the herb is still popular in Mediterranean kitchen. Leaves and flowering tops (dried or fresh) of hyssop are commonly used in cooking. The leaves have a strong, pungent taste, so could be used only in limited amounts. Hyssop is extremely useful in meals with a high fat content. Flowers of the plant are useful for green salads or vegetables, in various sauces, and gravies, and for baking pita bread. Whole herb chopped in small pieces is used for soups, stews, and herbal teas, while fresh leaves alone are suitable for seasoning of soft cheeses (feta and cottage cheese, in particular), flavored butters, sandwiches, and pasta dishes. For example, in Lithuanian kitchens the cultivated plant is used as the flavoring and bitter ingredient for salads, fatty meats (especially of wild animals), fish, herring, potato dishes, and various sauces.

Bright and aromatic flowers of hyssop attract honeybees, and honey from these bees is of high quality: clear, golden in color, with a specific and very pleasant taste. *Hyssopus officinalis* could be cultivated as a honey herb. A medicinal preparation of hyssop, so-called hyssop oxymel, is made from the fresh or dried herb, macerated in honey and some apple cider vinegar.

Hyssop EOs, being light-yellow liquids, have herbaceous, camphor-like odors with warm and spicy undertones. A literature review on the chemical aspects of volatile oils of the plant indicates a long list of principal constituents and oil chemotypes. The most characteristic and important components in hyssop oils are isopinocamphone (syn. cis-, (3)-pinanone), pinocamphone (syn. trans-, (3)-pinanone), and their precursor β-pinene. Among other principal constituents are pinocarvone, sabinene, germacrene D, germacren D-4-ol, α-, β-phellandrene, 4-carvomenthenol, thymol, carvacrol, elemol, limonene, linalool, 1,8-cineole, α-terpinene, myrtenol, myrtenyl acetate, (methyl) eugenol and other compounds. For earlier published data concerning chemotaxonomy of *H. officinalis* oils, see some review articles (Lawrence (1980, 1984, 1994, 1995, 2003, 2008); Fathiazad and Hamedeyazdan, 2011; Ogunwande et al., 2011; and references therein cited). Some of the newest data on principal composition (five major constituents, or at least over 5%) are presented in Table 1 (from the

TABLE 1 Principal Constituents (at least over 5%) of Hyssop Essential Oils from the Largest European Mediterranean Countries (France, Italy, Spain)[a]

Plant Material	EO Yield, %	Main Composition, %	Origin	References
Cp, Wp, flowering tops *Hyssopus officinalis, H. officinalis* var. *decumbens*	0.25–0.36, SD	*H. officinalis*: β-pinene 11.1, limonene ≤12.2, isopinocamphone 43.3; var. *decumbens*: linalool ≤51.7, 1,8-cineole ≤14.9, limonene ≤6.0	*Hyssopus officinalis* var. *decumbens:* France (High-Provence Alps, Banon); *H. officinalis:* Italy (Piedmont)	Fathiazad and Hamedeyazdan (2011) (therein cited Salvatore et al. (1998)), Mazzanti et al. (1998), Renzini et al. (1999)
Wp, at flowering, subsp. *aristatus* (Godron) Briq, fresh	0.2–0.4, SD 3 h	Three chemotypes: **1.** myrtenol 32.6, β-pinene 19.3, isopinocamphone 10.0; **2.** β-pinene 24.7, 1,8-cineole 23.1, methyl eugenol 12.2, (Z)-β-ocimene 5.2, linalool 5.0; **3.** methyl eugenol 43.9, limonene 15.9, 1,8-cineole 12.1, β-pinene 8.4, (Z)-β-ocimene 5.9	Italy (Central, Abruzzi region)	Fraternale et al. (2004) (therein cited Piccaglia et al. (1999))
Cp, ssp. *officinalis*	0.23–0.31, SD 3 h	Pinocamphone ≤34.0, isopinocamphone ≤29.0, β-pinene ≤10.8, α-phellandrene ≤9.6, linalool ≤7.9	Italy (Urbino, Marche)	Fraternale et al. (2004)
Cs	HD	S-Pinocamphone 36.7, pinocamphone 19.3, β-pinene 13.1	Italy	Tampieri et al. (2005)
Cs		β-Pinene 18.2, isopinocamphone 29.1, pinocamphone 11.1	Italy	De Martino et al. (2009)
Cp, full flowering, freshly picked	0.41, HD 3 h	Isopinocamphone 29.1, β-pinene 18.2, pinocamphone 11.2	Italy	de Almeida et al. (2010)
At flowering; leaves, flowers	1.14, HD 3 h	1,8-Cineole 52.9, β-pinene 16.8	Spain (Cuenca, Cañameros)	Fraternale et al. (2004) (therein cited Vallejo et al. (1995))
	CO_2-supercritical extraction, HD	1,8-Cineole ≤75.0, terpinen-4-ol ≤10.0, pinocarvone ≤6.0, β-pinene ≤6.0	Spain	Langa et al. (2009)
Cp	≤0.47, HD-extraction	β-Pinene ≤20.5, iso-pinocamphone 24.6, pinocamphone 19.8	Spain (Castilla-La Mancha)	Moro et al. (2011)
Cp		1,8-Cineole 53.0, β-pinene 12.0, pinocamphone 6.0	Spain	Fe Andrés et al. (2012)

EOs obtained from air-dried aerial parts of hyssop.
Cs, commercial sample; Cp, cultivated plants; Wp, wild-growing plants; SD, steam distillation; HD, hydrodistillation; h, duration of the procedures.
[a]*Data published after 1990.*

TABLE 2 Major Composition (≥5%) of Some Hyssop Essential Oils from Various European Countries[a]

Plant Material	EO Yield, %	Main Composition, %	Origin	References
Cp		β-Pinene ≤14.2, 1,8-cineole ≤12.0, pinocamphone ≤46.5, camphor ≤12.5	Bulgaria	Lawrence (2008) (therein cited Stoyanova and Grozeva (2006))
Cp, during flowering	0.36, HD 3 h	Isopinocamphone ≤33.6, pinocarvone ≤28.1, β-pinene ≤11.4, hedycaryol ≤9.1, germacrene D ≤ 5.5	Lithuania (Eastern)	Bernotienė and Butkienė (2010)
Cp, vegetative variation	0.5–1.8, SD 3 h	cis-Pinocamphone ≤65.4, trans-pinocamphone ≤53.0, β-pinene ≤13.9, germacrene D ≤ 6.2	Poland (Lublin)	Zawiślak (2013)
Cp, ssp. *officinalis*, at flowering	0.18, SD 3 h	cis-Pinocamphone 48.6, elemol 7.4, β-pinene 6.1, caryophyllene oxide 5.2	Poland (Lublin)	Baj et al. (2010)
Cp	0.4–0.8, SD, HD, HD by Dean–Stark equipment, 2 h	Isopinocamphone ≤45.5, elemol ≤17.2, myrtenol ≤6.0	Poland (North-Western, Szczecin)	Wesołowska et al. (2010)
Cs		β-Pinene 15.2, isopinocamphone 47.8, pinocamphone 14.7	Poland	Michalczyk et al. (2012)
Cs	Microwave extraction	Isopinocamphone 25.5, pinocamphone 22.1	Romania	Moro et al. (2013)
Wp, ssp. *pilifer* (Pant.) Murb.	0.6, HD 3 h	1,8-Cineole 36.4, β-pinene 19.6, isopinocamphone 15.3	Serbia (Eastern, Sicevo Gorge)	Džamić et al. (2013)
Cs		Isopinocamphone 43.3, pinocamphone 16.8, β-pinene 16.3	Serbia	Glamočlija et al. (2005)
Cp	0.5, HD 2.5 h	Pinocamphone 42.9, isopinocamphone 14.1, germacrene-D-11-ol 5.7, elemol 5.6, sabinene 5.2	Serbia	Mitić and Dordević (2000)
Cp, *H. off.* "Perlay," ssp. *canescens*, ssp. *officinalis*, seasonal variation	0.8–1.3	Pinocamphone ≤60.0, isopinocamphone ≤30.0, β-pinene ≤15.0	Switzerland (Valais)	Rey et al. (2004)
Cp	HD	Isopinocamphone ≤57.7, pinocamphone ≤22.1, β-pinene ≤8.9	UK (Auchincruive)	Letessier et al. (2001)
Cp, forms: *albus Alef.*, *cyaneus Alef.*, *ruber Mill*	0.6–1.1	Pinocamphone ≤64.9, isopinocamphone ≤59.9, pinocarvone ≤16.9, β-pinene ≤13.2, β-phellandrene ≤7.9	Yugoslavia (Novi Sad)	Lawrence (2008) (therein cited Chalchat et al. (2001))

EOs obtained from air-dried aerial parts of hyssop.
Cs, commercial sample; Cp, cultivated plants; Wp, wild-growing plants; SD, steam distillation; HD, hydrodistillation; h, duration of the procedures.
[a]*Countries listed in alphabetic order, data published after 2000.*

largest European Mediterranean countries as published since 1990), Table 2 (from various European countries since 2000), and Table 3 (from some American, African, and Asian countries since 2000).

Considering the interest in natural products for different commercial fields, including agriculture and agro foods, application of *H. officinalis* should be discussed more extensively. In veterinary medicine, hyssop preparations and EOs are given to domestic animals as anthelmintic remedies and for treatment of gastrointestinal disorders. Hyssop extracts and EOs possess insecticidal properties against flies, flea beetles, green caterpillars, mites, larvae of some worms, cabbage moths, slugs, etc., but they attract hummingbirds, butterflies, bees, etc., who are good pollinators for the plants growing in the hyssop's neighborhood. Hyssop oils, exhibiting toxic, antibacterial, and antifungal properties are used against many plant diseases, for example, potato's late blight.

TABLE 3 Main Constituents (over 5%) of Some Hyssop Essential Oils from American, African, and Asian Countries[a]

Plant Material	EO Yield, %	Main Composition, %	Origin	References
Cp, flowering, fresh, dried	0.19, SD 1 h, extraction	Pinocamphene + isopinocamphene 57–75.0, β-pinene ≤15.0	USA (Mississippi)	Zheljazkov et al. (2012)
		β-Pinene 19.6, pinocamphone 19.2, camphor 16.3	Egypt	El Sawi and El Din (2002)
Dried leaves	0.24, HD 4 h	α-Pinene 70.9, β-pinene 10.9	Nigeria (Western Lagos)	Ogunwande et al. (2011)
Cp, ssp. *officinalis*	1.18	trans-Pinocamphone 49.1, β-pinene 18.4, cis-pinocamphone 9.7	India (Lucknow)	Lawrence (2008) (therein cited Garg et al. (1999))
Cp, fresh, seasonal variation	≤0.4, HD 3 h	Pinocamphone ≤64.9, β-pinene ≤32.4, 1,8-cineole ≤11.9, germacrene D ≤ 11.3, limonene ≤9.8	India (Kashmir)	Khan et al. (2012)
Cp	0.5, HD 4 h, supercritical fluid extraction (SFE)	HD: sabinene 11.0, iso-pinocamphene 10.9, pinocamphene 8.2; SFE: sabinene ≤17.1, iso-pinocamphene ≤16.5, pinocamphene ≤13.6	Iran (Karaj)	Kazazi and Rezaei (2009)
Wp, flowering	1.3, 3 h	Myrtenyl acetate 74.1, camphor 6.8	Iran (North)	Fathiazad and Hamedeyazdan (2011)
Cp		Isopinocamphone ≤53.1, pinocamphone ≤14.4, elemol 8.6, β-pinene 7.8, α-terpineol ≤7.4	Iran	Ogunwande et al. (2011) (therein cited Nazari et al. (2008))
Wp, leaves	2.29, HD 4 h	Pinocarvone 29.2, trans-pine camphone 27.2, β-pinene 17.6	Turkey (Gümüshane, 1700 m)	Figueredo et al. (2012)
Wp, ssp. *angustifolius*	1.13, HD 3 h	Pinocarvone 36.3, pinocamphone 19.6, β-pinene 10.6, 1,8-cineole 7.2, isopinocamphone 5.3	Turkey (Gümüshane, 1800 m)	Lawrence (2008) (therein cited Özer et al. (2006))
Wp		Isopinocamphone 57.3, (–)-β-pinene 7.2, (–)-terpinen-4-ol 7.1, pinocarvone 6.5	Turkey (Southeast Anatolia)	Kizil et al. (2010)
Cp, phenological stages	0.4–1.2, HD	Isopinocamphone 56.7, β-pinene ≤19.4, 4-carvomenthenol ≤14.0, carvacrol ≤8.7, pinocarvone ≤6.8	Turkey (different parts)	Kizil et al. (2010) (therein cited Kizil et al., 2008)
Wp, flowering	HD 3 h	Thymol 19.0, β-bisabolol 10.6, carvacrol 7.7, n-dodecane 5.2, caryophyllene oxide 5.0	Iran (Southwestern)	Dehghanzadeh et al. (2012)
Wp, fresh leaves, flowers	≤1.38, HD 3 h	Isopinocamphone ≤40.3, pinocamphone 14.9, n-decane ≤8.7, pinocarvone ≤6.8	Iran (Ardabil, 1843 m)	Moghtader (2014)

Cp, cultivated plants; Wp, wild-growing plants; SD, steam distillation; HD, hydrodistillation; h, duration of the procedures.
[a]*EOs obtained from aerial parts of hyssop.*

Nematicidal and toxic effects of hyssop EOs (for main composition see Table 1) have been tested against some plant parasitic nematodes, including root-knot nematode (*Meloidogyne* spp.), which is one of the most economically damaging genera of plant parasites. Those have a very negative impact on yields of various horticultural and field crops (Fe Andrés et al., 2012 and therein cited). It should be mentioned that hyssop oils exhibited a weak nematicidal activity (or only at high concentrations) in the above review study.

Many EOs, containing phytotoxic components, are widely used as allelochemicals. Allelopathic activity of hyssop oils at different doses was evaluated against seed germination and early initial radicle growth of the crop species *Raphanus sativus* L. cv. Saxa (radish), *Lepidium sativum* L. (garden cress), and *Lactuca sativa* L. (lettuce) (de Almeida et al., 2010) (for EO composition, see Table 1).

Generally, herbal EOs, containing compounds with low molecular weights and possessing highly lipophilic properties, can easily penetrate through cell membranes to induce biological reactions. Antibacterial, antifungal, and antioxidant activity of some *H. officinalis* oils is presented in Table 4.

Hyssopus officinalis (collected from the wild in the Sepidan mountains) possesses high antimicrobial activities in vitro against *Klebsiella* sp. and *Erwinia amylovora*, two important plant pathogens, and can be used in treatment of some plant diseases (Dehghanzadeh et al., 2012) (for details, see Tables 3 and 4).

Additionally, the antifungal activity in vitro and in vivo experiments was evaluated against two phytopathogenic fungi, *Pyrenophora avenae* and *Pyricularia oryzae*; the mycelial growth of the fungi was completely inhibited by 0.4% hyssop oil. Also, the oil reduced germination of *Botrytis fabae conidia* and uredospores of *Uromyces viciae-fabae* (Letessier et al., 2001). However, two EOs of Italian *H. officinalis* ssp. *officinalis* (for EOs composition see Table 1) have shown a significant antifungal activity against 13 strains of phytopathogenic fungi. Reports showed that 100% inhibition for all pathogens was achieved with 1600 ppm of oil (Fraternale et al., 2004); see Table 4.

TABLE 4 Antibacterial, Antifungal, and Antioxidant Activity of Some *Hyssopus officinalis* EOs[a]

Tested Activity, Organisms	Results	References
Antimicrobial, ATCC: 90028(Ca), 90030(Cg), 6258(Ck), 90906(Afu), 90113(Cn), 29213(Sa), 33591(MRS), 35218(Ec), 27853(Pa), 23068(Mi)	MIC: 0.008–1.25 µg/mL; antioxidant activity 2039 µmol of TE/L	Zheljazkov et al. (2012)
Antimicrobial, ATCC: 25922, 27853, 25923, 19615, 10231; antioxidant	Strong against *Staphylococcus pyogenes, Staphylococcus aureus, Candida albicans, Escherichia coli*, but not against *Pseudomonas aeruginosa*; low antioxidant activity	Kizil et al. (2010)
Antimicrobial, antioxidant	*C. albicans*, MIC: 15.625–250 µL/mL; no distinctive antioxidant properties	Özer et al. (2006)
Antibacterial, gram positive: DSM: 4313, 4384, 25923, 2352, gram negative: DSM 8579, ATCC 50071 and nonpathogenic bacterial strains of *Lactobacillus* (DSM: 20079, 9595, 20081, 20494, 20711); antifungal (strains DSM: 1997, 1097, 1994, 2429, 62074, 70238), mutagenic activity (*Salmonella typhimurium* strains TA98, TA100)	No mutagenic activity, no activity against lactic acid bacteria, no against *Debaryomyces hansenii, Penicillium expansum*; weak activity toward *P. aurantiogriseum*	De Martino et al. (2009)
Antibacterial, *Pseudomonas fluorescens*, *Xanthomonas axonopodis* pv *citri*, *Erwinia amylovora*, *Acidovorax* sp., *Streptomyces scabies*, *Bacillus subtilis*, *S. aureus*, *Klebsiella* sp.	High activity against *E. amylovora* and *Klebsiella* sp., MIC: 25–100 µL, MBC: 25–100 µL	Dehghanzadeh et al. (2012)
Antifungal ATCC: 6275, 12066, 11730, 9197, 13276,10509, 9112; TK 5318; IAM 5061, *C. albicans* yeast	MIC: 10.44–52.2 mg/mL, MFC: 26.1–104.4 mg/mL	Džamić et al. (2013)
Antifungal against *Mycogone perniciosa* (Mang)	MIC: 5 µL/mL, MFC: 15 20 µL/mL	Glamočlija et al. (2005)
Fungal plant pathogens: *Fusarium* strains (ATCC: 12656, 15624, 24383, 24362, 11853, 15659, 24630), *Fusarium oxysporum* Schl., *Phytophtora capsici* Leon., *Botrytis cinerea* Pers., *Rhyzoctonia solani* Ell. et Mart., *Phytophtora cryptogea* Pethyb. et Laff., *Alternaria solani* Ell. et Mart.	MIC: 300–1600 µg/mL, MFC: 2.4–4.8 mg/mL	Fraternale et al. (2004)
Antifungal	Complete inhibition of *Aspergillus niger* at 0.5–1.5% v/v	Fathiazad and Hamedeyazdan (2011) (therein cited Motiejūnaitė and Kalėdienė, 2003)

[a]*MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration.*

The antifungal effects of oil, whose composition is detailed in Table 2, against the major pathogenic diseases *Mycogone perniciosa* (Mang) of the cultivated mushrooms (*Agaricus bisporus* Lange (Imb)) was studied by microatmosphere method by Glamočlija et al. (2005).

Hyssop oils could be useful in food technology, mainly to improve the aroma and organoleptic properties of different types of foods. The presence of toxigenic fungi and pathogenic bacteria in foods (especially stored for a long time) raises a major risk to human and animal health. EOs help to inhibit growth of food-borne and spoilage microbes in raw and processed foods. Michalczyk et al. (2012) have examined the effects of hyssop EOs (for EO composition, see Table 2) on the microbiological and biochemical characteristics of stored ground beef. The EOs inhibited undesirable sensory changes and growth of *Enterobacteriaceae* bacteria, which contain a large number of pathogens.

In addition, hyssop oils and extracts also inhibit degradation of pigments (caused by cooking and storage of meat) and may be a useful additive against discoloration in meat processing.

Antifungal activity of hyssop volatile oils was estimated by De Martino et al. (2009) against several fungal strains of the genus *Penicillium*, an important contaminant of foods and agricultural commodities; against *Aureobasidium pullulans*, usually known as a contaminant of vegetables; and against *Debaryomyces hansenii* (a ubiquitous unicellular haploid yeast, as a parasite in humans, fish, and vegetables). In totally, six pathogenic (four of them gram-positive and two gram-negative strains) and five nonpathogenic bacterial strains of *Lactobacillus* were used in order to evaluate antimicrobial activity. Using the disk diffusion method, EOs were much more efficient against micromycetes than against bacteria (De Martino et al., 2009); for details, see Tables 1 and 4.

The in vitro antimicrobial (on *Staphylococcus aureus* (ATCC 6538 P) and *Streptococcus sanguis* (ATCC 10556)) and cytotoxic (using the brine shrimp (*Artemia salina* Leach) tests) activity of hyssop oil was shown by Renzini et al. (1999). In another study, conducted by G. Mazzanti et al. (1998) the effects of *H. officinalis* oil were tested on several gram-positive and gram-negative bacteria. It also evaluated antimycotic action against yeasts, including seven strains of *Candida albicans*, *Candida krusei*, and *Candida tropicalis*. The results demonstrated that the oil antimicrobial activity was negligible, while growth of all the yeasts was strongly inhibited. Antibacterial activity of hyssop EOs was evaluated in vitro by disk volatilization method (Nedorostova et al., 2009) against five food-borne bacteria (two gram-positive bacteria: *Listeria monocytogenes* and *S. aureus*, three gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*).

Antifungal effect of hyssop oil against *Penicillium verrucosum*, a common cheese spoiler, was confirmed by Moro et al. (2013). The effect of 50% inhibition of fungal growth was achieved for hyssop EOs (for EO composition see Table 2) at concentrations below 0.02 μL/mL. Even more, the innovative usage of the plant as a natural cheese cover was proposed in order to prevent mycotoxicogenic fungal growth.

Antimicrobial and antioxidant activities of EO of Turkish wild *H. officinalis* was tested on *E. coli*, *P. aeruginosa*, *S. aureus*, *Staphylococcus pyogenes*, and *C. albicans*, while the antioxidant activity was evaluated by using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method (Kizil et al., 2010). The oil showed relatively low antioxidant activity as compared to butylated hydroxytoluene and ascorbic acid, but demonstrated good antimicrobial properties against some tested organisms in the above study. *Aspergillus niger* was found to be the most resistant fungus, while *Cladosporium* species was the most sensitive to hyssop oil (Table 4).

EOs and extracts of *H. officinalis* ssp. *pilifer* (the EO composition presented in Table 2) were tested in order to evaluate their potential antioxidant activity by DPPH radical scavenging method and antifungal properties against several strains of *Aspergillus*, *Cladosporium*, *Penicillium*, *Trichoderma viride*, and *C. albicans* yeast. EOs possessed the lowest activity compared to the extracts (Džamić et al., 2013). Antimycotic properties of hyssop oils (see Table 1) were tested in vitro by using a semisolid agar antifungal susceptibility method against a strain of *C. albicans* in another study (Tampieri et al., 2005), where minimum inhibitory concentration (MIC) value of 1000 ppm was determined after 48 h and after 7 days. EOs of hyssop cultivated in the USA did not show very significant antimicrobial (against 10 different strains of *Candida*, *Aspergillus*, *Cryptococcus*, *Staphylococcus*, *Escherichia*, *Pseudomonas*, and *Mycobacterium*), antileishmanial (in vitro on a culture of *Leishmania donovani* promastigotes), antimalarial, and antioxidant activity, and did not alter ruminal fermentation (Zheljazkov et al., 2012), see Tables 3 and 4. In vitro antimicrobial activity was exhibited against 8 bacteria, 10 fungi, and a yeast (*C. albicans*) by Özer et al. (2006), see Table 4. Results of antioxidant activity showed that the oil was inactive, while the methanol extract provided 50% inhibition at a concentration of 117.0 μg/mL in DPPH assay.

Generally, it should be mentioned that hyssop extracts exhibit much stronger antioxidant activity than EOs, because inhibition of lipid oxidation is mainly responsible by the presence of various phenolic compounds in the extracts, compared to volatile oils, being mainly rich in oxygenated monoterpenes, which are known to have a low radical scavenging activity.

EOs of the plant are applied as flavoring agents in many packaged and processed foods: teas, candies, chewing gums, snack foods, crackers, cookies, chips, desserts, gelatins, puddings, baked goods, ice creams, frozen meals,

alcoholic and nonalcoholic beverages, liqueurs, aperitifs, etc. Hyssop is used to flavor the famous French liqueurs Bénédictine and Chartreuse. Bénédictine, developed as a medicinal aromatic herbal beverage, contains more than 20 plants and spices, among them hyssop, angelica root, arnica flowers, orange peel, thyme, cardamom, peppermint, cassia and cloves. Chartreuse is an aromatic green, yellow, or white liqueur flavored with hyssop and peppermint oils. However, *H. officinalis* oils are included in the recipe of the famous absinthe drink. Even more, due to its pungent taste, hyssop (by infusion of the herb in alcohol) is used in the composition of some other bitters drinks. There is information that in some Arab countries, fermented hyssop leaves are used to make a drink sherbet, a refreshing, mildly alcoholic beverage.

Despite all of its advantages and the wide application of hyssop, toxic properties of its EOs should be discussed. The chemical composition of hyssop oil is now controlled worldwide by the ISO (9841:1991. Oil of Hyssop *Hyssopus officinalis* L. ssp. *officinalis*). In this regulatory document, 13 compounds are recognized as standards; pinocamphone, isopinocamphone, and β-pinene are declared as the most abundant (40–90%) constituents in hyssop EOs. Several published cases reported that hyssop oils at high doses exhibited convulsing, epileptogenic, and neurotic effects to experimental animals. Powerful convulsing properties of EOs are related to the content of highly reactive monoterpene ketones, such as isopinocamphone, pinocamphone, and 1,8-cineole. Carcinogenic properties might arise due to methyl eugenol, but hyssop EOs rich in this constituent are quite rare.

SUMMARY POINTS

- Principal chemical variability of *H. officinalis* EOs has been shown.
- Numerous research studies have demonstrated that hyssop oils are active against many plant and food-borne pathogens.
- Hyssop could play a significant role in preservation of agricultural fields, seeds, vegetables, fruits, grains, and other stored products against many fungi and microbes.
- Hyssop and its EOs are used to improve some organoleptic properties, spoilage resistance, and shelf life of some food products (fish, meat, cheese, etc.).
- Antioxidant activity of hyssop EOs was mild, in contrast to herbal extracts.
- Hyssop EOs are used as flavoring agents in nonalcoholic and alcoholic beverages, different liqueurs, aperitifs (including a bitter spirit, absinthe), and for many packaged and processed foods (candies, snack foods, baked goods, chewing gums, ice creams, frozen meals, etc.). The plant is a principal flavoring component in the production of the famous French liqueurs Chartreuse and Bénédictine.
- Toxicity of hyssop volatile oils is mainly related to the presence of such compounds as pinocamphones, 1,8-cineole, limonene, linalool, methyl eugenol, and pinenes.

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Chapter 54

Iris (*Iris germanica*) Oils

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List of Abbreviations

BHA Butylated hydroxyanisole
BHT Butylated hydroxytoluene
DPPH 2,2-Diphenyl-1-picrylhydrazyl
MIC Minimum inhibitory concentration

INTRODUCTION

Modern food processing must ensure the safety of food during storage, processing, and transport. Although a fair number of new preservation techniques have been developed, there are still a few safety concerns to the general food industry for the storage and processing of vegetables, meat, fruits, and dairy products (Sultanbawa, 2011). Due to the drawbacks associated with synthetic additives, demand for plant-derived food additives has grown in the last few decades. Employment of plant extracts and essential oils as antibacterial, antifungal, antialgal, and antioxidant agents has been documented by various researchers worldwide (Sultanbawa, 2011). Researchers from the Ohio State University found that in the last decade, natural antimicrobials and antioxidants have been used preferably in meat products due to their health advantages and safety benefits when compared with synthetic additives, viz., butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). According to some reports, natural antioxidants (viz., plant extracts, vitamins C and E) have played vital roles as food preservatives compared to synthetic antioxidants (Daniells, 2006).

Essential oils (EOs) are crude natural mixtures of volatile natural products and it has been often been reported that mono- and sesquiterpenes form the major components of essential oils. Furthermore, these terpenes are responsible for the antimicrobial properties of these essential oils. Due to their antimicrobial properties, EOs have been used as flavoring agents as well as preservatives (Kalemba and Kunicka, 2003). This chapter gives an overview of the antimicrobial and antioxidant activity of the EOs derived from *Iris germanica* L.

BOTANICAL ASPECTS

The word *Iris* originates from Greek word for "rainbow" referring to the many different colored flowers found in the *Iris* species. The genus *Iris* belongs to the family Iridaceae (Figure 1), which contains about 260 species (Kaššák, 2012). *Iris germanica* L. is extensively distributed all over the world, viz., North America, Eurasia, and North Africa. Furthermore, most *Iris* spp. are found in desert (dry) or rocky habitats. Relatively few *Iris* spp. occur in wetland environments (Kaššák, 2012). Moreover, out of 260 *Iris* species, 13 are found in Turkey (Burcu et al., 2014), 58 in China (Deng et al., 2009), and 16 in Pakistan (Rahman et al., 2002). *Iris* spp. are identified through their basal fan of unifacial leaves and also from the colorful perianth of three upright colorful petals and three horizontal sepals (Kaššák, 2012). Moreover, *Iris* spp. are also identified from base-fused-style branches and three stamens that are opposite to the sepals and petaloid style (Kaššák, 2012).

USAGE AND APPLICATIONS

Plant-derived products play a most important role as a food source and health vector in individuals and communities. The medicinal value of these plants is essentially due to the secondary metabolites contained in them, which display specific physiological actions on the human body. Most of these plants are employed as spices and a basic food source,

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Kingdom	Plantae (Plantes, Planta, Vegetal, plants)
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta (Land plants)
Superdivision	Embryophyta
Division	Tracheophyta (Vascular plants, tracheophytes)
Subdivision	Spermatophytina (spermatophytes, seed plants)
Class	Magnoliopsida
Superorder	Lilianae (Monocots, monocotyledons, monocotylédones)
Order	Asparagales
Family	Iridaceae
Genus	*Iris* L. – iris
Species	*Iris germanica* L.
Synonym(s):	*Iris flavescens* Delile
	Iris florentina L.
	Iris germanica var. *florentina* (L.) Dykes

FIGURE 1 Taxonomy and nomenclature of *Iris germanica* L.

and on occasion are added to the diets of pregnant and nursing mothers (Kaššák, 2012). Plants of the *Iris* genus are extensively employed in traditional medicine, viz., *I. germanica* rhizomes are employed as laxatives, diuretics, and carminatives in Turkish folk medicine. It has been reported that *I. germanica* rhizome juice is used to remove freckles and also to heal certain sores (Burcu et al., 2014). Moreover, root decoctions of *I. germanica* are employed as aperients, antispasmodics, diuretics, stimulants, as well as emmenagogues. Furthermore, some reports mentioned that an extract of the root of *I. germanica* has been employed for treatment of edema and gall bladder diseases as well as for certain fevers and enlargement of the liver. *Iris germanica* has also been used for treatment of catarrhal disease in children, for venereal diseases, and for purifying blood (Kaššák, 2012). Additionally, *I. germanica* has economic importance as a consequence of its use in the perfume and cosmetic industries, and some selected plants from the genus *Iris* are also grown for ornamental purposes (Burcu et al., 2014).

Certain plants of the genus *Iris* have demonstrated anti-inflammatory, anti-ulcer, anticancer, and antibacterial activities (Deng et al., 2009). Interestingly, most of the species of *Iris* have in general been employed in dentifrices and perfumes (Choudhary et al., 2005). Numerous compounds have been reported to be present in *Iris* plants and include flavonoids, isoflavonoids, triterpenoids, xanthones, stilbene glycosides, and quinones (Kaššák, 2012; Rahman et al., 2002). These compounds demonstrated cytotoxic, antimalarial, antioxidant, anti-inflammatory, antiangiogenic, antiproliferative, anti-neoplastic, antifungal, and antineoplastic activities (Kaššák, 2012). In addition, some further natural products isolated from *Iris* plants include xanthinoxidase, aldose reductase, quinone reductase (QR), COX-1 (cyclooxygenase-1), and COX-2 (cyclooxygenase-2) inhibitors (Kaššák, 2012). Reports of secondary metabolites used as diuretics, germination inhibitors, hepatoprotectives, immunoenhancers, insecticides (termites and other insects), and also showing cytochrome P450 1A (Cyp1A) activity have been published (Kaššák, 2012).

Members of the Iridaceae family showed antifungal, antibacterial, anticancer, anthelmintic, anti-HIV, antidepressant, and antiviral properties (Nath et al., 2013). Furthermore, some plants of the Iridaceae family are employed for the treatment of intestinal disorders, coronary disorders (angina pectoris), dermal, gut, urogenital, and respiratory infections. Plants from the Iridaceae family are also used as a diuretic, purgative, stimulant, detoxicant, expectorant, and hypertensive agent (Nath et al., 2013).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Antimicrobial Activity

Essential oils are volatile oils extracted from plants and have been employed both as food preservatives as well as additives to food as part of the most vital spice industry. Additionally, these oils are employed in perfumery, cosmetics, and medicines (Hyldgaard et al., 2012). It has been reported that the initial utilization of essential oils was in medicine, but later in the nineteenth century, their use as flavor constituents increased. It has been reported that about 3000 various essential oils are known, and interestingly only 300 of these oils are employed in the flavor and fragrances industry (Hyldgaard et al., 2012). Essential oils are considered important since they are believed to be used by the plant for its defense against being either eaten or destroyed by disease—this is why the plants developed EOs that have antimicrobial properties. It has been reported that the first antibacterial potential of secondary metabolites were tested using EO vapors by De la Croix in 1881 (Hyldgaard et al., 2012). Since then, EOs or their secondary metabolites have been shown to demonstrate antibacterial, antiparasitic, insecticidal, antiviral, antifungal, and antioxidant properties. Interestingly, the food industry primarily utilizes

FIGURE 2 *Iris germanica* L.

FIGURE 3 Rhizomes of *Iris germanica* L.

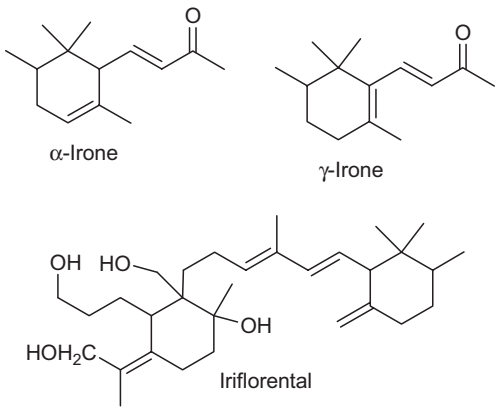

FIGURE 4 Structures of α- and γ-irones and iriflorental.

EOs as flavorings and as food preservatives (Hyldgaard et al., 2012). *Iris germanica* L. (Figure 2) has been cultivated as an ornamental plant in most countries due to its brilliantly colored flowers.

The rhizome of *I. germanica* L. (Figure 3) contains pleasantly smelling substances called irones (α- and γ-irones) (Figure 4), which are formed from iriflorental during the rhizome aging process (Krick et al., 1984).

In order to isolate the essential oils, the rhizomes of *I. germanica* L. have to be dried three years, during which the irone formation takes place to provide a good concentration of product. Although hydrodistillation of *I. germanica* L. rhizomes is a comparatively long procedure, the usual yield of oil is 0.2–0.35%, which is considered low.

TABLE 1 Antimicrobial Activity of the Essential Oils of *Iris germanica*

Sample	*Pasteurella multocida*	*Escherichia coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Ganoderma lucidum*	*Aspergillus flavus*	*Aspergillus niger*	*Alternaria alternate*
Oil	11[a]	10	10	12	10	12	10	12
Standard[c]	16[a]	18	28	26	28	26	22	22
Oil	754[b]	821	811	699	804	706	803	702
Standard	496[b]	447	207	253	213	256	352	356

[a]*Agar disc diffusion method, diameter of inhibition zone (mm) including disc diameter of 6 mm.*
[b]*Data represent the MICs (μL/mL) ± standard error of the means for four experiments.*
[c]*Standard: Rifampicin/Terbinafen.*

TABLE 2 Antioxidant Activity of the Essential Oils of *Iris germanica*

Sample	DPPH Inhibition (%)	DPPH IC_{50} (μg/mL)	Inhibition in Linoleic Acid Oxidation (%)
Oil	89.7	32.6	58.3
BHT	89.5	19.2	93.0

For this reason, the iris essential oil is considered to be one of the most expensive oils on the market and sells for about €15,000/kg (Roger et al., 2012).

Asghar et al. (2011) evaluated the antibacterial potential of the essential oils of *I. germanica* toward two Gram-positive bacteria, viz., *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative bacteria, viz., *Pasteurella multocida* and *Escherichia coli* (Table 1). These same researchers evaluated the antifungal potential of *I. germanica* L. oils toward four pathogenic fungi, viz., *Ganoderma lucidum*, *Aspergillus flavus*, *Aspergillus niger*, and *Alternaria alternate*. Gram-positive and Gram-negative bacterial strains were grown at 37 °C in nutrient agar (NA, Oxoid, Hampshire, UK) and pathogenic fungal strains were grown at 28 °C using potato dextrose agar (PDA, Oxoid, Hampshire, UK). The antimicrobial potential of oils of *I. germanica* L. was performed via two methods, viz., disc diffusion method and minimum inhibitory concentration (MIC) method (Rahman et al., 2001; Asghar et al., 2011).

The antimicrobial results demonstrated that essential oils of *I. germanica* showed significant antibacterial potential toward *P. multocida*, *E. coli*, *B. subtilis*, and *S. aureus*. Similarly, *I. germanica* EOs also demonstrated promising inhibitory activity toward the four pathogenic fungi, viz., *G. lucidum*, *A. flavus*, *A. niger*, and *A. alternate*. Interestingly, *I. germanica* EOs demonstrated a more powerful antimicrobial potential than the standard rifampicin/Terbinafine. In particular, EOs possessed strong activity toward two bacteria, viz., *E. coli*, *B. subtilis*, and two fungi, *G. lucidum* and *A. niger*, with growth inhibition of 10 mm. Similarly, MIC values of *I. germanica* EOs were higher than the standard drug (Asghar et al., 2011).

Antioxidant Activity

Antioxidant activities of *I. germanica* EOs were performed via two assays, viz., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and percent inhibition in linoleic acid assay. The DPPH assay was performed by Mimica-Dukic et al. (2003) and percent inhibition in linoleic acid assay was performed by using Singh et al. (2005) methodology. Asghar et al. (2011) reported on the antioxidant potential of the essential oils of *I. germanica*, and the results indicated strong DPPH inhibition (Table 2) compared to standard BHT (BHT: IC_{50}: 19.2 μg/mL (98.5%); Oil: IC_{50}: 32.6 μg/mL (89.7%)). However, essential oils of *I. germanica* demonstrated moderate inhibition in the linoleic acid oxidation assay as compared to standard BHT (BHT: 93.0%; Oil: 58.3%).

Chemical Composition of Essential Oils from *I. germanica*

Asghar et al. (2011) investigated the chemical composition of *I. germanica* L. EOs by gas chromatography-mass spectrometry (GC-MS) and identified 11 compounds (Table 3). On the other hand, Dulgheru and Burzo (2011) investigated oils of the

TABLE 3 Composition of the Terpenes in the Essential Oils Extracted from *Iris germanica*

No.	Compound (Dulgheru and Burzo, 2011)	(%) Flowers	(%) Buds	Compound (Asghar et al., 2011)[a]
1	Acid-γ-linolenic	1.1	–	9-Hexadecanoic acid methyl ester
2	Farnesol	1.3	–	9-Octadecenoic acid methyl ester
3	β-Cedrene	1.1	–	8-Octadecenoic acid methyl ester
4	β-Caryophyllene	1.0	–	11-Octadecenoic acid methyl ester
5	β-Farnesene	4.7	–	10-Octadecenoic acid methyl ester
6	Z-β-Farnesene	1.1	–	13-Octadecenoic acid methyl ester
7	Heptadecane	1.1	–	16-Octadecenoic acid methyl ester
8	β-Sequiphellandrene	2.1	–	1,2-Benzenedicarboxylic acid diisooctyl ester
9	Heptadecadiene	14.2	3.0	Bis (2-ethylhexyl) phthalate
10	Benzyl benzoate	–	32.8	Methyl 6-methyl heptanoate
11	*trans*-Farnesol	3.2	–	Nonanoic acid, 9-oxo-methyl ester
12	Nonadecane	5.0	–	
13	Methyl palmitate	3.4	–	
14	Tricosane	18.2	14.3	
15	Methyl tricosane	5.1	5.4	
16	Heptacosane	7.4	10.5	
17	Tetracosane	2.3	2.7	
18	Octacosane	5.0	11.3	
19	Heneicosane	6.4	–	
20	Docosan	1.6	–	
21	Phenylpropyl acetate	–	1.8	

[a]*Percentage composition of EOs are not reported in Asghar et al. (2011).*

flower buds and perfume of the mature flowers of *I. germanica*. Interestingly, bud oils of *I. germanica* were reported to have eight components, viz., heptadecadiene (3.0%), benzyl benzoate (32.8%), tricosane (14.3%), methyl tricosane (5.4%), heptacosane (10.5%), tetracosane (2.7%), octacosane (11.3%), and phenylpropyl acetate (1.8%). EOs found in mature flowers, however, contained 26 components of which 12 were hydrocarbons (67.9%), eight were sesquiterpenes (15.6%), three were fatty acids (5.2%), two were monterpenes (0.9%), and one was an aldehyde (0.5%). Furthermore, heptadecadiene, nonadecane, tricosane, methyl tricosane, heptacosane, octacosane, and heneicosane were the most abundant components produced by mature flower EOs. Interestingly, seven components, viz., heptadecadiene, *trans*-farnesol, tricosane, methyl tricosane, heptacosane, tetracosane, and octacosane were present in the two investigated *I. germanica* oils (Dulgheru and Burzo, 2011).

SUMMARY

- Essential oils of *I. germanica* L. possess significant antimicrobial potential against the growth of food poisoning organisms.
- Oils of *I. germanica* L. demonstrate a most promising antioxidant activity.
- The antimicrobial and antioxidant activities of *I. germanica* L. essential oils confirm their potential use in the food industries.
- The antimicrobial and antioxidant activities of *I. germanica* L. essential oils also confirm their potential use in the preservation of foodstuffs against bacteria and fungi.
- These oils may thus be valuable for increasing the shelf life of foodstuffs.
- The reviewed studies clearly demonstrate that *I. germanica* L. essential oils present a great potential for the food industry.

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Chapter 55

Jasmine (*Jasminum sambac* L., Oleaceae) Oils

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List of Abbreviations

m Meter
ft Foot
cm Centimeter

INTRODUCTION

Many microorganisms are responsible for food spoilage. They can originate from its own natural microflora or from the processing conditions like in the course of harvesting/manufacturing, storage, and transport. In some cases, the microflora has no discernible effect on the food quality and food safety, while in others, this may affect the quality in several ways like causing food spoilage, food-borne illness, or food fermentation. While food fermentations are desirable transformations of food, food spoilage, food-borne infections and intoxications may result in huge economic losses as in cases where a particular batch of food has been found to be involved in an outbreak of a disease or has a short shelf life and hence the complete batch has to be recalled back from the market and destroyed. The usage of biological preservation is increasing due to consumer demand, minimum undesirable effect, less environmental drawbacks, etc. Change in life style has forced people to rely on fast food, processed food, etc. So this factor is increasing the risk of microorganisms to be involved in food spoilage. To counter the complexity of microbial interactions with food products, there is a great deal of emerging interest in improving understanding and knowledge of biological preservation.

The word *jasmine* is derived from the Arabic word meaning "yasmin" or "gift from God." *Jasmine sambac* is popularly known as "Queen of the night" as well as "moonlight of the grove" because the flowers open up at midnight to release their full fragrance (Rao and Rout, 2003). In the Philippines, *J. sambac* is traditionally called *Sampaguita*, meaning "I promise you" and its flower is a symbol of fidelity, purity, and eternal love. It is the country's national flower and is considered as a symbol of honor and dignity. *Jasmine sambac* flowers are a rich source of essential oil (Fernando et al., 2010).

There are more than 200 species of jasmine now cultivated in the subtropics worldwide mainly for the fragrance for the perfume industry. The major jasmine absolute or concrete producing countries are Egypt and India; smaller producing countries include Morocco, Algeria, France, and Italy. Egypt produces approximately 70–80% of the world's jasmine absolute alone. Jasmine is used in gardens as an ornamental plant. Jasmine oil prepared from the flower is used traditionally in treatment of various diseases like arthritis, hepatitis, conjunctivitis, gastritis, and diarrhea. It has stimulant, relaxant, analgesic, anti-inflammatory, anticonvulsant, and aphrodisiac activity. The jasmine plant is one of the commercially important plants for its fragrance for the perfume and food industries. Jasmine oil is one of the main ingredients of aroma used in aromatherapy for creating a feeling of well-being and mental relaxation. It is used in beverages for its flavor. Jasmine-scented tea, prepared by absorbing the fragrance of flowers onto dried green tea leaves, is famous in China and Thailand. It is also used to add flavor to foodstuffs such as sweets and candies, cookies, snacks, and chewing gum. It is also used in perfumes, lotions, soaps, air fresheners, and skin care products. Jasmine oil has been used as a preservative in food preparations for antimicrobial and antioxidant actions.

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Jasmine oil is a viscous, clear yellow-brown liquid with the characteristic odor of jasmine, produced from the concrete extract of the flowers. One kilogram of the jasmine absolute is obtained from about 1000 kg of jasmine flowers (about 7.6 million blossoms; Holmes, 1998). Jasmine oil is extracted from the flowers by solvent extraction and enfleurage (mainly in France) methods (Scott, 2005). The main constituent of jasmine absolute, prepared from flowers, is benzyl acetate. Flowers have a unique flowery and sweet clove-like aroma. Volatile components include epoxylinalool, linalool, benzyl acetate, indole, E-E-α-farnesene, Z-3-hexenyl benzoate, benzyl alcohol, and methyl anthranilate (Holmes, 1998; Lasekana and Lasekan, 2012). Although limited studies have been carried out for jasmine essential oil for its antimicrobial activity against various gram-negative, gram-positive bacteria and fungi, sufficient evidence can be correlated with existing literature about the antimicrobial activity of components with structural similarity as of jasmine essential oil (e.g., linalool). Linalool is found in many essential oils in varying percentages. Linalool is a monoterpenoid alcohol, the major component of jasmine essential. Due to their lipophilic character, monoterpenes will preferentially partition from an aqueous phase into membrane structures. The mode of action of linalool is associated with membrane expansion, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and alteration of ion transport processes of microorganisms.

BOTANICAL ASPECTS

Jasminum sambac (L.) Aiton, also known as *Nyctanthes sambac* (L.) is classified under the genus *Jasminum* under the tribe Jasmineae. It belongs to the olive family Oleaceae (USDA, 2013). *Jasminum sambac* is commonly called Arabian Jasmine in English, though it is not native to Arabia. This plant is native to southwestern and southern Asia, mainly the Philippines, India, Myanmar, and Sri Lanka (Lasekana and Lasekan, 2012). *Jasminum sambac* and other species of the genus spread into the Middle East, Persia, and then Europe in the eighteenth century. It is now cultivated nearly throughout the tropical and subtropical parts of the world.

Jasmine is a small evergreen vine or shrub reaching up to 0.5–3 m (1.6–9.8 ft) tall with attractive glabrous leaves producing attractive white sweet-scented flowers in great profusion in the hot season. This species is highly variable in terms of plant propagation. Only a few varieties can be reproduced by seed. Once cultivated, it does not bear seeds and the plant is reproduced solely by cutting, layering, marcotting, and other methods of asexual propagation. Though flowering occurs throughout the year, peak season starts from March and lasts until July. Flowers are produced in clusters of 3–12 together at the end of branches, about 2–3 cm in diameter with 5–9 lobes, strongly scented with a white corolla. Flowers open at night and close in the morning (Leonhardt and Teves, 2002). Gunfumalli, Motia, Virupakshi, Sujimalli, Madanabanam, and Ramabanan are a few varieties of *J. sambac* cultivated in South India (Skaria et al., 2007). It is widely cultivated for its attractive and sweet fragrant flowers. It is used in gardens as an ornamental plant. The flowers are also used for perfumes and making tea. Other commercially important species grown for the perfumer industry are *Jasminum officinalis*, *Jasminum grandiflorum*, and *Jasminum auriculatum*. The main chemical components are benzyl acetate, linalool, benzyl alcohol, indole, benzyl benzoate, *cis*-jasmone, geraniol, methyl anthranilate, and trace amounts of p. cresol, farnesol, *cis*-3-hexenyl benzoate, eugenol, nerol, ceosol, benzoic acid, benzaldehyde, y-terpineol, nerolidol, isohytol, phytol, *cis*-3-hexenyl benzoate, etc. Major constituents are linalool and benzyl acetate (Rath et al., 2008).

USAGE AND APPLICATIONS

As a fresh flower, jasmine is used in bouquets and garlands to honor guests. The different parts of *J. sambac* such as the leaf, stem, bark, and roots are important as sources of chemicals that are useful in the pharmaceutical industries with different pharmacological activities (Sabharwal et al., 2011). Extracts prepared from the fragrant flowers of the plant are used traditionally in the treatment of various ailments. In China and Thailand, jasmine flowers are used as a folk remedy for the treatment of arthritis, hepatitis, conjunctivitis, gastritis, and diarrhea (Fernando et al., 2010). Jasmine oil is one of the main ingredients of aroma used in aromatherapy and dermatological applications (Holmes, 1998; Stevensen, 1998).

Jasmine is used along with lavender, rosemary, rose, and chamomile oils for the treatment of depression as inhalants. It has a stimulating effect for the relief of depression and uplifting mood in humans (Hongratanaworakit, 2010). Romans and Egyptians used this oil along with oils of lavender or ylang-ylang in aromatherapy, and baths infused with the oils cause feelings of well-being and mental relaxation (Wei and Shibamoto, 2010). Oil massage has shown to decrease the spike-wave in electroencephalogram and has anticonvulsant activity (Howes et al., 1998). Oil of ylang-ylang and jasmine are used as an aphrodisiac (to increase sexual desire; Buckle, 2006). It is also used to reduce stress and anxiety in pregnant women during labor. It is massaged onto the abdomen, thighs, and lower back to ease contractions of the uterus. It is also used to treat amenorrhea and spasmodic dysmenorrhea (Holmes, 1998; Steflitsch and Steflitsch, 2008).

USAGE AND APPLICATIONS IN FOOD INDUSTRIES

Essential oils possess a number of activities like antimicrobial, antiviral, antioxidant, anticancer, etc. (Burt, 2004; Bakkali et al., 2008; Gutierrez et al., 2008). Jasmine oil is extensively used by food industries to prevent food spoilage and to flavor foods. Food spoilage is the process in which food deteriorates such that it is no longer fit for human consumption or its edible quality is reduced. Many external forces are responsible for food spoilage. It occurs at the moment foods are harvested due to attacks from microorganisms, enzymes, and oxidation (atmospheric oxygen). Therefore, jasmine oil is incorporated in food products and beverages for its antimicrobial, antioxidant, and flavoring properties (Tull, 1997).

As Antimicrobial for Food Preservation

Various microorganisms responsible for food spoilage include bacteria, fungi, and yeast. Bacteria generally attack low-acid foods like vegetables and meat. When bacteria attack the foods and break down the food, acids, and other waste products are generated. The bacteria themselves may or may not be harmful to humans, but the products generated are unpleasant to taste and sometimes harmful to humans, therefore they are no longer fit for consumption.

Yeasts are responsible for the decomposition of food in which the sugar content is high. They metabolize the sugars to produce alcohols and carbon dioxide, a process called fermentation. They also grow as a dry film on food surfaces such as on pickle brine (Tull, 1997). A fungi or mold grows in the form of multicellular filaments called *hyphae*. There is a large, diverse number of fungal species that grow on the surface of foods leading to discoloration and fuzzy appearance. Mold can cause illness, when taken in the body or inhaled, especially if the person is allergic. Like yeasts, molds also flourish in high-acid foods like fruit, tomatoes, jams, jellies, and pickles. The attack by these microorganisms on foods causes a change in appearance like change in color, texture, an unpleasant odor or taste. Table 1 summarizes various microorganisms involved in food spoilage (Samson et al., 2004).

Essential oils and their components have been exploited for antibacterial properties and are used as dental root canal sealers, antiseptics, and feed supplements for lactating sows and weaned piglets (Burt, 2004). Lang et al. (2012) have reviewed the antimicrobial and antifungal activities of various essential oils. The main ingredient of jasmine oil, linalool, exhibits antimicrobial and antifungal activity against various microbes and fungi. For the same reason, Jasmine oil has been used as a preservative in food preparation for its antimicrobial action. It is found to be active against many bacterial and fungal strains as listed in Table 2.

Rath et al. studied the antibacterial activity of hydrosteam distilled essential oil of *J. sambac* L. flower (natural oil), synthetic oil (combination of major components), synthetic blends (linalool, benzyl acetate, methyl anthranilate, and methyl salicylate) and six major individual components (linalool, benzyl acetate, methyl salicylate, methyl anthranilate, and benzyl benzoate) against *Escherichia coli* (MTCC-443) strain. They determined minimum inhibitory concentration (MIC) by tube dilution technique. They reported that MIC of 31.25, 7.8, 15.62, and 15.62 μL/mL for natural oil, synthetic oil, synthetic blend, and linalool, respectively (Rath et al., 2008). Abdoul-Latif et al. evaluated the essential oil and methanol extract of *J. sambac* for its antimicrobial activity using disc diffusion and microdilution methods. They found that the essential oil and methanol extract showed better activity against bacterial species than against yeast. The zone of inhibition (mm) and MIC are listed in Table 2 (Abdoul-Latif et al., 2010).

Singh et al. studied antifungal activity of Mogra (Jasmine oil) prepared from *J. sambac* against *Aspergillus flavus*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Epidermatophyton floccosum*, and *Microsporum nanum* isolated from soil. Jasmine oil showed good activity against all these strains. The zone of inhibition by jasmine oil is listed in Table 2 (Singh et al., 2011).

Park et al. have tested linalool on various strains of periodontopathogens and *Streptococci* for its antimicrobial action and found that linalool possesses strong antimicrobial activity. The periodontopathogens and *Streptococci* cause oral infectious diseases such as dental caries and periodontal diseases by colonizing on the tooth surface. *Streptococcus mutans* and *Streptococcus sobrinus* are mainly responsible for dental caries while gram-negative bacteria such as *Porphylomonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* cause periodontal diseases. Linalool is one of the components used in toothpaste and mouthwashes for its antimicrobial action. Antimicrobial activity of linalool is enlisted in Table 3 indicating the MIC and minimum bactericidal concentration (MBC) of linalool against various strains of microbes. Linalool is also used in sweet candies to prevent infection, especially in children (Park et al., 2012). Bagamboula et al. reported the antibacterial activity in terms of zone of inhibition for the commercially pure linalool compound against *Shigella sonnei* (10% of linalool with 1.2 mm of zone inhibition), *Shigella flexneri* (10% of Linalool with 2.2 mm), and *E. coli* (1% of linalool with 3.8 mm of zone inhibition) (Bagamboula et al., 2004).

TABLE 1 Types of Food Spoilage and Microorganisms Involved (Samson et al., 2004)

Food	Type of Spoilage	Microorganisms Involved
Bread	Moldy	*Rhizopus nigricans*, *Penicillium*, *Aspergillus niger*
	Ropy	*Bacillus subtilis*
Maple sap and syrup	Ropy	*Enterobacter aerogenes*
	Yeasty	*Saccharomyces*, *Zygosaccharomyces*
	Pink	*Micrococcus roseus*
	Moldy	*Aspergillus*, *Penicillium*
Fresh fruits and vegetables	Soft rot	*Rhizopus*, *Erwinia*
	Gray mold rot	*Botrytis*
	Black mold rot	*A. niger*
Pickles, sauerkraut	Film yeasts, pink yeasts	*Rhodotorula*
Fresh meat	Putrefaction	*Alcaligenes*, *Clostridium*, *Proteus vulgaris*, *Pseudomonas fluorescens*
Cured meat	Moldy	*Aspergillus*, *Rhizopus*, *Penicillium*
	Souring	*Pseudomonas*, *Micrococcus*
	Greening, slime	*Lactobacillus*, *Leuconostoc*
Fish	Discoloration	*Pseudomonas*
	Putrefaction	*Alcaligenes*, *Flavobacterium*
Eggs	Green rot	*P. fluorescens*
	Colorless rots	*Pseudomonas*, *Alcaligenes*
	Black rots	*Proteus*
Concentrated orange juice	"Off" flavor	*Lactobacillus*, *Leuconostoc*, *Acetobacter*
Poultry	Slime, odor	*Pseudomonas*, *Alcaligenes*

Linalool is a monoterpenoid alcohol and is the major component of jasmine essential oil. The antimicrobial activity of most terpenoids is linked to their functional groups, and it has been shown that the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are important for antimicrobial activity. The terpenoids are a large group of antimicrobial compounds that are active against a broad spectrum of microorganisms. Their lipophilic character, monoterpenes will preferentially partition from an aqueous phase into membrane structures. The mode of action of linalool is associated with membrane expansion, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and alteration of ion, transport processes of microorganisms (Trombetta et al., 2005).

Other major linalool-containing essential oils have been found to be active against a wide range of microorganisms. Examples include *Coriandrum sativum* essential oil (linalool: 25.9–64.4%, active against *Candida* species and *Listeria monocytogenes*), *Cinnamomum verum* essential oil (linalool: 3.55%, active against *Campylobacter jejuni*, *Enterobacter aerogenes*, *E. coli*, *L. monocytogenes*, *Pseudomonas aeruginosa*, *S. enteritidis* and *Staphylococcus aureus*), Cit*rus hystrix* essential oil (linalool: 5.8%, active against *Aspergillus flavus* and *Aspergillus parasiticus*), *Satureja Montana* (linalool: 11%, active against *Candida albicans*, *S. cerevisiae*, *E. coli*, *L. monocytogenes*, *S. aureus*, *S. Typhimurium*, and *Clostridium perfringens*), *Gnaphalium affine* essential oil (linalool: 10.6%, active against six bacteria and five fungi). So it is possible that isolated linalool from jasmine essential oil can be effective against the above-mentioned microorganisms. Linalool combined with eugenol showed the highest synergy, suggesting that a monoterpenoid phenol combined with a monoterpenoid alcohol is an effective combination (Hyldgaard et al., 2012). All of the above data explain that jasmine oil or its components, that is, linalool, can help in food preservation.

TABLE 2 Inhibitory Activity of Jasmine Oil against Bacterial and Fungal Strains

Sr. No.	Bacteria/Fungi	MIC (μg/mL)	Zone of Inhibition (mm)	References
1	*Bacillus cereus* LMG 13569	0.5	17	Abdoul-Latif et al. (2010)
2	*Enterococcus faecalis* CIP 103907	1	22	
3	*Escherichia coli* CIP 105182	0.5	31	
4	*Listeria innocua* LMG 1135668	0.5	28	
5	*Salmonella enterica* CIP 105150	1	31	
6	*Shigella dysenteriae* CIP 5451	1	29	
7	*Staphylococcus aureus* ATCC 9244	1	23	
8	*Staphylococcus camorum* LMG 13567	8	8	
9	*Enterococcus faecalis*	4	25	
10	*Pseudomonas aeruginosa*	1	23	
11	*Staphylococcus aureus*	8	23	
12	*Streptococcus pyogenes*	0.5	41	
13	*Escherichia coli*	31.25 (μL/mL)	–	Lang et al. (2012)
14	*Aspergillus flavus*	>8	10	Abdoul-Latif et al. (2010)
15	*Candida albicans* ATCC 10231	0.5	15	
16	*Candida albicans*	0.5	13	
17	*Aspergillus niger*	>8	9	Singh et al. (2011)
18	*Aspergillus flavus*	–	13	
19	*Microsporum nanum*	–	17	
20	*Epidermatophyton floccosum*	–	21	
21	*Trichophyton tonsurans*	–	30	

MIC, Minimum inhibitory concentration, determined by microdilution method.

As Antioxidant

Atmospheric oxygen is one of the sources for oxidation process. Oxidation of food ingredients degrades the quality of the foods. It produces free radicals, which in turn are responsible for cell damage or death. It leads to rancidity or color changes in the foods. Antioxidants are agents that inhibit the oxidation of other molecules. Antioxidants terminate free radical chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves. Antioxidant activity measurement can be done based on the ability of a compound to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, to inhibit the cycle of lipid oxidation by preventing the formation of hexanoic acid from hexanal (aldehyde/carboxylic acid assay), or malonaldehyde from UV-irradiated squalene (malonaldehyde/gas chromatography (MA/GC) assay).

Jasmine oil has been evaluated for antioxidant activity by DPPH free radical scavenging and β-carotene-linoleic acid assays. Jasmine oil showed IC50 value of 7.43 μg/mL and RAA value of 96.6% in DPPH assay and β-carotene-linoleic acid assay, respectively (Abdoul-Latif et al., 2010).

Wei et al. tested antioxidant activity of 13 essential oils by using three different antioxidant assays, namely aldehyde/carboxylic acid assay, DPPH assay, and MA/GC assay. They have found that jasmine essential oil exhibited 100%, 92%, and 21% antioxidant activity in aldehyde/carboxylic acid assay, DPPH assay, and MA/GC assays, respectively, at 500 μg/mL after 40 days. In Table 4, antioxidant activities of different essential oils are tabulated and jasmine oil has shown antioxidant activity with similar potency to that of α-tocopherol. Jasmine oil is used for preservation of food and to prevent degradation (Wei and Shibamoto, 2007, 2010). Many other essential oils containing linalool have been reported to possess antioxidant activity (Miguel, 2010). So jasmine oil as a whole or linalool can be used for the prevention of oxidation of foods and food products.

TABLE 3 Antimicrobial Activity of Linalool against Periodontopathogens and *Streptococci species* (Park et al., 2012)

Species and Strains	MIC (mg/mL)	MBC (mg/mL)
Porphylomonas gingivalis ATCC 33277^T	0.8	1.6
P. gingivalis ATCC 49417	0.1	0.2
P. gingivalis ATCC 53978	0.1	0.1
Prevotella intermedia ATCC 25611^T	0.2	0.4
P. intermedia ATCC 49046	1.6	1.6
Prevotella nigrescens ATCC 33563^T	0.8	0.8
P. nigrescens ATCC 25261	0.8	0.8
Fusobacterium nucleatum subsp. *nucleatum* ATCC 25586^T	0.2	0.4
F. nucleatum subsp. *polymorphum* ATCC 10953^T	0.2	0.4
F. nucleatum subsp. *vincentii* ATCC 49046^T	0.1	0.1
F. nucleatum subsp. *fusiforme* ATCC 51190^T	0.2	0.4
F. nucleatum subsp. *animalis* ATCC 51191^T	0.2	0.2
Aggregatibacter actinomycetemcomitans ATCC 33384^T	0.1	0.2
A. actinomycetemcomitans ATCC 43717	0.1	0.1
A. actinomycetemcomitans ATCC 43718	0.1	0.1
Streptococcus mutans ATCC 25175^T	1.6	3.2
S. mutans KCOM 1054	1.6	3.2
S. mutans KCOM 1111	0.8	1.6
S. mutans KCOM 1113	0.4	0.4
S. mutans KCOM 1116	0.4	0.8
S. mutans KCOM 1126	0.8	0.8
S. mutans KCOM 1128	3.2	3.2
S. mutans KCOM 1136	0.1	0.1
S. mutans KCOM 1197	0.4	0.8
S. mutans KCOM 1202	1.6	1.6
S. mutans KCOM 1207	0.8	1.6
S. mutans KCOM 1217	1.6	1.6
Streptococcus sobrinus ATCC 33478^T	1.6	1.6
S. sobrinus KCOM 1157	1.6	1.6
S. sobrinus KCOM 1196	1.6	1.6
S. sobrinus KCOM 1221	0.4	0.8

ATCC, America Type Culture Collection; KCOM, Korean Collection for Oral Microbiology.

As Flavoring Agent

Jasmine is a commercially important plant. It is extensively used for fragrance in aromatherapy and the perfume industry. Linalool along with other volatile components is one of main ingredient responsible for aroma of oil obtained from flowers. Linalyl β-D-glucopyranoside and its 6′-*o*-malonate are the glycosidic precursors synthesized in flower buds that undergo enzymatic hydrolysis releasing the linalool (Moon et al., 1994). Traditionally, scented tea is produced in China and Thailand by absorbing the fragrance of various flowers onto dried green tea leaves. Jasmine oil rich in linalool is used for this

TABLE 4 Antioxidant Activities of Essential Oils (Wei and Shibamoto, 2007)

Sr. No.	Essential Oil	Inhibitory Effect (%)		
		Aldehyde/Carboxylic Acid Assay (500 μg/mL)	DPPH Scavenging Assay (200 μg/mL)	Malonaldehyde/Gas Chromatography Assay (500 μg/mL)
1	α-tocopherol (Standard)	100	88	75
2	Ylang-ylang	100	65	20
3	Rose	100	70	50
4	Parsley seed	100	50	65
5	Jasmine	100	92	21
6	Celery seed	98	55	20
7	Juniper berry	76	50	10
8	Patchouli	60	48	−9
9	Angelica seed	58	40	–
10	Lavender	23	48	–
11	Ginger	21	52	–
12	Sandal wood	13	48	–
13	Chamomile	8	50	–
14	Peppermint	6	55	–

purpose. Jasmine tea is the most famous and researchers are looking to produce more varieties of scented green tea to meet consumer preferences (Samakradhamrongthai et al., 2009). Scented Jasmine green tea is also familiar in Japan because it has been served in Chinese restaurants for a long time and also as polyethylene terephthalate-bottled and canned jasmine (Ito et al., 2002). Flowers added to green tea stimulate blood circulation and help the respiration system. In Japan, jasmine oil along with leaves of *Diospyros kaki* is used in antismoking candies (Krishnaiah et al., 2011).

Jasmine oil leaves a pleasant memory after use. This quality is used as a marketing device to sell edible and cosmetic products. The most common essential oils used in foods are listed in Table 4. Jasmine oil is used to add flavor to foodstuffs such as sweets and candies, cookies, snacks, and chewing gum. It is also used in perfumes, lotions, soaps, air fresheners, and skin care products (Hernandez, 2000).

SUMMARY POINTS

- Arabian Jasmine plant is widely cultivated throughout the tropical and subtropical parts of the world for its attractive and sweetly fragrant flowers.
- Linalool and benzyl acetate are main ingredients of jasmine oil.
- Jasmine oil is used traditionally in the treatment of various diseases like arthritis, hepatitis, conjunctivitis, gastritis, and diarrhea.
- Jasmine plant is one of the commercially important plants for fragrance of flowers for perfume and food industries.
- It has stimulant, relaxant, analgesic, anti-inflammatory, anticonvulsant, aphrodisiac activity.
- It is used in beverages, teas, sweets and candies, cookies, snacks, and chewing gum for its flavor.
- Linalool has been proven to be active against a wide range of microorganisms, so it can be used as a preservative in food preparations.
- The mode of action of linalool is associated with membrane expansion, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and alteration of ion transport processes of microorganisms.
- US-FDA qualifies linalool as safe and that it can be used as a flavoring agent for food products.
- Linalool can be used with other components of essential oils to form an effective preservative agent.

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Chapter 56

Juniper (*Juniperus communis* L.) Oils

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INTRODUCTION

Juniperus communis L. is a volatile secondary metabolite essential oil-bearing and wide-spreading conifer. The high ecological amplitude is typical of this species (Adams, 1998), it is frequent in dry pinewoods, on river valleys, and also occurs on wet soils. It is light-demanding, but can grow in rather little daylight. Wide ecological amplitude is the reason for the quantitative and qualitative diversity of essential oils. *Juniperus communis* has been used in folk medicine and also in folk veterinary medicine. Nowadays, the cones of *J. communis* and their essential oils are recognized by the European Pharmacopoeia and are pharmaceutical raw material. This chapter aims to provide an overview of current knowledge about the quantitative and qualitative composition of essential oil of common juniper, its properties, and its use in the food industry as a spice and natural food preservative.

BOTANICAL ASPECTS

Juniperus communis L (*Cupressaceae* Rich. Ex Bartl.) is an evergreen, perennial, long-lived (to 600 years or more) coniferous plant having the largest range of any woody plant in the cool temperate geographical regions of Northern Hemisphere, from the Arctic south, in mountains, to around a latitude of 30° north in Europe, Asia, and North America. *Juniperus communis* is a globally distributed species exhibiting a wide range of ecological adaptation. They grow as scattered single individuals of the undergrowth in dry pinewoods or mixed forests or may form a stand (Figure 1). The plant is light demanding but also tolerates the shade (Navasaitis, 2004). A wide geographical distribution is the main reason for the remarkable variation in the morphological characteristics and chemical composition of the secondary metabolites of *J. communis* (Adams, 2004).

Juniperus communis has green and sharp leaves (needles) in whorls of a three, which are 10–15 mm long and 1–2 mm wide; the leaves remain on the branch for up to 4 years. *Juniperus communis* is a dioecious species: male and female cones grow on separate plants which are wind-pollinated. The male plants of common junipers are often similar to trees, occasionally reaching 10 m tall, while the female plants as more sprawling better resemble the shrubs. The plant blooms in April–May; however, the cones of *J. communis* mature in late autumn of the second year. Therefore, the unripe second year and ripe third year berries may be collected from the same plant simultaneously (Figure 2). The spherical, 5–12 mm diameter, cones are berry-like, blue-black with a waxy coating and usually have three (or sometimes six) scales, each scale with a single seed. *Juniperus communis* is a very slowly growing plant reaching approximately 20 and 50 cm in height after 5 and 10 years, respectively.

Intraspecific variation is characteristic for *J. communis*; two subspecies are distinguishable, namely subsp. *communis* and subsp. *alpina* (Suter) Čelak (syn. *J. communis* subsp. *nana*, *J. communis* var. *saxatilis* Pallas, *J. sibirica* Burgsd.) (Adams and Pandey, 2003). Subsp. *communis* is usually an erect shrub or small tree with 8–20 mm (sometimes reaching 27 mm) long leaves and small 5–8 mm diameter cones; common in low and moderate altitude of temperate latitude of northern hemisphere. This subspecies includes several varieties such as var. *communis*, var. *depressa* Pursh, var. *nipponica* (Maxim.) E.H. Wilson (syn. *J. rigida* var. *nipponica*) and var. *hemisphaerica* (J. Presl & C. Presl) Parl. that are indigenous to Europe, most of northern Asia, North America, Sierra Nevada, and California, Japan, and Mediterranean mountains, respectively. Subsp. *alpina* is a prostrate ground-hugging shrub with shorter leaves (3–8 mm long) and larger cones (7–12 mm diameter), It is common in subarctic region and higher altitudes of temperate latitude. Several varieties have been described for this subspecies: var. *alpina*, var. *jackii* Rehder and var. *megistocarpa* Fernald & H. St. John, growing in Greenland, Europe and Asia, Western North America and Eastern Canada, respectively.

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FIGURE 1 *Juniperus communis* L. stand (Lithuania, Skuodas mun., Šaukliai, Šaukliai landscape reserve).

FIGURE 2 Unripe second year and ripe third year cones of *Juniperus communis* L.

USAGE AND APPLICATIONS

Juniper has been used by man since prehistoric times. Ancient people attributed a mystical potency to junipers and their sprigs were thrown into fires to protect against evil spirits. In Lithuania, Poland, and other countries of the Baltic region the sprigs of *J. communis* have been used for blessing during Palm Sunday as they are believed to protect houses against thunder and evil, and crops against showers of hails, droughts, and rodents. In olden times, the needles of hallowed juniper sprigs were admixed to the first seeder of cereals, while the sprigs were spiked in the field for a good crop yield; they were also placed in hives to protect bees against disease (Mardosa, 2011). The mystical potencies attributed to junipers were associated with strong disinfectant properties of this species which were observed a long time ago. Therefore, the sprigs of junipers were even burned to warding off the plague during different epidemics such as cholera, dysentery, and ship fever. The cones were also used as a diuretic, an abortifacient, for diabetes treatment, and for foot complaints, while decoctions of *J. communis* branches and cones were used to treat kidney infections and to prevent or cure helminthiasis (Chevallier, 2000). *Juniperus communis* has also been used in folk veterinary medicine as a feed to prevent worms in ruminants (Viegi et al., 2003).

Nowadays the cones of *J. communis* and their essential oils are recognized by the Official Pharmacopoeias of Austria and Switzerland, as well as the European Pharmacopoeia. The strong antibacterial, antifungal, antiviral, antioxidant, and antiinflammatory properties are characteristic of the essential oil of *J. communis* cones; therefore, it is used in contemporary pharmacy for healing cold or as a bacterial and fungal antiseptic (Filipowicz et al., 2003; Pepeljnjak et al., 2005).

TABLE 1 Main Terpenes (Percentage in Essential Oil ≥10%) of Essential Oil of Leaves and Cones of *Juniperus communis* L. Determined in Some Countries of Asia and Europe

	Main Compounds of *Juniperus communis* L. Essential Oils	
Country	Leaves	Cones
Iran	Sabinene (40.7%), α-pinene (12.5%), terpinen-4-ol (12.3%)	Sabinene (36.98%), α-pinene (20.0%), limonene (10.6%)
India	α-Pinene (49.5%), limonene (19.5%) δ-3-Carene (14.7%)	
Mongolia	α-Pinene (58.2%)	
Italy	Sabinene (61.09%), limonene (36.2%), β-selinene (15.2%), terpinen-4-ol (10.67%)	α-Pinene (52.26%), limonene (40.1%), germacrene D (17.2%)
Greece	α-Pinene (41.3%), sabinene (17.4%)	
Croatia	α-Pinene (16.9%), sabinene (12.1%)	
Poland	α-Pinene (89.7%), sabinene (42.5%), γ-terpinene (11.8%), limonene (15.8%)	α-Pinene (90.0%), myrcene (46.3%), sabinene (15.8%)
Lithuania	α-Pinene (75.8%), sabinene (40.8%), β-phellandrene (11.4%)	α-Pinene (75.8%), myrcene (24.4%), terpinen-4-ol (13.1%), β-caryophyllene (11.3%), sabinene (10.2%)
Sweden	α-Pinene (56.8%)	
Estonia	α-Pinene (36.4%)	α-Pinene (47.9%)
Russia	α-Pinene (21.7%), sabinene (13.4%)	

Shamir et al. (2003), Lohani et al. (2013), Adams (1998, 2000), Angioni et al. (2003), Marongiu et al. (2006), Silva et al. (2000), Mastelic et al. (2000), Ochocka et al. (1997), Ložienė et al. (2010), Ložienė and Labokas (2012), Butkienė et al. (2006, 2009), and Orav et al. (2010).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Juniperus communis is the only pot herb among coniferous plants. For food purposes mostly the cones are used, while the applications of sprigs are rarer. Flavoring of gin is probably the most well-known culinary use of juniper cones. The name gin is derived from *genièvre* (French), *jenever* (Dutch), and *ginepro* (Italian), all of which mean juniper. This juniper-based spirit was first produced in the sixteenth century in Holland. In the late seventeenth century, gin spread to England causing the so-called "gin epidemic". In France and Scandinavian countries a fermented alcoholic beer–like drink, *genevrette*, flavored with juniper berries was produced from barley (Vichi et al., 2005). Until the mid-twentieth century a beer-like drink with juniper, called *piwo kozicowe*, was commonly fermented together with hops and honey/sugar in north and north-east Poland. Today the popularity of this kind of beer has been relived in the Kurpie region (north-east of Poland) and is mainly served to tourists. Until the mid-twentieth century, juniper cones were occasionally used for making wine and liqueur in Poland (Łuczaj and Szymański, 2007). Syrup produced from *J. communis* and *Taraxacum* spp. is sold in health food shops in Estonia (Łuczaj et al., 2012). Juniper cones are included in a peppery sauce and can be added to casseroles, marinades, and stuffing. In Poland, juniper cones have been widely used as a spice, especially in a sauerkraut stew, called *bigos* and in *kiełbas jałowcowa* sausages (Łuczaj and Szymański, 2007).

The aromatic properties of juniper cones depend on essential oil. The unripe cones have been reported to accumulate more essential oil (up to 4.2%) than ripe cones (up to 1.1%) (Butkienė et al., 2006; Ložienė and Labokas, 2012); however, the qualitative composition of essential oils of unripe and ripe cones of the same shrub were found to be similar (Ložienė et al., 2010). Two terpene compound groups, namely monoterpenes and sesquiterpenes, constitute the main part of juniper essential oils although the balance between them is rather variable (Butkienė et al., 2009). Terpenes determine the strong and distinctive aroma of essential oils of cones (Vaičiulytė and Ložienė, 2013). Their total content in juniper volatile oils exceeds 10% (Table 1). However, commonly the predominant essential oil constituent in the cones of *J. communis* is a bicyclic monoterpene α-pinene, the content of which may reach 90% of the total oil (Angioni et al., 2003; Shamir et al., 2003; Butkienė et al., 2006, 2009). Its isomer, β-pinene, is present in the essential oils of *J. communis* cones in lower amounts, for instance, in the presented typical chromatogram the content of β-pinene was to 32 times lower than that of α-pinene (Figure 3). Sabinene was also reported as a major (36.8%) or quantitatively second (13.2%) component in juniper berry oils from Iran

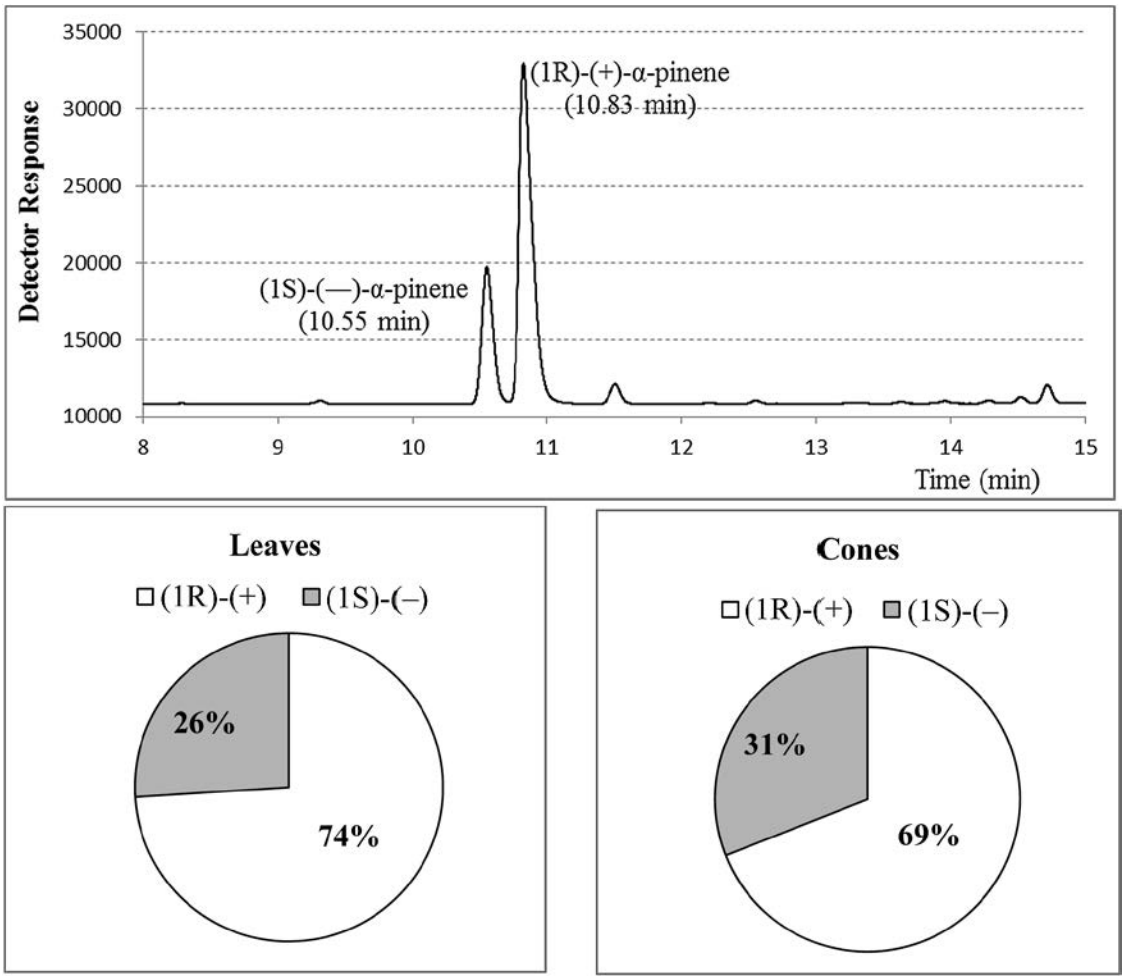

FIGURE 3 (1R)-(+)-α-Pinene prevailed over (1S)-(−)-α-pinene in essential oils of leaves and cones in *Juniperus communis* L. growing wild in Lithuania.

(Shamir et al., 2003) and mountain areas of Sardinia in Italy (Angioni et al., 2003), respectively. Myrcene, limonene, and terpinen-4-ol were reported to be other quantitatively important constituents in juniper berry essential oils (Shamir et al., 2003; Filipowicz et al., 2003; Mastelic et al., 2000).

Several studies have investigated the antimicrobial properties of *J. communis* essential oils and other preparations. Volatile extract isolated from common juniper was a stronger antibacterial or antifungal agent against all tested microorganisms than petroleum ether and ethanol extracts. The minimal inhibitory concentrations of the applied extracts were in the following order: ethanol extract > petroleum extract > volatile extract (El-Ghorab et al., 2008). Hydrosol of juniper, which is an aqueous distillate, demonstrated low antibacterial activity against *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Escherichia coli*, and *Pseudomonas fluorescens* which play role especially in the spoilage of freshwater fish (Oral et al., 2008).

Juniper essential oil applied at 0.1, 0.5, and 1% concentrations was found to possess weak inhibitory effects against vancomycin-resistant *Enterococci* and *E. coli* O157:H7 that were experimentally inoculated in feta soft cheese and minced beef meat and stored at 7 °C for 14 days (Selim, 2011). However, juniper oils were active against *Saccharomyces cerevisiae* (Schelz et al., 2006). Common juniper from the southern part of Serbia and its fractions of different composition demonstrated weak antimicrobial activity, while the fractions with a high content of α-pinene, and a mixture of α-pinene and sabinene showed the highest antimicrobial activity, especially against fungi. In comparison with the commercial antibiotics, the oil fractions showed more extensive spectra of antimicrobial activity, as well as wider inhibition zones (Glišić et al., 2007).

In general, terpenes, which are the dominant constituents of juniper essential oils, are not efficient antimicrobials when applied as single compounds. For instance, large scale experiments in vitro demonstrated that α-pinene, β-pinene,

(+)-sabinene, γ-terpinene, β-myrcene, and limonene possessed no or low antimicrobial activity against food spoilage bacteria such as *E. coli*, *Staphylococcus aureus*, and *Bacillus cereus* (Dorman and Deans, 2000). However, the cones of *J. communis* were used not only as a flavoring and spice, but also as a meat preservative in Poland (Łuczaj and Szymański, 2007). It is assumed that the antimicrobial activity of juniper essential oil may depend not only on its major constituents, but also on the interactions between the major and the minor constituents, resulting in additive or synergistic antimicrobial effects of several compounds (Hyldgaard et al., 2012). For instance, it was shown that the combinations of juniper and clary sage essential oils, α-pinene, and linalool resulted in additive effects in the assays with *S. cerevisiae* and *Geotrichum candidum*, whereas the combination of α-pinene and limonene demonstrated synergistic effects (Tserennadmid et al., 2011).

Burning the branches (needles) of *J. communis* produces a strongly smelling smoke that has been used for smoking meat and fish since ancient times. Although the needles accumulate less essential oil (up to 0.9%) than the cones (Ložienė and Labokas, 2012; Butkienė et al., 2006), their chemical compositions are very similar, composed of monoterpenes α-pinene and sabinene as dominating constituents (Table 1).

Chirality of odor compounds may be an important factor in fragrances and flavors because perception of odor and flavor depends on the exact conformation of the isomers (Vaičiulytė and Ložienė, 2013). Different isomers of the same compound may possess rather different odor qualities. From this point of view, one of the most important components of juniper essential oils, α-pinene, naturally exists in two enantiomeric forms, dextrorotatory (1R)-(+) and laevorotatory (1S)-(−). The (1R)-(+) enantiomer of α-pinene contains some minty fragrance notes, whereas the (1S)-(−) enantiomer exerts a characteristic pine tree aroma (Zawirska-Wojtasiak, 2006). Studies of juniper essential oil samples collected from different locations in Europe showed that the ratio of α-pinene enantiomers may be very different. It was reported that *J. communis* berries and leaves collected from different populations in Italy, Austria, France, and central Poland accumulated more (1S)-(−)-α-pinene than (1R)-(+)-α-pinene: in some cases the (1S)-(−) enantiomer amounted to 80% in the berries and 100% in the leaves (Sybilska et al., 1994; Ochocka et al., 1997). However, in the samples collected in Lithuania (Figure 3), Finland, and the northern part of Poland the (1R)-(+) enantiomer prevailed over the (1S)-(−)-α-pinene (Hiltunen and Laakso, 1995; Ochocka et al., 1997; Labokas and Ložienė, 2013).

SUMMARY POINTS

- Common juniper is an evergreen, perennial coniferous growing wild in the cool temperate geographical regions of Northern Hemisphere.
- The amount of essential oil in the unripe cones was slightly higher than those in the ripe cones of the common juniper.
- The commonly predominant essential oil constituent in common juniper is a bicyclic monoterpene α-pinene, as well as other compounds, such as sabinene, myrcene, limonene, and terpinen-4-ol, as well as other quantitatively important constituents.
- The studies of juniper essential oil samples collected from different locations in Europe showed very different ratios of α-pinene enantiomers.
- Common juniper oils are aromatic and possess antibacterial and antifungal properties, making then a potential food flavoring and preservative.

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Chapter 57

Lavender (*Lavandula angustifolia*) Oils

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List of Abbreviations

EO Essential oil
GABA Gamma-aminobutyric acid

INTRODUCTION

Lavenders are a diverse group of plants in the *Lamiaceae* (mint) family. Generally native to the Mediterranean region, lavenders are now a significant commercial crop around the world with over 2000 metric tons of lavender essential oil (EO) produced every year, primarily in Europe (Lis-Balchin, 2004). The oil of *Lavandula* genus has been used for centuries as a therapeutic and aromatic agent in traditional medicine due to its carminative, sedative, and antidepressant properties and has gained popularity in the flavor and fragrance industries. Lavender EO is composed of mainly mono- and sesquiterpenes, aromatic 10- and 15-carbon compounds, with backbones formed from the condensation of five-carbon isoprene units (Vranova et al., 2012). Different species and cultivars of lavender have unique oil chemotypes, and to date one of the most accurate ways to identify an individual is to characterize its oil composition (see Table 1 for a comparison of the oil some of the most common lavenders). These volatile compounds are produced in specialized structures on the leaf and flower surface, though specific morphology may vary among species and developmental stage. These glandular trichomes are composed of three main parts: a secretory cell connected to an oil storage sac by a stalk (Figure 1) (Huang et al., 2008). With increasing concerns about antibiotic resistance and environmental impact, traditional antibiotics, pesticides, and preservation methods are being replaced by naturally occurring alternatives. Due to its antimicrobial, antioxidant, antifungal, insecticidal, and insect repellant properties, lavender EO is becoming a popular target product to replaced traditional, synthetic treatments.

BOTANICAL ASPECTS

Lavenders are members of the genus *Lavandula* in the *Lamiaceae* family, a family of aromatic plants that also includes rosemary, mints, basil, and sage among others. Named for their unique flower morphology, the petals of the Lamiaceae are fused to form an upper and lower "lip". *Lavandula angustifolia*, or English Lavender, is composed of over 50 cultivars and is native to the Mediterranean region. As the name suggests, lavender grows readily in England, Europe, North America, and Australia often being found in ornamental and herb gardens, or commercially cultivated for its high quality EO. Some of the most popular cultivars include Lady, Munstead, and Hidcote; all vary slightly in EO composition and plant morphology. Blooming in late spring and early summer, though flowering will continue through to the end of August, lavender produces fragrant flowers from which EO is distilled. Flowers are borne in a spike on a distinct peduncle, possess a multiflowered cyme, and are generally purple to violet in color; however, some cultivars possess unique white or pink colored flowers. Leaves are narrow, opposing blades that remain evergreen in temperate climates. Although both leaves and flowers produce EO, only the floral EO is of commercial value, since flowers produce significantly more EO than leaves. Floral oils are richer in desirable EO compounds such as linalool and linalyl acetate and have lower amounts of undesirable compounds, such as camphor. These shrubs, though generally considered to have the highest quality oil, are also the smallest of the commercial lavenders, generally growing to only 1–1.5 m in height and width. English lavender is difficult to propagate, so plants are often cultured from cuttings off mature plants. Grown around the world, lavender grows best in well-drained soil in plant hardiness zones 5–11. For an in-depth description of the species and a classification key refer to Upson and Andrews (2004) (Figure 2).

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TABLE 1 Classification of the Genus *Lavandula*. Hybrids Have Only Been Included for the Section *Lavandula*

Subgenus *Lavandula*	Section *Lavandula*	*Lavandula angustifolia*
		Lavandula latifolia
		Lavandula lanata
		Lavandula × *intermedia*
		Lavandula × *aurigerana*
		Lavandula × *losae*
		Lavandula × *chaytorae*
	Section *Dentatae*	*Lavandula dentata*
	Section *Stoechas*	*Lavandula stoechas*
		Lavandula pedunculata
		Lavandula viridis
Subgenus *Fabricia*	Section *Pterostoechas*	*Lavandula multifida*
		Lavandula canariensis
		Lavandula minutolii
		Lavandula bramwellii
		Lavandula pinnata
		Lavandula buchii
		Lavandula rotundifolia
		Lavandula maroccana
		Lavandula tenuisecta
		Lavandula rejdalii
		Lavandula mairei
		Lavandula coronopifolia
		Lavandula saharica
		Lavandula antineae
		Lavandula pubescens
		Lavandula citriodora
	Section *Subnudae*	*Lavandula subnuda*
		Lavandula macra
		Lavandula dhofarensis
		Lavandula samhanesis
		Lavandula setifera
		Lavandula qishnensis
		Lavandula nimmoi
		Lavandula galgalloensis
		Lavandula aristibracteata
		Lavandula somaliensis
	Section *Chaetostachys*	*Lavandula bipinnata*
		Lavandula gibsonii
	Section *Hasikenses*	*Lavandula hasikensis*
		Lavandula sublepidota
Subgenus *Sabaudia*	Section *Sabaudia*	*Lavandula atriplicifolia*
		Lavandula erythraecea

Subgenus, section, and species of lavender identified to date. Hybrids have been omitted except for the section *Lavandula* as this is the only section widely grown for commercial essential oil production.
Classification complied from information outlined in Upson and Andrews (2004).

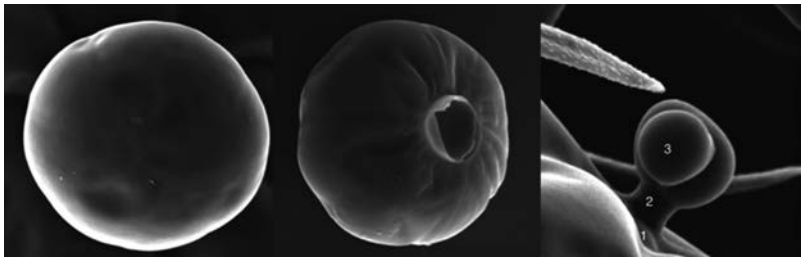


FIGURE 1 Scanning electron microscope image of glandular trichomes on the lavender leaf surface. The scanning electron microscope image shows the following views of a lavender leaf glandular trichome: top (left), bottom (middle), and side view (right), the secretory cell, stalk and oil sac are identified by numbers 1, 2, and 3, respectively.

FIGURE 2 Mature *Lavandula angustifolia* in bloom, Kelowna, BC. An English lavender plant grown commercially at the Okanagan Lavender Herb Farm in Kelowna, British Columbia, essential oil is collected from the flowers in late Spring.

USAGE AND APPLICATIONS

The floral EO from lavender has been used as far back as Greek and Roman times, where lavender was added to wash water for its pleasant scent. The first to report the use of lavender in medieval times was Abbess Hildegard (1098–1179) in what is now Germany and was later followed by physicians such as the English Nicolas Culpepper. Due to its strong odor, many of the traditional healers and physicians felt the EO was too strong for direct use and instead the hydrodistillate or very small quantities of diluted oil were used. Lavender is described in medieval herbals and documents for the treatment of many diverse maladies including palsy, head lice, migraines, epilepsy, fainting, panting, passions of the heart (likely panic attacks, heart palpitations, or other heart problems), colds, apoplexy, bites, cramps, and congestion. Lavender EO produced in England was also used during the Second World War with sphagnum moss to help disinfect wounds and aid in healing (Lis-Balchin, 2004). In spite of the extensive history of lavender use, until recent times the action of lavender EO was purely anecdotal; however, studies have supported some of these claims, while others such as the use of lavender as an abortifactant remain completely unfounded. Lavender's traditional uses for antifungal, antibacterial, antidepressant, antiinflammatory, carminative, analgesic, and sedative properties show promise and are leading to a resurgence of interest in lavender oil (Cavanagh and Wilkinson, 2002). Studies have found lavender EO to have significant effects on the central nervous system (Chioca et al., 2013; Landelle et al., 2008). In particular, the monoterpenes, linalyl acetate and linalool, have been shown to have a neurological effect when inhaled or absorbed through the skin, particularly affecting the limbic system. Though the

TABLE 2 Percent Floral Oil Composition of the Major Lavender Species Commercially Grown for Their Oil: *Lavandula angustifolia*, *Lavandula latifolia*, and Their Hybrid *Lavandula × intermedia*

	Lavandula latifolia	*Lavandula × intermedia*	*Lavandula angustifolia*
1,8-cineole	25–36%	4–10%	–
Camphor	5.3–15.3%	6–12%	–
Borneol	0.8–4.9%	1.5–3.7%	–
Linalool	26–44%	20–35%	10–50%
Linalyl acetate	0–1.5%	19–38%	12–54%
Lavandulyl acetate	0.2–1.5%	0.5–3%	0.1–14%

The floral essential oil composition of major constituents of commercially grown lavender species, major mono- and sesquiterpene constituents are defined as those with greater than 1% of oil composition in one or more species. Composition is given as ranges to account for variation between cultivars.
Data compiled from Lis-Balchin (2004).

exact cellular mechanism of action of lavender EO and its constituents have not been determined, studies suggest that activity is due to their effect on serotonergic neurotransmission and ionotrophic γ-aminobutyric acid type A receptors (Chioca et al., 2013; Lis-Balchin, 2004). Lavender essential oil also shows potential as a replacement for traditional antibiotics and antifungals having been found to be active against resistant strains of bacteria such as the hospital acquired infections methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* and difficult to treat fungal infections such as *Candida* (Cavanagh and Wilkinson, 2002). In bacteria, this activity is generally attributed to oxygenated terpenes, which act to disrupt bacterial membranes, however, minor oil constituents have been shown to have synergistic roles and play a significant role in determining bioactivity of EOs. In fungi, EOs have been found to inhibit germ tube formation and conidial development. Although lavender is a particularly mild EO in terms of application to human skin, its ability to disrupt membranes has been found to effect human cells in vitro suggesting that prolonged exposure could lead to side effects, though in vivo studies are required to determine potential side effects and dosage or application limits (Prashar et al., 2004). As a result of its diverse activity, lavender has become a popular natural health product, being used directly or incorporated into creams, oils, and formulations (Table 2).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Extensive use of lavender EO in traditional medicine and the pharmaceutical industry has led to an increased interest in lavender EO in a diversity of other fields. In particular, the use of EO in food sciences has increased since the 1990s, due to its pleasant fragrance and flavor, as well as its ability to control the growth of infectious agents. According to reports from the Food and Agricultural Organization of the United Nations food spoilage and deterioration of food quality leads to the loss of 10–40% of all food produced worldwide with the highest losses from fruit and vegetable crops (Gustavsson et al., 2011). Increasing concerns about the effect of synthetic control and preservation agents on the environment and human health has led to a push for safer alternatives to chemical pesticides, herbicides, antimicrobials, and preservatives, with lavender, in many cases, being able to fill this niche.

In addition to being a useful agent in the treatment and prevention of human pathogens, lavender EO has been found to be effective in the inhibition of food spoilage microorganisms. Lavender EO has been tested for the preservation of produce, meat and fish. In the case of ground beef, lavender was found to be effective in preventing growth of *Escherichia coli*, a major contaminant in meat processing, at levels as low as 0.50 mg/L and was even more effective against *S. aureus*, being effective at 0.25 μg/mL (Djenane et al., 2012). Evidence from in vitro studies has suggested that lavender EO can be effective against a wide range of food-borne pathogens including, but not limited to, *Salmonella*, *E. coli*, and Enterobacteriaceae at levels as low as 10% (Imelouane et al., 2009; Lodhia et al., 2009). In some cases direct application of EO to a food product was not practical, and in the case of fish a novel biodegradable gelatin–citosan film was developed in which EO was incorporated and then applied to the fish (Gómez-Estaca et al., 2010). As new methods for use of EOs are developed and their activity is better characterized, the probability of mainstream use of products such as lavender EO, which are safer and still an effective means of control, will increase, hopefully leading to a decrease in resistant and persistent infections.

Fungal infections are a particularly devastating problem in food production with few products registered for control of fungal pathogens. The increased difficulty in treating fungi has led to a push for safer, more effective fungicides as even

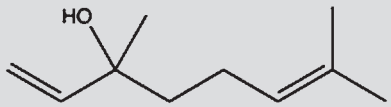
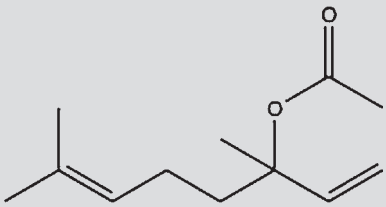
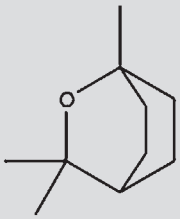
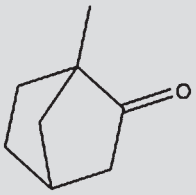
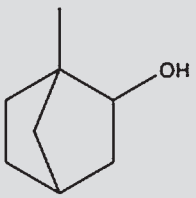
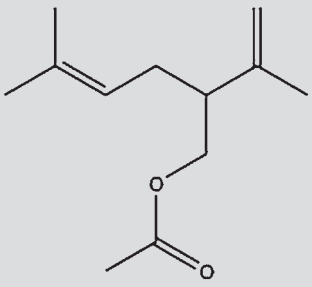
those fungicides registered for use on food crops have limited applications in a season due to exposure risk or have low effectiveness, like powdered sulfur for control of powdery mildew, and must be applied frequently. In contrast, lavender EO has been shown to be effective in the control of both postharvest and agricultural fungal infections. Lavender EO has been shown to cause cytoplasmic coagulation, vacuolation, hyphal shriveling, loss of conidiation, degradation of hyphae, inhibition of spore tube germination, and germ tube elongation in fungi. Potential control of important agricultural disease agents such as *Phytopthora*, *Sclerotina*, and *Botyritis*, the causal agents of blight, white mold, and gray mold, respectively, could significantly decrease crop loss (Moon et al., 2007; Soylu et al., 2010; Zambonelli et al., 1996).

Both volatile applications and direct contact applications have been found to be effective in the control of bacterial and fungal pathogens in both pre and postharvest situations. Unfortunately, EOs have been found to breakdown or evaporate rapidly when exposed to high temperature, oxidation, or ultraviolet light, and so application or dispersal methods that can maintain or enhance the activity of the agrochemical are desirable. Naturally, a plant combats EO degradation by storage in glandular trichomes, storage cavities that burst upon contact with pathogens, or insect pests, and new application methods seek to replicate this strategy by creating what can be considered artificial glandular trichomes. Microscopic beads of oil are captured inside a coating of biodegradable polymer such as polyethylene glycol. These beads can then be applied to the surface of crops either in the field or after harvest imitating the natural distribution of glandular trichomes (Varona et al., 2010) (Table 3).

Lavender possesses significant antioxidant potential, though specific potential varies greatly with species. Antioxidants that are safe for consumption are important in the food industry because lipid breakdown due to oxidation reduces food quality and flavor. This breakdown can be induced by cooking or heating process, such as in microwave heating, or can be the result of storage. In the case of soybean oil undergoing microwave heating, addition of spike lavender (*Lavandula latifolia*) EO can be added in small quantities as a stabilizer to prevent oxidation of the lipid fraction of the oil, production of undesirable secondary oxidation products, and maintain vitamin content (Rodrigues et al., 2012). Many flavoring ingredients in lavender, including the monoterpenes, are degraded by oxidation, leading to loss or changes in flavor. The presence of antioxidants helps maintain this flavor and, as a result, maintains food quality over a longer period of time (Da Porto et al., 2009). In the case of minced beef, addition of lavender EO not only decreased lipid oxidation, but also maintained the fresh odor of the beef for three days longer than untreated beef (Djenane et al., 2012).

Perhaps of all its applications in food science, the most promising activity is its insect repellency in both agricultural settings and stored products. Lavender has, to date, been shown to be active against *Crytolestes ferrugineus*, *Sitophilus zeamais*, rice weevil (*Sitophilus oryzae*), lesser grain borer (*Rhyzopertha dominica*), bean weevil (*Acanthoscelides obtectus*), rusty grain beetle, and the red bud borer (*Resseliella oculiperda*) (Cosimi et al., 2009; Papachristos et al., 2004; Rozman et al., 2007, 2006; van Tol et al., 2007). The interest in new botanical pesticides continues to increase as botanical products continue to be found to be effective and the licensing of products such as neem oil, pyrethrum, and rotenone have paved the way for the entrance of new botanical products for insect control. Generally, a new botanical pesticide must cause less damage to human health and the environment than currently available pesticides, must degrade rapidly (to prevent bioaccumulation), and be pest specific. Both lavender EO and its constituents alone have been tested in insect repellency and toxicity assays and have been found to be effective. Generally, monoterpene ketones have been found to be more active than alcohols with some of the most potent monoterpenes including terpineol, camphor, and cineole. Though the individual constituents on their own do show potent activity, generally, the whole oil has shown even greater activity indicating synergistic activity of the constituents (Papachristos et al., 2004). Additionally, lavender EO has shown varying effects on different ages and sexes of pests, making an understanding of the pest of concern and specific testing necessary before lavender EO could be used in the field. Lavender products for field use have been developed including the development of a grafting strip impregnated with lavender EO. Grafting allows for the development of crops such as apple trees with hearty root stocks joined to commercially relevant or profitable canopies; however, insect infestation can result in failure to graft costing significant amounts of both time and money. Researchers have found that impregnation of rubber budding strips with lavender EOs can decrease bud infestation by the red bud borer by 95%. This leads to better unions during grafting and more efficient production of the desirable hybrids (Papachristos et al., 2004). In addition to its use in field applications, lavender has been shown to be an effective fumigant for the preservation of stored food products such as flour, rice, and wheat, with complete insect mortality being demonstrated 24 h after exposure in the rice weevil and lesser grain borer (Rozman et al., 2007, 2006). This is of significant interest because current fumigants to treat infested dry goods and grains required the use of phosphine or methyl bromide. With growing pest resistance to phosphine and the now established ozone depleting effects of methyl bromide, a safer more effective strategy is well needed (Shaaya and Kostyukovsky, 2009). The contact activity of lavender EO as an insecticide is attributed to its solvent properties on the insect cuticle, while fumigant activity effects the respiratory and digestive systems (Rozman et al., 2007). Biochemical studies have shown that lavender EO results in the depletion of protein due to decreased protein synthesis and aggregation of insect proteins around foreign

TABLE 3 The Structures and Bioactivities of the Major Monoterpene Constituents of Lavenders in the Section *Lavandula*

Monoterpene	Structure	Bioactivity	References
Linalool		Insect repellent, insecticide, antifungal	van Tol et al. (2007), Rozmann et al. (2006), Sokovic and van Griensven (2006)
Linalyl acetate		Antifungal	Sokovic and van Griensven (2006)
1,8-Cineole		Insect repellent, insecticide, antibacterial, antifungal	Rozmann et al. (2007), Sokovic and van Griensven (2006), Carson and Riley (2008)
Camphor		Insect repellent, insecticide, antibacterial, antifungal	Rozmann et al. (2007), Sokovic and van Griensven (2006)
Borneol		Insect repellent, insecticide	Rozmann et al. (2006)
Lavandulyl acetate		Bacteriostatic	Sokovic and van Griensven (2006)

The biological activity of the major constituents of commercially grown lavenders. The biological activity of lavender essential oil is a result of its individual constituents. Although synergistic activity is present, the activity of individual constituents can help explain the observed bioactivity of the whole oil.

compounds as a defense mechanism, as well as depletion of carbohydrates resulting from decreased amylase activity, likely due to the stress of oil application and depletion of lipids. Lavender EO treatment has been shown to decrease amino acid metabolism by effecting glutamic oxaloacetic transaminase activity and glutamic pyruvic transaminase activity, as well as effecting neurotransmission by decreasing acetyl cholinesterase activity (El-Sheikh et al., 2005). Perhaps the biggest obstacle in establishing lavender oil as a new botanical insecticide is determining cost-effective commercial availability of

large amounts of oil; unfortunately lavender EO is unlikely to become part of an integrated pest management system until this has been accomplished.

Lavender, in addition to being a biologically active plant, is also used as a flavoring and aroma additive in foodstuffs. The flavor of lavender EO is described as earthy with undertones of citrus, rosemary, and mint and is considered to be a gourmet food ingredient. The majority of lavender food products are produced by small local companies or lavender growers; however, the niche lavender market is growing with the popularization of lavender as a food ingredient. Many online companies now sell "edible" lavender that has specifically been produced for consumption and can be used in home cooking and baking. Lavender is also available in teas and spice blends like the popular "*herbes de provence*". In general, only English lavenders are used for consumption as they have a milder more pleasant taste and odor. Lavender EO alone is also seeing an upswing in use as a flavoring agent, having been used for chewing gums, chocolates, candies, and other confections; beverages such as tea, coffee, hot chocolate, lemonade, and cocktails; diverse condiments including chutneys, salsas, jams, jellies, and syrups; baked goods and dry mixes; cooking spices; sugars; and salts, among many others. EOs for cooking can be purchased both pure and as components in flavoring formulations. Entire recipe books have been published on the use of lavender and its EO in cooking with recipes for almost anything from chicken to pizza to ice cream (Shipley, 2004; Hander Lyons, 2007).

Before any product, botanical or otherwise, can be used in the food industry, the safety of the product must first be thoroughly investigated. Some studies have been performed on lavender EO to establish the toxicity to mammalian cells, humans, and the environment. Although centuries of use have demonstrated lavender to be a particularly mild oil, high exposure rates to lavender EO has led to some negative side effects. Lavender EO is a mild solvent and has been shown to have limited cytotoxicity in mammalian cell lines in vitro, and ingestion of large quantities has been linked with cardiac events in children, but has been shown to have no mutagenic activity in vitro (Evandri et al., 2005; Landelle et al., 2008; Prashar et al., 2004). Lavender oil is degraded in response to prolonged exposure to heat and ultraviolet light and readily undergoes oxidation, making it easily biodegradable and is therefore an unlikely environmental contaminant. Historical use and modern studies indicate that lavender can be considered a safe product, particularly in the food industry where only very limited volumes of EO are generally used as part of complex mixtures.

SUMMARY POINTS

- Lavender essential oil is effective in controlling bacterial growth on food products.
- Lavender essential oil shows promise in controlling plant fungal infections in both pre and postharvest situations.
- The quality of lipid-containing foods can be preserved through storage and cooking by the addition of lavender essential oil due to its antioxidant properties.
- Due to its strong insect repellency, and contact and fumigant toxicity, lavender essential oils are effective means of insect control.
- The flavor and fragrance of lavender essential oils has made it a popular food additive, flavorant, and cooking ingredient both commercially and in home kitchens.
- Both modern scientific studies and historical data show lavender essential oil to be a safe botanical product when applied topically or consumed at low levels.

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Chapter 58

Lemongrass (*Cymbopogon* spp.) Oils

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List of Abbreviations

EO Essential Oil
SFE Supercritical Fluid Extraction

INTRODUCTION

From ancient times, herbs and spices have been part of the human diet used to enhance the flavor, color, and aroma of foods. These spices, together with their essential oils (EOs) were also a major form of therapy due to their antimicrobial and antiseptic properties (Moore-Niebel et al., 2011). Lemongrass EO is still regarded as one of the most important EOs produced in the world, even though it has lost its commercial importance due to the production of synthetic citral (the oil was formally the main source of natural citral used in the production of vitamin A) (Attokaran, 2011). The EO of lemongrass is generally regarded as safe (GRAS) (Smith et al., 2005) and, like other EOs, contains aromatic and flavoring components. Also, its antimicrobial properties have been widely investigated (Mishra and Dubey, 1994; Tzortzakis and Economakis, 2007). When added to food stuffs, the oil helps retain organoleptic properties and retard microbial activity by food spoilage microorganisms responsible for reducing food quality, spoilage, and economic loss (Masniyom et al., 2012). These properties suggest the potential use of lemongrass oil as a food preserving agent in an industry that has become complex due to the increasing number of food products requiring longer shelf life and the negative consumer perceptions of artificial preservatives.

The main active ingredients of lemongrass EO, citral (65–85%), neral, and geraniol have been shown to inactivate foodborne pathogens (Moore-Niebel et al., 2011). The potency of lemongrass EO in conserving the organoleptic characteristics of food and food products has also been reported (Masniyom et al., 2012). In this chapter we discuss the renewed interest in plant-based preservatives, in this case lemongrass EO.

BOTANICAL DESCRIPTION

Lemongrass is a tall perennial C_4 grass belonging to the family Poaceae (Gramineae), commonly known as the "sweet grass family." *Cymbopogon* is a genus of about 55 species (Table 1) indigenous to the tropical and semitropical areas of Asia and cultivated in South and Central America, Africa, and other tropical countries. Lemongrass grows as dense clumps to about 1.8 m (6 ft) in height and 1.2 m (4 ft) in width, having short rhizomes with strap-like leaves of 1.3–2.5 cm (0.5–1.0 in) wide and 0.9 m (3 ft) long (Figure 1). Lemongrass plants do not typically produce flowers or flowering panicles (Reitz, 1982).

Lemongrass is propagated both from seeds and rooted slips. The seeds germinate within 5–6 days under optimum conditions of temperature and moisture, and seedlings are usually ready for transplanting at about 60 days old. Propagation of lemongrass by slips is achieved by dividing well-grown clumps. The upper part of the clump is cut off within 20–25 cm of the root, and then divided into slips. A total of 125,000 plants per ha are planted on irrigated lands or areas with high rainfall, while 60,000 plants per ha is an advisable density in areas with lower rainfall. The rows are spaced 20 cm apart with a width of 40 cm (Vermeulen et al., 1998). The plant grows on a wide range of soils, but the best yields are obtained from well drained sandy to loam soils, with a pH value of 4.3–8.4. The soil can give three to four harvests throughout the year when water is readily accessible. The yield and oil content of lemongrass are influenced by the soil conditions, such that under dry-land conditions, it produces one to two cuts a year in the raining season, and survives till the next raining season without water as a result of its deep rooting system (de Boer, 2005).

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TABLE 1 List of Some *Cymbopogon* Species and Their Native Countries

Species	Native
Cymbopogon ambiguous	Australia
Cymbopogon bombicynus	Australia
Cymbopogon citratus	China
Cymbopogon citriodora	West India
Cymbopogon flexuosus	East India
Cymbopogon nardus	Thailand
Cymbopogon schoenanthus	South Asia and North Africa
Cymbopogon procerus	Australia
Cymbopogon obtectus	Australia
Cymbopogon. refractus	Australia
Cymbopogon proximus	Egypt

Cymbopogon is a genus of over 55 different species found in tropical and subtropical regions of the world. Often different species of lemongrass are found in different countries but with similar physical and chemical characteristics.

FIGURE 1 Lemongrass (*Cymbopogon flexuosus*) plant. Lemongrass grows as a dense clump of rhizomes with strap-like leaves. Photographed at the National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria.

Lemongrass contains about 1–2% EO dry weight with a wide variation of the chemical composition as a function of genetic diversity, habitat, and agronomic treatment of the culture (Carlson et al., 2001). Also, the drying method adopted has been shown to affect the chemical composition of lemongrass oil. According to Hanaa et al. (2012), oven-drying of the leaves of *Cymbopogon citratus* results in higher oil content (2.34%) compared with sun-drying (2.10%) and shade-drying (2.12%), while shade-drying results in higher percentage of citral (Table 2). Lemongrass oil is characterized by a high citral content (>45%) (Moore-Niebel et al., 2011) and its quality is generally determined by the amount of citral

TABLE 2 Effect of Drying Methods of *Cymbopogon citratus* on Its Essential Oil Components

Compound	Fresh	Sun-drying	Shade-drying	Oven-drying
Myrcene	15.69	16.16	14.49	15.42
Limonene	0.41	0.42	0.43	0.40
E,E-Cosmene	0.20	0.23	0.21	1.26
Z-β-Ocimene	0.97	t	0.17	0.22
E-β-Ocimene	0.41	0.28	0.26	0.27
α-Terpinolene	1.02	1.09	1.09	1.06
Citronellal	0.60	2.06	2.03	3.01
Cis-verbenol	0.15	0.15	0.15	0.18
Linalool	1.03	2.06	2.03	2.44
Cis-carveol	1.18	1.49	1.35	1.47
Atrimesol	0.26	0.15	t	0.19
Nerol	0.17	0.27	0.22	0.29
Neral	34.98	30.08	34.52	31.28
Geraniol	0.53	0.86	0.95	1.31
Geranial	40.72	31.53	39.86	37.24
Carveol	0.18	0.65	0.38	0.73
Geranyl acetate	0.51	0.7	0.49	0.67
Caryophellene	0.28	0.23	0.20	0.21
	99.29	**88.41**	**98.83**	**97.63**

t < 0.05. The essential oil content (%) of the major components of lemongrass is affected by the methods used in drying the leaves.
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present. Citral is composed of two stereoisomeric monoterpene aldehydes: geranial (*trans*-citral) and neral (*cis*-citral). EO of *C. citratus* is mainly composed of citral (30–93.74%) with general predominance of geranial (Negrelle and Gomes, 2007).

USAGE AND APPLICATION OF LEMONGRASS

Lemongrass is primarily used as a spice to flavor foods in several South Asian cuisines, especially Thai and Vietnamese. It also serves as a flavoring agent for alcoholic and nonalcoholic beverages. Some fine cocktails and other alcoholic beverages are flavored with lemongrass for its subtle citrus flavor. It is commonly used to impact flavor in tea in African and Latin American countries. It is also used in Ayurvedic medicine for the treatment of infectious diseases and fever. Traditionally, it is used as an analgesic, antiinflammatory, antipyretic, diuretic, and tranquilizing properties in treating various digestive disorders, inflammation, diabetes, nervous disorders, as well as other health problems (Carlini et al., 1986; Negrelle and Gomes, 2007). Though there are limited scientific data on the medical claims of lemongrass, the infusion or decoction of aerial parts of lemongrass has been used in traditional medicine for treatment of several ailments in different parts of the world. In Brazil, the tea made from its leaves is popularly used as antispasmodic, analgesic, antiinflammatory, antipyretic, diuretic, and sedative (Leite et al., 1986; Souza Formigoni et al., 1986). Tea made from dried leaves is taken orally in Cuba as a hypotensive, for catarrh and rheumatism (Carbajal et al., 1989).

The oil may be of West Indian (*C. citratus*) or East Indian (*Cymbopogon flexuosus*) origin (Attokaran, 2011). Citral is used in the production of vitamin A and β-ionone (used as synthetic perfumery chemical and as modifier in many floral compositions). The EO is used in some toiletries and cosmetics as a low-cost fragrance material. It is also used in beverages, bakery and confectionary products. The EO has been reported to have a strong spasmolytic effect on smooth muscles (Attokaran, 2011).

USAGE AND APPLICATION IN FOOD SCIENCE

Essential Oil of Lemongrass as a Food Preservative

The increasing incidence of food-borne diseases as a result of contamination of foods with undesirable microorganisms during food processing, storage, and distribution in spite of advances in sanitation and inspection techniques poses a serious public health problem (Oussalah et al., 2007). Microorganisms grow on food products, producing toxins that are hazardous to human health, while some are capable of causing food spoilage and food-borne diseases in processed foods. Yeasts and molds are capable of producing toxins, such as afflatoxin and citrinin, generate off-flavors, and cause discoloration and proteolysis. Afflatoxins are produced by certain strains of *Aspergillus flavus* and are the most dangerous mycotoxins, thus the need to control fungal growth and mycotoxin formation. Preventing fungal decay in food products after harvest is increasingly challenging and novel preservative approaches that comply with food standards need to be developed. The use of synthetic food preservatives to curb the deleterious effects of microorganisms is beset by problems of microbial resistance and food safety concerns; creating renewed interest in the preservation of foods by natural preservatives. Preliminary experiments have shown that the EOs of various plants possess antifungal and antibacterial activities with little or no risk of microbial resistance as they contain different antimicrobial substances having different modes of action; hence they are presently exploited as current control methods are either expensive and/or pose significant threats to human health and environment (Paranagama et al., 2003; de Silveira et al., 2012).

The antifungal and antibacterial activities of EO of lemongrass against different microbial species have been documented (Mishra and Dubey, 1994; Tzortzakis and Economakis, 2007; de Silveira et al., 2012). In a report by de Silveira et al. (2012), lemongrass EO was found to be most active compared with other EOs against 12 bacterial strains: *Staphylococcus aureus*, *Lactobacillus plantarum*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, and *Yersinia enterocolitica*. Their results were consistent with reports by Naik et al. (2012) who reported that lemongrass EO was effective against both Gram-positive and Gram-negative bacteria. These results demonstrate that the application of lemongrass EO in food products may provide better food safety and longer shelf life.

Preservatives for Natural Food Products

Mishra and Dubey (1994) demonstrated that lemongrass EO was an effective postharvest fungitoxicant for the protection of foodstuffs against storage fungi. The oil was shown to completely inhibit the growth of 35, 45, and 47 species at 500, 1000, and 1500 ppm, respectively. The fungitoxic potency of the oil remained unaltered for 210 days of storage, after which it started to decline. In a separate experiment reported by Tzortzakis and Economakis (2007), the EO of lemongrass caused a significant reduction in the colony development of key postharvest pathogens: *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer*, and *Aspergillus niger*. The fungal spore production was inhibited by 70% at 25 ppm of lemongrass oil concentration compared with equivalent plates stored in ambient air. At the highest oil concentration (500 ppm), fungal sporulation was completely retarded. Also, it reduced spore germination and germ tube length in *C. coccodes*, *B. cinerea*, *C. herbarum*, and *R. stolonifer* in a concentration-dependent manner, but accelerated spore germination in *A. nig*er at concentrations up to 100 ppm.

A study by Paranagama et al. (2003) using *A. flavus* isolated from Sri Lankan rice demonstrated that lemongrass EO could be used to manage aflatoxin formation and fungal growth of *A. flavus*. The EO was fungistatic and fungicidal against *A. flavus* at 0.60 and 1.0 mg/mL, while sporulation was completely inhibited at 2.8 mg/mL.

Lemongrass EO has also been reported to prolong the shelf life of sea food. Treatment of mussels during prolonged refrigeration with 0.5% lemongrass EO and a combination of 0.25% turmeric and 0.25% lemongrass EOs was shown to retard bacterial growth and lipid oxidation. Lemongrass EO was found to cause exudate loss with increasing storage time of the mussels, but maintained high sensory qualities (flavor and odor) compared with the control. The exudate loss was attributed to the ability of EOs to induce denaturation of muscle proteins (Masniyom et al., 2012).

In a study by Moore-Neibel et al. (2011), the concentration of *Salmonella enterica* inoculated into organic leafy greens significantly reduced in a concentration and time-dependent manner when treated with lemongrass EO, demonstrating the potential of the EO as an alternative decontaminant for fresh leafy greens in the organic fresh produce industry.

Preservatives for Processed Food Products

Several studies have been carried out to determine the efficacy of lemongrass EO in preserving different types of finished food products and the possibility of using them as substitutes for synthetic preservatives in food industries (Abd-El Fattah et al., 2010; Belewu et al., 2012).

TABLE 3 Effect of Various Concentrations of Lemongrass Essential Oil on Mycotoxin Production in Yoghurt Medium at 5 and 28 °C

	Mycotoxin Production (μg per 50 mL Yeast Extract Sucrose Medium)					
	5 °C			28 °C		
	Total Afflatoxins			Total Afflatoxins		
Essential Oil Level (%)	*Aspergillus flavus*	*Aspergillus parasiticus*	*Ochratoxin A*	*Aspergillus flavus*	*Aspergillus parasiticus*	*Ochratoxin A*
Control 0.0	35.3	84.0	116.0	108.6	142.5	185.0
0.05	0.0	0.0	0.0	65	56.5	0.0
0.1	0.0	0.0	0.0	43	40.15	0.0
0.3	0.0	0.0	0.0	0.0	0.0	0.0
0.5	0.0	0.0	0.0	0.0	0.0	0.0
1.0	0.0	0.0	0.0	0.0	0.0	0.0

Each value represents mean of three replicates. Incorporation of lemongrass essential oil in yoghurt stored at 5 °C completely inhibits production of mycotoxins, while at 28 °C, mycotoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* decreased with increasing oil concentration, such that at 0.3% lemongrass oil, mycotoxins were not detected.
Reprinted from Abd-El Fattah et al. (2010) with permission.

Abd-El Fattah et al. (2010) reported that incubation of yoghurt with 0.1% lemongrass oil inhibited mold and mycotoxin production by *A. flavus*, *Aspergillus parasiticus*, and *Aspergillus ochraceus*, thus preserving yoghurt for over 28 days at 5 °C (Table 3). It also improved the physicochemical properties as well as sensory characteristics of yoghurt relative to the control, suggesting the possible use of lemongrass EO for decontamination of mycotoxigenic fungi and prevention of mycotoxin formation in dairy products. In another study conducted by Belewu et al. (2012) to compare the effect of different levels of eucalyptus and lemongrass EOs on the shelf life of fresh West African soft cheese called "*wara,*" the application of eucalyptus and lemongrass EOs at a ratio of 3:1 significantly improved the nutrient composition, acceptability, and shelf life of cheese for 4 weeks compared with three days observed for the control sample.

The antifungal activities of lemongrass EO against fungi known to cause the spoilage of bakery products have also been demonstrated. Guynot et al. (2003) reported the total inhibition of *Eurotium amstelodami*, *Eurotium herbariorum*, *Eurotium rubrum*, *A. flavus*, *A. niger*, *Eurotium repens*, and *Penicillium corylophilum* in wheat flour agar by volatile components of lemongrass EO. The inhibition was, however, limited when tested in sponge cake analogues, with the oil only delaying the growth of *Eu. repens* on cake analogues. In another study, Suhr and Nielson (2003) demonstrated the antifungal activity of the EO against rye bread spoilage fungi. Introducing lemongrass EO into rye bread media initially inhibited *P. corylophilum* and *Endomycopsis fibuligera* but it gradually lost its effect, whereas the volatile components of the EO completely inhibited *Penicillium roqueforti* and *En. fibuligera* in baked rye bread. This highlights the possible application of the EO of lemongrass for the preservation of bread and other bakery products. The antifungal properties of lemongrass EO has been attributed to its antioxidant components, such as phenols, flavonoids, and flavones. Phenols isolated from the leaves of lemongrass have been shown to inhibit fungal growth in dairy products (Abd-El Fattah et al., 2010).

It has also been reported that lemongrass EO may reduce or completely inhibit the bacterial population of fruit juices, pastries, cocoa, and chocolate products. Based on results by Vazirian et al. (2012), lemongrass EO at 1 μL/mL was found to completely inhibit four food-borne pathogens: *E. coli, Candida albicans, B. cereus,* and *S. typhimurium* added to cream-filled cakes after 72 h of baking. Also, Kotzekidou et al. (2008) demonstrated the ability of lemongrass EO to inhibit *S. typhimurium* inoculated in freshly produced chocolate bars, but not effective against *S. aureus*. They reported that EOs may not provide complete protection against chocolate containing high concentration of pathogens at low temperatures. Raybaudi-Massilia et al. (2006) demonstrated that lemongrass EO could inhibit *Salmonella Enteritidis, E. coli,* and *Listeria innocua* in apple, pear, and melon juices.

The antibacterial properties of EOs have been attributed to several mechanisms, including the breakdown of phospholipid bilayer resulting in loss of cellular constituents, enzymes, and destruction of genetic materials (Kotzekidou et al., 2008). According to Moore-Niebel et al. (2011), lemongrass EO exerts its effect by inhibiting formation of biofilms, eliminating preformed biofilms, and targeting the bacterial cell.

Safety of Lemongrass EO as Food Preservative

Natural flavor complexes (NFCs) such as lemongrass oil exert their effects by binding at extremely low levels of exposure to the gustatory or olfactory systems receptor proteins to produce an identifiable sensation. However, at higher levels, these molecules and their metabolites may become toxic; hence, NFCs are evaluated for safety based on the chemical composition and variability of their composition in commercial products (Smith et al., 2005).

According to the 22nd publication of Federal Emergency Management Agency expert panel, lemongrass oil is GRAS affirmed. This is because very low amounts of the oil are used as flavoring, hence the toxic congeneric components of the oil consumed are usually below threshold (1.80 mg/person/day) and, even at higher concentrations, these congeneric components are effectively detoxified to yield metabolites that are eliminated from the body (Smith et al., 2005).

Taking into account the various reports on the antimicrobial, antifungal, and organoleptic conserving activities of the lemongrass EO against many pathogenic or spoilage microorganisms, it can be concluded that the oil possess features that make it a good candidate for the biopreservation and storage of food.

Extraction of Essential Oil of Lemongrass

The EO of lemongrass has a spicy, pleasant lemon aroma, and appears as a yellowish liquid, although most are reprocessed to fit their specific use (Figure 2). It is optically active with a refractive index of 1.483–1.489 and specific gravity of 0.890–0.904 for east Indian and 0.869–0.894 for west Indian lemongrass oils (Attokaran, 2011). The oil is mostly extracted by steam distillation, where the leaves are chopped and filled into a distillation still, followed by the introduction of steam. The upcoming stream extracts the oil in the leaf into the condenser. The resulting mixture of both oil and water is then separated in the separators (Koul et al., 2004). Another method is supercritical fluid extraction (SFE) which has been shown to produce oil of better quality compared with steam distillation. In a study by Ha et al. (2008), the composition of oil obtained by SFE (94.4% citral, 1.14% myrcene, and 0.5% limonene) was not different from the reference standard, while the oil extracted by steam distillation was found to be significantly different in terms of purity and composition from the reference standard.

FIGURE 2 Lemongrass oil packaged for export and local consumption with trade name "Citronella Grass oil" obtained in Thailand. This oil has been processed for use as a cosmetic, as lemongrass EO is a light-yellow colored liquid. The photograph was taken by Miss Sopitsuda Maivun of the Faculty of Fine Arts and Architecture, Rajamangala University of Lanna, Chiang Mai, Thailand.

SUMMARY POINTS

- Lemongrass is a tall perennial C_4 grass belonging to the family Poaceae.
- Lemongrass is primarily used as a spice to flavor various dishes.
- It contains about 1–2% EO dry weight.
- Lemongrass EO exhibits broad spectrum fungitoxicity on some postharvest fungi.
- It also inhibits several bacteria known to have deleterious effects when consumed in foods.
- Addition of lemongrass EO and its volatiles to food products prevents food contamination, deterioration, and maintains the sensory properties.
- Apart from steam distillation, the oil can be obtained by SFE.
- Lemongrass EO is GRAS affirmed.

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Chapter 59

Lemon Myrtle (*Backhousia citriodora*) Oils

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INTRODUCTION

Lemon myrtle leaves are used in food applications as a fresh or dried herb, while the essential oil (EO) is used as food flavoring because of its distinct aroma and flavor. In 1889, Joseph H. Maiden reported on the potential use of lemon myrtle for commercial production and a German company, Schimmel & Co., was the first to identify citral as an active component (Robbins, 2008). Citral is a major chemical component in the leaf and also the EO. Citral derives its name from *Backhousia citriodora* F. Muell from where it was originally extracted and isolated, and is responsible for the characteristic lemon flavor (Southwell et al., 2000). Lemon myrtle EO has over 90% citral, compared with the other lemon-flavored EOs, such as citrus (3–10%), lemon grass (75%), and tropical verbena (74%) (Robbins, 2008).

Lemon myrtle has also been used in herbal teas which have been gaining popularity in western countries, with a major market in the United States followed by Europe. Lemon myrtle teas are available as pure or blended samples and the antioxidant properties are comparable to the black teas from *Camellia sinensis* (Chan et al., 2010). Lemon myrtle is one of the most cultivated and commercially mature species in the native food industry in Australia (Clarke, 2012). This chapter reviews the application of lemon myrtle essential oil in food and its general usage, botanical aspects, and chemical properties (Figure 1).

BOTANICAL ASPECTS

The plant genus *Backhousia* is endemic to Australia and belongs to the family Myrtaceae. The most common chemotype, *B. citriodora* F. Muell, is also known as lemon-scented myrtle, lemon ironwood, and sweet verbena tree. It is a large shrub to medium-sized tree endemic to rainforest areas of coastal Queensland between Brisbane to Cairns and is a very aromatic plant (Taylor, 1996). It is a perennial tree crop and typically planted in rows. The leaves contain about 95% citral, which gives the characteristic lemon flavor and the flowers and seeds also have lemon flavors (Ahmed and Johnson, 2000). Horticultural development has progressed through the years with the selection of clones for growth with potential for high citral and oil capacity. There are two main commercial clones being planted: the line commonly referred to as Limpinwood shows superior ornamental presentation, high biomass, high oil yield, and citral content; and the variety commonly referred to as Line B or Eudlo clone is slightly lower in biomass, oil yield, and citral content (Robbins, 2008). In Australia, lemon myrtle can be harvested all year round; however, harvesting during the monsoonal rainy periods may be restricted in Northern Queensland. This species exists in two chemical forms: a variety characterized by an essential oil with a high content of citral, and a variety characterized by an essential oil rich in laevo-citronellal, which is rare. The leaves contain 1.1–3.2% oil. The citral chemotype, is the most common one used in food, cosmetics, personal care, and aromatherapy, and is grown commercially in Queensland as well as the Lismore area of northern New South Wales (Lassak, 2012; Brophy et al., 1995).

USAGE AND APPLICATIONS

Lemon myrtle is an Australian native herb and is used as the dried and milled product or steam distilled to obtain lemon myrtle essential oil (Clarke, 2012). The indigenous Australians have used lemon myrtle traditionally for cooking and healing purposes. In Europe too, lemon myrtle is becoming popular both for use in cuisine and phytotherapy (Horn et al., 2012). The aroma and flavor of the lemon myrtle as a dried milled leaf has been described as the aroma of lemon candy, perfumed with some menthol notes. The flavor is strong lemon with some sweetness and cooling on the palate (Smyth et al., 2012). Since the mid-1990s, the essential oils, foliage, flowers, and seeds of lemon myrtle have been used widely

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FIGURE 1 Lemon myrtle shrubs (picture kindly provided by Australian Rainforest Products).

as ingredients in perfumes, food flavorings, herbal teas, and personal care products such as soaps, creams, shampoos, and conditioners (Taylor, 1996; Clarke, 2012). The antimicrobial activity of lemon myrtle has also been used in the treatment of certain diseases such as molluscum contagiosum in children, which is a common contagious viral disease affecting children globally, a 10% (v/v) solution of lemon myrtle EO as a topical application has shown a reduction of greater than 90% in the number of lesions in children (Burke et al., 2004). Lemon myrtle EO has also been effective in inhibiting methicillin-resistant *Staphylococcus aureus*, which has been identified as a multidrug-resistant bacterium (Chao et al., 2008) and also shown very good antifungal activity, indicating the potential of using this EO as an antiseptic (Wilkinson et al., 2003).

USAGE AND APPLICATIONS IN FOOD SCIENCE

For essential oil production from *B. citriodora*, specialized machines cut the stems and leaves into smaller particle sizes. The cut leaves are placed in stainless steel bins and then steam distilled to obtain the essential oil, which predominantly contains citral (3,7-dimethyl-2-7-octadienal) with two main isomeric aldehydes: neral and geranial. The essential oil is stored in stainless steel or glass containers and not in plastic as the EO is very corrosive to this material (Robbins, 2008). Lemon myrtle is used as a dried herb, flavoring agent, and herbal tea in Australia and is approved in the European Union under the novel food category. Food products to be approved under this category must be excluded from potential health risks and thorough testing of these plants and their parts in food preparations is a prerequisite (Horn et al., 2012). The chemical composition of the lemon myrtle EO is given in Table 1 (Hayes and Markovic, 2002; Southwell et al., 2000; Brophy et al., 1995), the physical properties assessed were relative density (0.888–0.910), refractive index (1.4853–1.4909), and optical rotation (−1.5° to+0.4°) (Southwell et al., 2000).

ANTIMICROBIAL PROPERTIES

The antimicrobial activity of *B. citriodora* EO is believed to be directly related to the high citral content (Lis-Balchin et al., 1998); however, the importance of other minor components such as linalool, citronellal, and β-myrcene in combination with citral for enhanced antimicrobial activity has also been reported. A study by Sultanbawa et al. (2009) revealed complete inhibition of *Escherichia coli* at 0.313% and *S. aureus* at 0.156% when evaluated against lemon myrtle EO; however, the levels of citral alone required to completely inhibit *E. coli* and *S. aureus* were 2.5% and 0.625%, respectively. The higher levels of citral on its own, compared with lemon myrtle EO suggests an enhanced antibacterial activity with the EO possibly due to synergies between the major and minor chemical components. A study on four lemon myrtle EOs with citral contents between 93% and 98% were evaluated for antibacterial and antifungal activity against 21 organisms. This study in general reported that antimicrobial activity of lemon myrtle EO was greater than citral alone. In addition, the EO was an effective antibacterial agent and an excellent antifungal agent. This indicates the potential of using lemon

TABLE 1 Chemical Composition of the Essential Oil of Lemon Myrtle

Component	Percentage
β-Myrcene	0.1–0.7
6-methyl-5-Hepten-2-one	0.1–2.5
(±)Linalool	0.3–1.0
Citronellal	0.1–0.9
Iso-neral (*Cis-iso* citral)	0.6–2.7
Iso-geranial (*Trans-iso* citral)	1.0–4.2
Neral (citral A)	32.0–40.9
Geranial (citral B)	46.1–60.7
Trans-geraniol	0.4–0.7

myrtle EO as a surface disinfectant and as a natural preservative in the food industry (Wilkinson et al., 2003). The shelf-life of barramundi fillets was extended when treated with both the oregano and a mix of oregano and lemon myrtle EO compared with the controls. Based on microbiological data, the shelf-life of barramundi fillets during vacuum storage was 10–12 days. Dipping in antimicrobial solutions of 0.1% (v/w) of oregano and a mixture of 0.05% oregano (v/w) + lemon myrtle 0.025% (v/w) extended the shelf-life of the fish fillets by an additional 4 days. This study also indicates the potential of reducing the concentration of essential oils used by developing blends that have synergies (Sultanbawa et al., 2012). One of the challenges of using EO as natural preservatives is the strong flavor it can impart to the food, and if the concentration can be reduced by blending then it becomes more attractive to use as a natural antimicrobial in food applications. The effectiveness of lemon myrtle against postharvest fungal diseases in nectarines has been reported. The essential oils, lemon myrtle (*B. citriodora*), cinnamon bark (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*), thyme oil (*Thymus vulgaris*), clove bud (*Eugenia caryophyllata*), valerian (*Valeriana officinalis*), and Australian tea tree oil (*Melaleuca alternifolia*) in controlling brown rot caused by the fungus *Monilinia fructicola* in nectarines was evaluated. This study revealed lemon myrtle essential oil as the most potent fumigant to control the mycelium growth and spore germination (Lazar-Baker et al., 2011).

Clostridium perfringens is the causative agent of necrotic enteritis in poultry. Economic production of poultry meat with intensive growing techniques is threatened by bacteria showing multiple resistances to antibiotics compounded by the restriction in the European Union of in-feed use of antibiotic growth promoters. This has resulted in the increase of necrotic enteritis and the search for natural alternatives to reduce gut colonization of *C. perfringens*. Use of four essential oils, lemon myrtle (*B. citriodora*), eucalyptus oil (*Eucalyptus citriodora*), lemon tea tree oil (*Leptospermum petersonii*), tea tree oil (*M. alternifolia*), and other secondary plant metabolites have indicated that the fermentative activity of *C. perfringens* is inhibited by all essential oils tested with the most effective being lemon myrtle with a minimum inhibitory concentration of 0.05%. This indicates the possibility of using lemon myrtle EO as a feed additive in poultry to control the gut colonization of this pathogen (Zrustova et al., 2006).

FLAVORING AGENT

Lemon myrtle EO in Australia is used as a source of lemon flavoring in the food and beverage industries. A study of the sensory properties of lemon myrtle EO assessed the main contributing compounds to the lemon and sweet aromas of lemon myrtle to be neral and geranial, citronellal, and linalool (Forbes-Smith and Paton, 2002). Lemon myrtle EO is used as a lemon flavor replacement in milk-based products, such as cheesecake or ice cream to prevent curdling associated with the acidity of lemon fruits (Horn et al., 2012). However, like other essential oils lemon myrtle is sensitive to light and oxygen and this can affect the shelf-life and limits its application in the food industry (Buchaillot et al., 2009). To stabilize the EO, encapsulation by spray drying to powder form has been reported by The Vien et al. (2008). In this study, the optimized microencapsulated blend contained 18% of the essential oil. The benefit of such powders is the solubility in aqueous phase which is an advantage when used as a flavoring agent in food applications. These encapsulated flavors can also be used in shortbread, pasta, and macadamia and vegetable oils (Horn et al., 2012). Lemon myrtle has been used as a herb in speciality

cheeses in Australia; these cheeses are semihard, vacuum packed, and are matured for less than 3 months. This market is growing as the consumer is looking for new flavors (Agboola and Radovanovic-Tesic, 2002).

SAFETY OF LEMON MYRTLE ESSENTIAL OILS

Lemon myrtle essential oil has a high content of the aldehyde, citral (>90%), which is known to be a potential skin sensitizer; however, the risk is relatively low (Pengelly, 2003). A study by Hayes and Markovic (2002), where an in vitro toxicity test was performed based on the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, or MTT, carried out cytotoxicity assay. This test revealed that products containing 1% lemon myrtle oil were found to be low in toxicity and could potentially be used in the formulation of topical antimicrobial products. There is a lemon myrtle EO standard for Australia, AS 4941 published in 2001, that is under revision to reflect the current commercial quality of the oil. Three aldehydes: caprylic aldehyde, pelargonic aldehyde, and caprinic aldehyde, that are not present in lemon myrtle essential oil, have been found in some of the lemon myrtle oils in the global market. In order to protect the Australian lemon myrtle EO and also to prevent adulteration the Australian standard is currently under revision. These three aldehydes must not be present in lemon myrtle EO and the range for geraniol is recommended to be amended to 0.6–2.5% (Lassak, 2012).

SUMMARY POINTS

- Leaves of lemon myrtle (*Backhousia citriodora*) are steam distilled for the production of the essential oil.
- Citral is the major chemical component contributing to the lemon flavor of the essential oil.
- Lemon myrtle EO is used in the food and beverage industry globally as a flavoring agent.
- It has broad spectrum antimicrobial activity and has the potential to be used as a natural food preservative.
- Antifungal activity enables the application in postharvest disease management.
- The characteristic lemon flavor in the EO can be used as a flavor in a wide range of food and beverage products.

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Chapter 60

Licorice (*Glycyrrhiza glabra* Linn.) Oils

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List of Abbreviations

EMLE Enzymatically Modified Licorice Extract
FDA Food Drug Administration
GRAS Generally Recognized as Safe
CFR Code of Federal Regulations
WHO World Health Organization
JECFA Join Expert Committee on Food Additives
FEMA Flavour and Extract Manufactures Association

INTRODUCTION

Nowadays, food and pharmaceutical products are based on ingredients from herbs and other plant tissues. In this sense, the licorice plant and its derivatives have been part of herbal prescriptions to diverse health-related problems; in addition, its essential oil is used in the food industry. Current scientific knowledge has given specific information about the phytochemicals responsible of licorice properties. The main bioactive compound identified in licorice essential oil is a triterpene known as glycyrrhizin, which is used as sweetening agent in food products. However, the bioactive compounds may vary among varieties, and this is one of the main reason to study its botanical aspects and chemistry. The sweetness of licorice oil makes it suitable to eliminate undesirable tastes of other food ingredients, enhancing its organoleptic properties. Therefore, the most common uses of licorice oil are as an additive in confectionary and tobacco industries, care products, food products, beverages, and cosmetics.

This chapter discusses some basic botanical aspects of licorice (*Glycyrrhiza glabra* L.) including different varieties and other species around the world. Also, this chapter reviews the current uses and applications of licorice essential oil in food science, highlighting their benefits to food products quality and human health.

BOTANICAL ASPECTS

Licorice is a tall shrub that is native to Asia and the Mediterranean region and belongs to the family Leguminosae (Hayashi and Sudo, 2009). Its botanical name *Glycyrrhiza* is derived from the ancient Greek words g*lykos* (sweet) and *rhiza* (root), which was later Latinized to *liquiritia* and eventually to licorice (Isbrucker and Burdock, 2006). Licorice is a perennial plant, growing about 1.5 m high. The wrinkled and woody rootstock is brown outside and yellow inside. Leaves are unequally branched in four to seven pairs and their flowers are pale, blue, violet, yellowish white, or purplish in color, arising from the axils of the leaves in racemes or spikes, followed by pods (Hayashi and Sudo, 2009) (Figure 1). This plant has a long and storied history of use as a food flavoring and is one of the oldest and most popular herbal medicines in the world, known in both Eastern and Western cultures since the time of ancient civilizations of Mesopotamia, predating the Babylonian and Egyptian empires (Russo et al., 2014).

There are about 30 known species of licorice that are natives of warmer temperature countries. Among the most studies species are *Glycyrrhiza uralensis*, *Glycyrrhiza inflate*, and *G. glabra*, the latter being the most widely used in the food industry (Isbrucker and Burdock, 2006). *Glycyrrhiza uralensis* and *G. inflate* are grown in Asia, whereas *G. glabra* is the main species in southern and central Europe (var. *typica* and var. *echinata*), central and southern Russia (var. *glandulifera*), and Iran and Iraq (var. *violacea*) (Isbrucker and Burdock, 2006; Jiang et al., 2013). Licorice also grows in the United States (var. *lepidota*) and England (var. *typica*) but neither represents a significant contribution to world production. The two principal forms in commerce are licorice root and the essential oil, and are mainly extracted from varieties of *G. glabra* (Isbrucker and Burdock, 2006).

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FIGURE 1 Licorice (*Glycyrrhiza*) plant.

Glycyrrhiza glabra is a member of the pea family and grows best in subtropical climates in deep, fertile, well-drained soils, with full sun, and is harvested in the autumn, 2–3 years after planting (Omar et al., 2012). Its growing area includes the Mediterranean countries where two varieties of this species are recognized, var. *typica* and var. *enchinata* (Russo et al., 2014). The most appreciated for its organoleptic and chemical characteristics is *G. glabra* var. *typica*, known by the common name of "cordara" due to its roots habitus and characterized by horizontal stolons whose outer bark is brown in color with a bright yellow pulp. *Glycyrrhiza glabra* var. *typica* is commonly used in industrial processing because of high juice yield and high quality features products. Whereas *G. glabra* var. *enchinata* (commonly called "chiovara") is characterized by low juice yield and lower quality features (Brinckmann, 2013).

USAGE AND APPLICATIONS

Licorice is a highly nutritional and therapeutic plant that has been used from ancient times in different ways, mainly in food as a sweetener and a thirst quencher, and in pharmaceutical applications as a medicinal herb. Egyptians and Assyrians used such a remedy in a sweet liquid beverage, while in the battlefields and the desert, soldiers and travelers drank it to quench their thirst (Omar et al., 2012). Due to the beneficial properties of the bioactive components within licorice root and plant, it has been adopted by traditional herbal medicine in many cultures. For example, licorice appears in approximately 60% of traditional Chinese medicine prescriptions as an effective agent in the treatment for asthma, fatigue, dry cough, bronchitis, excessive phlegm, and for relieving drug toxicity (Isbrucker and Burdock, 2006; Wang et al., 2013). Moreover, licorice extract is produced by shredding and extracting the root with water in a steam extraction plant (essential oil). Currently, licorice oil is widely used as sweetener agent to masks the bitter taste in the confectionery and tobacco industry (Carmines et al., 2005; Hayashi and Sudo, 2009).

Nowadays, studies into licorice oil, root, and plant have reported some of the main bioactive compounds with pharmacological activities, which include some flavonoids among others. The most important are chalcones, isoflavones, isoflavans (hispaglabridin A and B), flavonones (liquiritigenin, isoliquiritigenin, and their corresponding aglycones), flavonols, isoflavenes, arylcoumarins, formononetin, saponoids, sterols, amino acids, and gums (Fenwick et al., 1990; Isbrucker and Burdock, 2006). Isoliquiritigenin in licorice with a chalcone structure and some isoflavan derived flavonoids, such as glabridin, licoricidin, and licorisoflavan A, were reported to exhibit a variety of biological properties, such as antiinflammatory, antioxidative, and antitumor activities (La et al., 2011; Yokota et al., 1998). Licochalcone A, a major chalcone in licorice, was reported to possess antitumor activity (Fu et al., 2004).

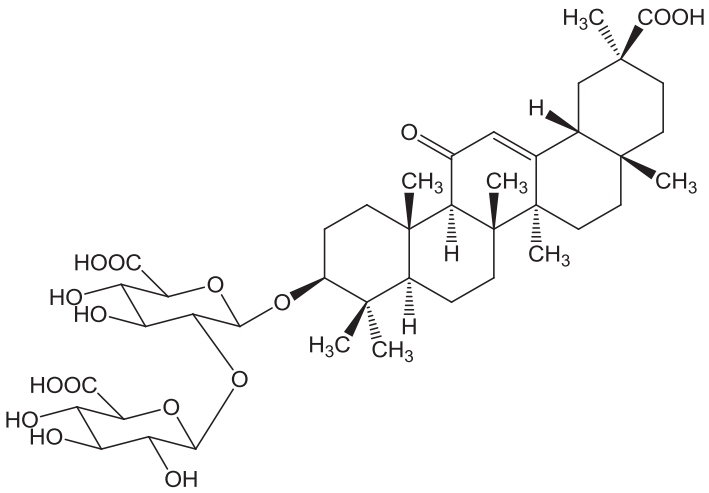

FIGURE 2 Structure of glycyrrhizin.

USAGE AND APPLICATION IN FOOD SCIENCE

The trend of the food science toward multifunctional and high quality foods is driven by consumers requiring food with health benefits as well (Jo et al., 2003). Emerging technologies applied in food science includes the incorporation of natural extracts in order to improve the food quality properties. In this sense, licorice oil has been used as additive in food industry to eliminate undesirable tastes and intensify and extend sweetness and flavors, as well as a foaming agent in alcoholic and nonalcoholic beverages. Once the licorice oil is obtained, it is then filtered and concentrated to produce a dark paste (a syrup-like material) which is cast into blocks, or may be spray-dried to obtain a powder (Ariño et al., 2007). Licorice extract powder is mostly used as a sweetener in the confectionery and pharmaceutical industry, while licorice paste is preferred for use as flavoring in the tobacco industry (Hayashi and Sudo, 2009). Furthermore, licorice oil is used as an agent in care products, food products, in chewing gum, beverages, toothpaste, cosmetics, and is one of the most widely used medicinal herb in China (Chin et al., 2007; Fenwick et al., 1990; Hayashi and Sudo, 2009). Such improvements in the food quality attributes are due to the sweet taste of licorice provided by the organic compound known as glycyrrhizin.

Glycyrrhizin is a triterpene saponin, and it is a conjugate of two molecules of glucuronic acid and glycyrrhetic acid (an oleanane-type triterpene) (Figure 2). The glycyrrhizin sweet taste is considered to be 50 to 200 times higher than sucrose and this important characteristic makes it a powerful natural sweetener and flavoring agent (Hayashi and Sudo, 2009; Tohma and Gulçin, 2010). However, structure–sweetness relationship studies revealed that the monoglucoronide derivative of glycyrrhizin showed higher sweet taste than glycyrrhizin itself (Liu et al., 2001). In this sense, enzymatically modified licorice extract, which is prepared by the treatment of licorice oil, is a natural sweetener that is mostly used in Japan, due to its unique properties such as higher solubility and better taste than licorice oil (Liu et al., 2001). The use of licorice oil in many food products has been regulated by the Food and Drug Administration (FDA), and its derivatives have been classed as generally recognized as safe (GRAS) with specific limitations since 1985 (Carmines et al., 2005).

The root contains about 20% of the components soluble in water, from which the primary active ingredient is the glycyrrhizin (Carmines et al., 2005; Wang et al., 2013). Also, it is noteworthy that around 10–25% of glycyrrhizin is found in the licorice oil (Carmines et al., 2005). As a tribasic acid, glycyrrhizin can form a mixture of potassium, calcium, and magnesium salts (Omar et al., 2012). On the other hand, the typical licorice aroma is likely to be mediated by a large collection of bioactive volatiles such as acetol, propionic acid, 2-acetyl-pyrrole, 2-acetyl-furan, and some phenols such as thymol and carvacrol (Table 1) (Farag and Wessjohann, 2012). Triterpene saponins and flavonoids are the main bioactive constituents of the root and the licorice oil, but several hundreds of other components have also been detected (Jiang et al., 2013).

The sweet-tasting flavor of licorice oil is applied in candies or sweets that contain around 2–10% of this oil (Herrera et al., 2009). The use of licorice oil in the confectionery industry may date from the eighteenth century when an English chemist, Dunhill, mixed molasses, sugar, flour, and licorice to made Pontefract cakes (Nieman, 1957). Licorice became famous in England and was added into candies, while in Northern America it started to be produced and imported. Nowadays, licorice confectionery is widely available, and large quantities of this oil are used in this kind of industry. Therefore, there are some patents were the licorice is used as a flavoring and sweetener in hard and soft candies (Koski, 2005; Stawski, 2008). However, the intake of licorice has been subjected to regulations by different organizations in Europe and USA (Fenwick et al., 1990).

TABLE 1 Relative Percentage of Volatile Compounds in Licorice Species Root Essential Oils Using Gas Chromatorgraphy–Flame Ionization Detection Measurements (n = 3)

		Concentration (%)						
Retention Index	Compound	*Glycyrrhiza glabra* (Egypt)	*Glycyrrhiza glabra* (Afghanistan)	*Glycyrrhiza glabra* (Syria)	*Glycyrrhiza inflate* (China)	*Glycyrrhiza echinata* (Bonn)	*Glycyrrhiza echinata* (Kiel)	Identification
951	(*E*)-2-heptenal	–	–	–	5.3	1.5	1.3	MS, RI
963	5-Methyl-Furfural	3.6	10.1	9.4	4.0	2.4	2.0	MS,RI
1011	(2*E*, 1*E*) heptadienol	–	–	–	–	4.2	3.4	MS, RI
1052	(*E*)-2-octen-1-al	–	–	–	–	7.4	5.5	MS, RI
1089	*o*-Guaiacol	2.2	tr	tr	3.4	–	–	MS, RI
1114	2-Phenylethanol	0.5	2.1	tr	1.6	tr	–	MS, RI, STD
1131	(*Z*)-pinene hydrate	tr	2.1	–	–	–	–	MS, RI
1134	Lavandulol<tetrahydro->	–	7.6	4.1	–	1.9	1.6	MS
1178	Terpinen-4-ol	tr	–	3.6	–	–	–	MS, RI
1179	(*E*)-linalool oxide	2.1	–	–	–	–	–	MS, RI
1185	p-Cymen-8-ol	–	2.7	3.0	–	–	–	MS, RI
1188	α-Terpineol	tr	–	2.4	–	–	–	MS, RI, STD
1199	Methyl chavicol		–	2.4	–	–	–	MS, RI
1201	(4*E*)-decenal	5.3	2.8	5.4	3.3	–	0.5	MS, RI
1205	Decanal	–	–	–	–	1.6	1.4	MS, RI, STD
1208	(2*E*, 4*E*)-nonadienal	–	–	–	–	2.1	1.0	MS, RI
1237	Cumin aldehyde	4.7	1.8	3.3	1.5	–	–	MS, RI
1241	Carvone	2.1	0.2	3.1	–	–	–	MS, RI, STD
1255	Piperitone	9.4	13.1	7.2	–	–	–	MS, RI
1272	(*E*)-cinnamaldehyde	3.6	4.5	6.2	–	–	–	MS, RI, STD
1285	(*E*)-anethole	1.3	–	–	2.9	–	–	MS, RI
1287	(2*E*, 4*Z*)-decadienal	–	–	–	–	6.4	5.6	MS, RI
1289	Thymol	27.2	6.0	5.5	–	–	–	MS, RI, STD
1294	Indole	–	7.4	1.8	4.5	–	–	MS, RI, STD

1301	Carvacrol	11.1	1.4	5.8	0.5tr	–	–	MS, RI, STD
1311	(2*E*, 4*Z*)-decadienal	–	–	–	–	20.8	18.0	MS, RI, STD
1313	p-Vinyl-guaiacol	8.5	8.5	9.5	10.3	–	–	MS, RI
1345	Unknown aldehyde	–	3.7	5.7	2.1	6.1	5.4	MS, RI
1360	Eugenol	9.4	7.5	8.8	2.8	tr	–	MS, RI, STD
1363	γ-Nonalactone	1.8	2.5	7.4	6.2	–	1.2	MS, RI
1405	Methyl eugenol	3.5	0.2	3.8	6.1	–	–	MS, RI
1418	β-Caryophyllene	0.5	0.5	1.1	2.1	1.5	–	MS, RI, STD
1440	β-Dihydro-ionone	–	–	–	7.7	–	–	MS, RI
1523	Himachalene epoxide	–	8.8	1.6	–	–	–	MS
1534	Spathulenol	–	–	–	–	4.3	3.7	MS, RI
1567	(1α, 10α)-Epoxy-amorph-4-ene				12.7			MS, RI
1589	β-Caryophyllene oxide	1.1		1.1	0.3	24.5	21.5	MS, RI
1615	Humulene epoxide II	–	–		–	11.7	10.3	MS, RI
Total alcohols		0.5	9.2	9.1	1.6	8.5	7.1	
Total aldehydes		17.2	22.9	30.0	16.1	48.3	40.7	
Total ethers/ epoxides		8.0	11.4	6.5	22.0	36.2	31.8	
Total ketones		11.5	13.3	10.3	7.7	–		–
Total phenols		58.5	23.4	29.7	16.9	0.0		

Relative concentration based on triplicate samples measurements; –, not detected; tr, trace; RI, retention index on DB5-MScolumn; MS, identification was based on comparison of mass spectra; STD, coanalysis relative to a pure compound and/or previous citing reference.
Source: Farag and Wessjohann (2012).

TABLE 2 Functional Uses and Levels of Licorice Permitted by the Food and Drug Administration

Type of Food	Maximum Level in Food (% Glycyrrhizin in Food)	Functional Use
Baked foods	0.05	Flavor enhancer, flavoring agent
Alcoholic beverages	0.1	Flavor enhancer, flavoring agent, surface-active agent
Nonalcoholic beverage	0.15	Flavor enhancer, flavoring agent, surface-active agent
Chewing gum	1.1	Flavor enhancer, flavoring agent
Hard candy	16.0	Flavor enhancer, flavoring agent
Herbs and seasonings	0.15	Flavor enhancer, flavoring agent
Plant protein products	0.15	Flavor enhancer, flavoring agent
Soft candy	3.1	Flavor enhancer, flavoring agent
Vitamin or minerals dietary supplements	0.5	Flavor enhancer, flavoring agent
All other foods except sugar substitutes	0.1	Flavor enhancer, flavoring agent

Source: United States Food and Drug Administration. Code of Federal Regulations Title 21, 184.1408 http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=184.1408.

The licorice oil and its main component glycyrrhizin are used as food additives in different foods. As mentioned, glycyrrhizin imparts a sweet taste to foods; moreover, it has salt-softening and flavour-enhancing properties and is also heat stable (Hayashi and Sudo, 2009). Glycyrrhizin may be used as a flavor enhancer for cocoa, replacing as much as 25% of the cocoa in manufactured products (Isbrucker and Burdock, 2006). Licorice can be used to reduce saltiness in soy sauce, other types of sauces, savoury snacks and sausages in Japan (Hayashi and Sudo, 2009). Moreover, for people who do not like the long-lasting sweet taste of glycyrrhizin, a more acceptable sweetness can be created by using a combination of glycyrrhizin and natural sugars or other sweeteners. Therefore, glycyrrhizin and licorice extracts are commonly used in sweet foods such as sweet snacks, ice cream, and sherbets to enhance their sweetness. Snacks like sticks and toffee bars, instant noodles, and sauces are others examples of foods containing licorice. Furthermore, licorice oil has been used in foods as an antioxidant and antimicrobial agent to increase food quality (Al-Turki et al., 2008; Jiang et al., 2013).

Licorice has been used in the beverage industry mainly by its property to enhance flavor and satisfy the thirst sensation (Omar et al., 2012). Also, the beer and ale industry are interested in the use of licorice oil and glycyrrhizin because of their foaming properties used to reduce potentially bitter-tasting compounds (Isbrucker and Burdock, 2006), for example, beverages containing licorice include Egyptian drink "*erk soos,*" Belgian beers, and anisettes (Raki, Ouzo, Pernod) (Omar et al., 2012). In addition, licorice is also a popular ingredient in herbal teas (Fiore et al., 2005). However, the characteristic flavor of licorice has apparently prevented its usage in various beverages because it is generally incompatible with other flavors. In this sense, efforts have focused on improving the use of licorice oil in the beverage industry. Koski (2005) developed a method to produce a licorice-containing citrus fruit beverage with additional flavourings, claimed to be used in the production of other beverages and associated products. According to this author, the resulting product has an unexpectedly refreshing taste and the beverage can be reduced, thinned, or combined with various other foods and beverages.

Licorice oil and its derivates are subjected to regulations such as maximum and minimum levels of use. As mentioned before, the FDA recognized licorice and its derivates as GRAS, stating that glycyrrhizin levels in food should not be hazard to human health according to the Code of Federal Regulations (21(3) CFR, 184.1408). However, depending of the food category, the CFR have reported maximum levels of glycyrrhizin contained in food (Table 2). Other organizations like the Council of Europe, the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives, and the Flavor and Extract Manufacturers' Association also approved licorice extract and its derivates as GRAS. The estimations of licorice extract and glycyrrhizin intake in USA are around 1.6 mg/day, or 0.027 mg/kg body weight, which is less of 2 mg/day (Isbrucker and Burdock, 2006). These regulations can be a contribution to the development of new safe products (Figure 3).

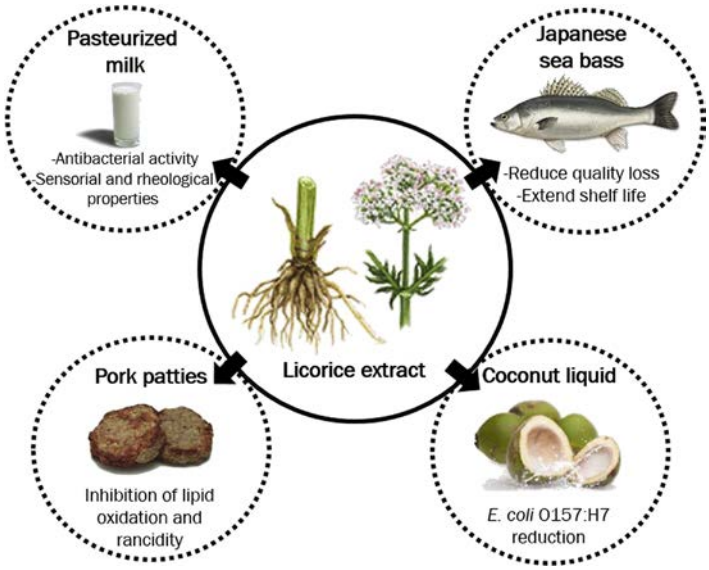

FIGURE 3 Safety–quality application of licorice in foods.

APPLICATION AND SAFETY-QUALITY USES IN FOOD

The assurance of food safety and quality by using licorice extracts have been reported by several authors. Qiu et al. (2014) showed that the combination of chitosan and licorice extract preserved the quality of Japanese sea bass fillets increasing antioxidant capacity, inhibiting lipid oxidation and microbial growth, and thus extending the shelf-life. These authors described how various phenolic compounds in the licorice extract could play an important role in the lipid oxidation and antimicrobial activity. Jiang et al. (2013) demonstrated that licorice ethanol extract inhibited lipid oxidation and protected sensory attributes of precooked pork patties during refrigerated and frozen storage. Such antioxidant activity is attributed to the high radical scavenging capacity of the extract, particularly against hydroxyl radicals, resulting in a suppression of protein and lipid oxidation.

Zhang et al. (2009) reported that ethanol extract from licorice roots contained strong antimicrobial activity against spoilage and pathogenic bacteria (*Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Lactobacillus sake*) and was used to preserve meat products quality (fresh pork and ham products) stored at 4 °C. Moreover, Al-Turki et al. (2008) reported that licorice could be applied successfully to control food-borne pathogens (*E. coli* and *Bacillus subtilis*) in some dairy products (milk and labneh). In addition, they suggest that licorice can be combined with different herbal extracts promoting antibacterial synergistic effects. On the other hand, Gabriel et al. (2014) demonstrated that the addition of vanillin (0–500 ppm), licorice root extract (0–420 ppm), and mild heat (55 °C) to young coconut liquid endosperm caused a decimal reduction of *E. coli* O157:H7. The combined efficacies of the additives were most significant at concentrations above 250 ppm vanillin and 210 ppm licorice root extract. These results concluded that these combinations may be used in the establishment of mild heat pasteurization processes for supplemented young coconut liquid endosperm, for better control of quality and safety.

On the other hand, licorice extract has been used to improve food technological properties. Mocanu et al. (2009) obtained a novel probiotic product named ROSALACT®, prepared from pasteurized milk with rosehip and licorice extract, using a mixed culture of probiotic bacteria. Results concluded that the application of natural extracts enhance the sensorial characteristics, including texture, taste, and mouthfeel. In this sense, the application of natural extracts diversifies the range of functional products with good sensorial and rheological properties.

SUMMARY POINTS

- Licorice roots of *Glycyrrhiz glabra* are the main tissue used to obtain essential oil and bioactive derivatives.
- Licorice oil represents an important source of bioactive compounds, including glycyrrhizin, volatile compounds, flavonoids, saponins, and others with pharmacological activities.
- Glycyrrhizin is responsible for the sweet-tasting properties of licorice and is commonly used as an emulsifier and gel-forming agent in foodstuff.
- Licorice extract application on foods offers good sensory properties, food quality, and safety.
- Licorice extract has antiviral, antiinflamatory, antihepatotoxic, and antimutagenic effects and its use as food additive could provide foods with health benefits.

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Chapter 61

Lime (*Citrus aurantifolia*) Oils

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List of Abbreviations

ABTS 2,2′-Azino-di-[3-ethylbenzthiazoline sulfonate (6)] diammonium salt
ATCC American Type Culture Collection
CFU Colony forming units
DPPH 2,2-Diphenyl-1-picrylhydrazyl
EO Essential oil
MBC Minimum bactericidal concentration
MIC Minimum inhibitory concentration
TE Trolox equivalent

INTRODUCTION

Lime (*Citrus aurantifolia*) is a famous citric fruit part of the family of Rutaceae with a smooth surface, a greenish-yellow color, and highly acidic juice. The massive production of this citric fruit is attributed to its organoleptic characteristics and functional properties as common ingredient of alcoholic and nonalcoholic cocktails and typical dishes of Mexican, Indian, Vietnamese, and Thai cuisine (Ubando-Rivera et al., 2005). In folk usage, it was recommended to prepare tea from its leaves and volatile oils to relieve common cold and flu, and it is suggested as an excellent source of vitamin C to prevent scurvy. The bioactive properties and characteristic flavor and odor of lime has been attributed to terpenes content that are present in lime essential oil (EO) (Gamarra et al., 2006). The main technique of lime EO production is steam distillation of fresh or dried limes. The oil obtained has an intense citric flavor with a yellow color and consist of a mixture of volatile components basically terpenes (75%), oxygenated compounds (12%), and sesquiterpenes (3%) (Gamarra et al., 2006). Lime EO is one of the most important flavoring oils, used widely in all kind of beverages, soft drinks, soft drinks powders and tablets, and in baked goods, such as cakes, pastries, pie fillings, confectionery, soft and hard center candies, gelatin desserts, ice creams, etc. (Gamarra et al., 2006).

In addition to its flavoring properties, it has been reported that lime EO and its constituents possess antimicrobial and bioactive properties as antioxidant and radical scavenging activities that make lime EO a potential additive for food industry that could enhance the quality and safety of the treated food. For this reason, this chapter discusses the applications of lime EO in food industry as flavor enhancer, antioxidant, and antimicrobial agent.

BOTANICAL ASPECTS

There are several species of lime tree, but *C. aurantifolia* is native to south-east Asia and is grown all over the world, mainly in America, Europe, Asia, and Indian regions. It has been cultivated since the fifteenth century (Calabrese, 2002). The greatest producers of lime are in North America, including Mexico, South Florida, and California (Calabrese, 2002). The massive production of this citric fruit is attributed to its organoleptic characteristics and functional properties as a common ingredient of alcoholic and nonalcoholic drinks and typical dishes of Mexican, Indian, Vietnamese and Thai cuisine, also, this fruit is also used as food additive for its preservatives properties (Ubando-Rivera et al., 2005).

There are diverse varieties of the genus *Citrus* including key lime or bartender's lime (*C. aurantifolia*) (Mexican, west Indian), Persian lime (*Citrus latifolia*) (Tahiti or Bears' lime), kaffir lime (*Citrus hystrix*) (kieffer lime; *makrut*, or *magrood*), Australian dessert lime (*Citrus glauca*), and sweet lime (*Citrus limetta*) (sweet limetta, Mediterranean sweet lemon). However, the most imported produced cultivars of this citric fruits are the Persian lime and key lime, particularly, the trees of this species can be founded in home backyards, being an ornament tree or for local consumption, and the principal difference between these limes are that the Persian lime is seedless, whereas key limes have a lot of seeds (Saunt, 2000).

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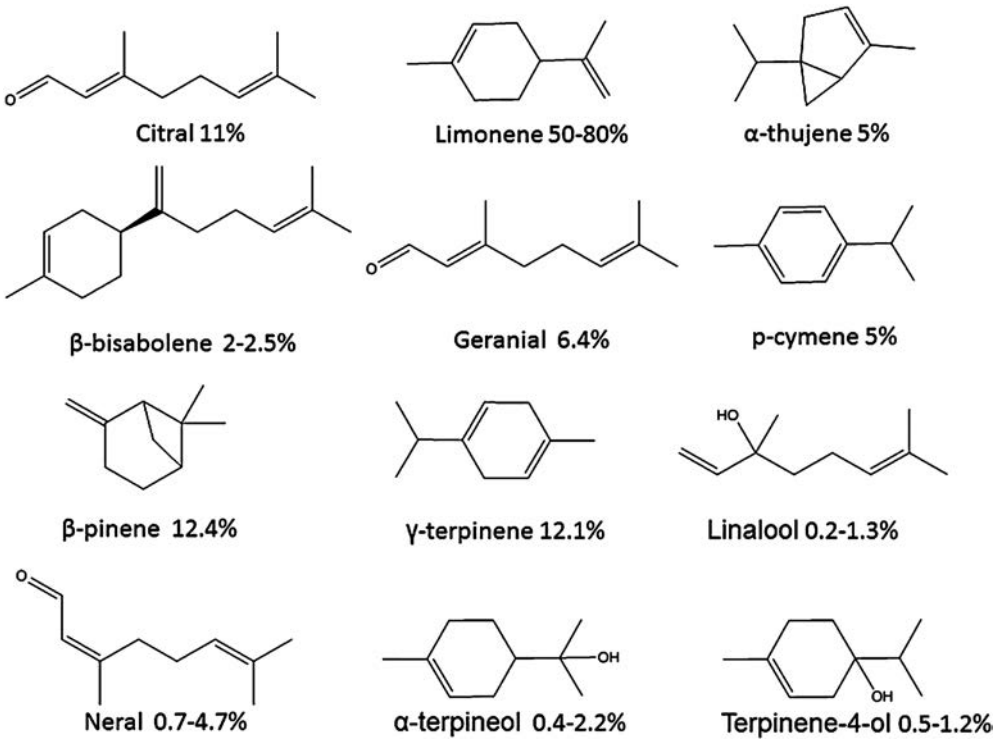

FIGURE 1 Main compounds identified in lime oil and relative abundance (%).

USAGE AND APPLICATIONS

The applications of lime are big. Generally, the seedless varieties dominate the local and international fruit markets, the juice of limes is used in the food industry reported than can be used as food additive for its organoleptic properties such as flavor and odor. Furthermore, lime is used as a principal component of refreshing drinks, tasty desserts, vegetables, fresh salads, sauces, and for seasoning meats and seafood (Francis, 2004). In folk usages it has been recommended to prepare tea from its leaves and volatile oils to relieve common cold and flu, and it is suggested as an excellent source of vitamin C to prevent scurvy (Bruneton, 1999). On the other hand, has been reported for the treatment of various diseases such as gastrointestinal disorders, insomnia, headaches, cardiovascular diseases, and cancer, and also as an antiseptic, antioxidant, antispasmodic, aromatic, astringent, carminative, sedative, and stimulant (Suryawanshi, 2011). In addition, pharmaceutical industries use this oil as flavoring agent in syrups and suspensions, and in perfumery its volatile composition is used as the base of many citric scents (Ruberto, 2002).

The bioactive properties and characteristic flavor and odor of lime have been attributed to terpenes containing as the main constituents of its EO (Gamarra et al., 2006) (Figure 1). The techniques of production of lime oil are steam distillation by fresh or dried limes. The oil obtained has an intense citric flavor with a yellow color and consists of a mixture of volatile components basically terpenes (75%), oxygenated compounds (12%), and sesquiterpenes (3%) (Gamarra et al., 2006). The yield of oil from lime varies from 0.2 to 0.99 mL per 100 g of fresh and dried fruits, respectively (Ramesh Yadav et al., 2004).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Citrus flavors are most commonly derived from citrus oils obtained from the peel of the citrus fruit or as a by-product obtained during the concentration of juice (Bringas-Lantigua et al., 2012). Flavors are complex mixtures of volatile and labile components of which the sensory perception can be changed as a result of oxidation, chemical interactions, or volatilization (Bringas-Lantigua et al., 2012). In the case of lime EO, citral and limonene are two of the most important citrus-like flavor compounds and are employed as flavoring agent in perfumes, creams, soaps, household cleaning products, and in some food products such as fruit beverages and ice creams (Espina et al., 2013).

Citral consists of neral and geranial, geometrical isomers that degrade rapidly at low pH and under oxidative stress. Acid-catalyzed cyclization of citral reduces the fresh lemon flavor of citrus oils and leads to the formation of compounds

that produce undesirable off-flavors. Citral degradation at low pH starts with the isomerization of geranial to neral, which then forms the monoterpene alcohols, *p*-menthadien-8-ol and *p*-menthadien-4-ol, which in turn can oxidize to form *p*-cymene-8-ol and through a dehydration reaction produces aromatic compounds such as *p*-dimethyl-styrene, *p*-cymene, and *p*-cresol (Djordjevic et al., 2007).

On the other hand, limonene is susceptible to degradation by oxidation reactions. Limonene oxidation initially results in the formation of hydroperoxides. As with the oxidation of unsaturated fatty acids, limonene hydroperoxides can undergo scission reactions that lead to the formation of numerous products including perillyl alcohol, perillyl acetate, carveol acetate, limonene oxide, carvone, and carveol. In citral degradation, limonene decomposition causes the loss of lemon-like odors and the formation of off-flavours described as flowery, piney, and minty (Djordjevic et al., 2007). To minimize these negative processes, microencapsulation could be used in the flavor industry to trap liquid flavoring materials, such as EOs, oleoresins, and flavoring mixtures in a protective layer of coating materials (Bringas-Lantigua et al., 2012). Spray drying is the most common way to perform microencapsulation of a flavor substance. The advantages of this technology are numerous, providing protection against oxidation and preventing the loss of flavors during storage (Bringas-Lantigua et al., 2012).

In addition to its flavoring properties, it has been reported that lime EO and its constituents possess antimicrobrial and bioactive properties as antioxidant and radical scavenging. Free radicals/reactive oxygen species (ROS) compromise food systems by decreasing their stability as well as causing chronic diseases in biological systems. Free radicals are chemical species having one or more unpaired electrons making them highly unstable and causing damage to other molecules. ROS include superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide which are often generated as by-products of biological reactions or from exogenous factors (Prakash et al., 2015).

Oxidation is a multilateral process that brings about undesirable changes related to food quality, organoleptic characteristics, safety, and nutritional value. Food oxidation causes major defects mainly affecting consumer acceptability. This process is manifested mainly through discoloration and generation of off-flavours, while alteration of principal components, such as vitamins, lipids, and proteins, is not always apparent. Because oxidation is associated with loss of nutritionally precious ingredients, as well as the formation of potentially toxic substances, the shielding of food matrices against oxidative deterioration is of technological importance (Prakash et al., 2015).

In this context, several studies have evaluated the antiradical and antioxidant activities of lime EO. Choi et al. (2000) showed that lime EOs have a radical scavenger activity of 158.5 mg TE/mL against the free synthetic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). In addition, films from fish skin gelatin containing lime EO possess DPPH and 2,2′-azino-di-[3-ethylbenzthiazoline sulfonate (6)] diammonium salt radical scavenging activity and ferric reducing antioxidant power of 0.09, 0.46, and 4.24 μmol TE/g dried film, respectively (Tongnuanchan et al., 2012). Also, lime EO showed inhibitory effects against linoleic acid peroxidation (Song et al., 2001). These results indicate that lime EOs have multiple antioxidant mechanisms.

The antioxidant properties of lime EO have been attributed to its terpenoid content mainly to γ-terpinene and terpinolene (Choi et al., 2000). Foti and Ingold (2003) revealed the mechanism by which γ–terpinene inhibits lipid peroxidation. The important fact is that the chain carrying peroxyl radicals are HOO· radicals that react rapidly with linoleylperoxyl radicals and thereby terminate the chain reaction. Because this mechanism is different to the mode of action of vitamin E, and because vitamin E becomes prooxidant at high concentrations, terpinene may provide a new stabilizing substance for edible lipids and may also provide a possibility to enrich foodstuffs and beverages with effective health benefit compounds (Foti and Ingold, 2003).

Regarding food safety, many antimicrobial compounds present in EOs have demonstrated their importance for microbial population control by targeting food-borne microorganisms (Sánchez Aldana et al., 2015). EOs have been extensively tested for their antifungal and antimicrobial properties. Also, EOs have been classified as generally recognized as safe compounds by the Unites States Food and Drug Administration and they have been considered an alternative of preservation techniques for foods, as nonthermal technologies and naturally derived antimicrobials (Ayala-Zavala et al., 2009).

In the case of citrus oils, lime EO showed a high antibacterial activity (see Table 1) against Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Shigella flexnerii*, *Salmonella* spp., *Citrobacter* spp., *Serratia* spp., *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*) bacteria with zone diameters ranging from 13 mm (*P. aeruginosa*) to 40 mm (*S. aureus* and *Salmonella* spp.) (Aibinu et al., 2007). Costa et al. (2014) evaluated the antimicrobial activity of the lime EO on a representative range of Gram-positive bacteria (*Bacillus subtilis*, *Enterococcus durans*, *Enterococcus hirae*, *Listeria monocytogenes*, *S. aureus*, *Staphylococcus epidermidis*) and Gram-negative bacteria (*Enterobacter cloacae*, *E. coli*, *P. aeruginosa*, *Serratia marcescens*, and *Salmonella typhi*), including ATCC and clinical strains, by broth microdilution method. Gram-negative bacterial strains were more resistant than the Gram-positive bacteria. Minimum inhibitory concentration values found for lime EO ranged from 0.25% to 1% (v/v) in standard Gram-positive bacteria, and from 0.5% to >1% (v/v) in Gram-negative bacteria. Minimum bactericidal concentration values were

TABLE 1 Antibacterial Activities of Lime Essential Oil

Test Organism	MIC (% v/v)	Inhibition Zone (mm)	References
Escherichia coli	ND	25	Aibinu et al. (2007)
Klebsiella pneumoniae	ND	19	
Staphylococcus aureus	ND	40	
Enterococcus faecalis	ND	25	
Shigella flexnerii	ND	30	
Pseudomonas aeruginosa	ND	13	
Bacillus subtilis	0.25	26.7	Costa et al. (2014)
Staphylococcus epidermis	0.25	19.2	
Staphylococcus aureus	0.25	24.7	
Listeria monocytogenes	0.25	ND	
Escherichia coli	1	13.3	
Pseudomonas aeruginosa	1	ND	
Salmonella typhi	0.5	15.4	

ND, Not determined; (% v/v), rate of oil volume to volume.
In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. Because a lower MIC value indicates that less of the compound is required in order to inhibit growth of the organism, compounds with lower MIC scores are more effective antimicrobial agents.

0.5 and >1% (v/v) for Gram-positive and Gram-negative bacteria, respectively (Costa et al., 2014). Gram-positive bacteria are more sensitive to EOs than Gram-negative bacteria because of their cell wall, consisting of only an outer peptidoglycan layer. The latter cannot act as an effective permeability barrier compared with Gram-negative bacteria, which possess outer membrane phospholipids (Costa et al., 2014).

To achieve more effective treatment of food products using EOs and its constituents, several technologies must be contemplated: nanoemulsions, nanocapsules, vapors, edible films based in different polymers or in conjunction with other preserving technologies like low temperatures, modified atmosphere packaging, and heat treatment (Table 2). In this context, the antibacterial activity of lime EO against the food-borne pathogen *L. monocytogenes* in apple juice was studied at two temperatures. The addition of 200 µL of lime EO per 100 mL of apple juice completely inhibited the growth of *L. monocytogenes* at 5 °C and at 37 °C. This concentration of EO extended the lag time by at least 292.7% compared with juice without lime EO. This is especially important considering that *L. monocytogenes* was able to grow in the juice at low temperatures in the absence of EOs (Carrizo et al., 2014).

Espina et al. (2014) investigated the effects of limonene combined with thermal treatments to pasteurize orange juice at different temperatures (54–60 °C). The addition of 200 ppm of limonene to commercial orange juice reduced the heating time to inactivate 5 $\log_{10}$ cycles of the target pathogen *E. coli* O157:H7 by 3.8 times, demonstrating that limonene and heat acted synergistically. In addition, orange juice with 100 ppm of (+)-limonene added maintained the sensory acceptance after a heat treatment able to inactivate 5 $\log_{10}$ cycles of *E. coli* O157:H7 (Espina et al., 2014).

Limonene (0.3%) incorporated into gellan-based edible coating was able to reduced total plate count and maintained the sensory characteristics of fresh-cut pineapple (Azarakhsh et al., 2012). Incorporating natural antimicrobial agents into edible coatings provides a novel way to increase the shelf-life and to maintain the quality of fresh-cut fruits. The inclusion of additives into these edible coatings to increase their effectiveness, such as EOs and their constituents with antimicrobial and antioxidant activities, has been reported and patented (Azarakhsh et al., 2012).

Limonene contained in this oil is considered to accumulate in the microbial plasma membrane and thus cause a loss of membrane integrity and dissipation of the proton motive force, this phenomenon occurred simultaneously with bacterial inactivation, identifying the cell envelopes as important limonene targets. In contrast to other terpenes, limonene did not cause sublethal injuries in any *E. coli* structure, cataloging its lethal action under the "quantal" effect ("all or nothing") (Espina et al., 2013). Similarly, citral acts by disrupting the cytoplasmic and the outer membrane of *E. coli* cells and also RNA polymerase, sigma S, which is involved in the *E. coli* response to citral damage (Somolinos et al., 2010).

TABLE 2 Antimicrobial Properties and Sensory Impact of Lime Essential Oil Applied on Different Food Systems

Food Matrix	Concentration Used	Antimicrobial Properties	Sensory Impact	References
Apple juice	200 µL of lime essential oil per 100 mL of apple juice	Inhibition of *Listeria monocytogenes* growth at 5 °C and at 37 °C Extension in the lag time at least 292.7% compared to juice without lime essential oil	NE	Carrizo et al. (2014)
Natural orange juice	Limonene at 100 ppm	Reduction in inactivation time of *Escherichia coli*	No differences in acceptance with untreated samples	Espina et al. (2014)
Fresh-cut pineapple	Gellan-based edible coating incorporated with limonene at 0.3%	Reduction in total plate count after 16 days of cold storage	No differences in acceptance with untreated samples	Azarakhsh et al. (2012)
Fresh tomato	Chitosan-based edible coating incorporated with lime essential oil at 0.1%	Inhibition of *Rhizopus stolonifera* growth	NE	Ramos-García et al. (2012)
Fresh papaya	Mesquite gum-based edible coating incorporated with lime essential oil at 0.05%	Reduction in disease incidence caused by *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* by 100% and 60%, respectively	NE	Bautista Baños (2013)

NE, Not evaluated.

TABLE 3 Antifungal Activities of Lime Essential Oil

Tested Organism	MIC (% v/v)	Inhibition Zone (mm)	Inhibition of Mycotoxin Production (%)	References
Aspergillus niger	ND	48	ND	Aibinu et al. (2007)
Candida albicans	ND	40	ND	
Candida albicans	0.125	ND	ND	Costa et al. (2014)
Candida parapsilosis	0.125	ND	ND	
Aspergillus parasiticus	ND	ND	89.6 (aflatoxin B1) 89.2 (aflatoxin G1)	Razzaghi-Abyaneh et al. (2009)

ND, Not determined; (% v/v), rate of oil volume to volume.

In addition, it has been reported that lime EOs possess antifungal properties and inhibit fungal toxin synthesis (Table 3). Plant pathogenic fungi are the main plant pathogens all over the word and they cause significant yield losses in numerous economically important crops (Sharma, 2014). All these negative effects of pesticides have indicated the need for alternative nonchemical methods in crop protection (Sharma, 2014). Lime EO at 1 mg/mL inhibited *Aspergillus parasiticus* growth by 47.8% and aflatoxin B_1 and G_1 production by 89.6% and 89.2%, respectively (Razzaghi-Abyaneh et al., 2009). The authors attributed the fungal growth and aflatoxin production inhibition to limonene because several researchers have been described the antifungal activity of limonene against *Aspergillus niger*, *Rhizoctonia solani*, and *A. parasiticus*. Also, it has been shown that limonene was able to decrease aflatoxin production by *A. parasiticus* in addition to fungal radial growth inhibition (Razzaghi-Abyaneh et al., 2009).

Bautista Baños (2013) evaluated the fungicidal effect of the thyme and lime EOs against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, and incorporated them in edible coatings to control postharvest diseases of papaya fruits. Papaya

fruit dipped in both the EOs experienced reduced decay caused by *C. gloeosporioides* and *R. stolonifer* by up to 50% and 40%, respectively. In addition, it was noticed that in papayas immersed in mesquite gum emulsion and formulated with lime EO, it was possible to reduce the disease incidence caused by *C. gloeosporioides* by 100% with lime EO at 0.5% (Bautista Baños, 2013). Similarly, Ramos-García et al. (2012) tested various chitosan-based formulations (1%) mixed with beeswax (0.1%), oleic acid (1.0%), and lime or thyme EO (0.1%) on tomato at three different maturity stages to control *R. stolonifer* at storage temperatures of 12 and 25 °C. The best coatings were chitosan (1%) + beeswax (0.1%) + lime EO (0.1%) and chitosan (1%) + oleic acid (1%) + lime EO (0.1%) because no growth of *R. stolonifer* took place. Observation with an electronic scanning microscope showed distorted mycelia and no development of *R. stolonifer* sporangiospores (Ramos-García et al., 2012). These results clearly show that lime EO may have potential for use as natural preservative in controlling fungal growth and mycotoxin contamination of foods, feeds, and agricultural commodities in practice.

SUMMARY POINTS

- The odor and flavor properties of lime essential oil can be used in the food industry to add traditional flavors in novel foods.
- Lime EO can stabilize free radicals and act as an antirancidity agent in food systems.
- Terpenes, compounds in lime essential oil, can inhibit growth of pathogenic and spoilage bacteria.
- Yeast and mold growth and mycotoxin biosynthesis can be inhibited by lime EO.

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Chapter 62

Lovage (*Levisticum officinale* Koch.) Oils

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List of Abbreviations

dw Dry weight
EO Essential oil
fw Fresh weight
MIC Minimum inhibitory concentration
t Traces

INTRODUCTION

Lovage (*Levisticum officinale* Koch.) is a perennial aromatic plant belonging to the Apiaceae family. It is native to some regions in Europe and Asia. Lovage has been long cultivated in Europe, the leaves being used as an herb, the roots as a vegetable, and the seeds as a spice (Blamey and Grey-Wilson, 1989). The roots and fruits are aromatic and stimulant, therefore in herbal medicine they have been used in disorders of the stomach and feverish attacks, especially for cases of colic and flatulence in children. The root of lovage has also been known for centuries as a medicine possessing carminative and spasmolytic activity and other physiological effects (Segebrecht and Scilcher, 1989). All anatomical parts of lovage yield yellowish aromatic Essential oil (EO) consisting mainly of terpenes and phthalides, β-phellandrene, α-terpinyl acetate, and *Z*-ligustilide in remarkably different proportions in the individual plant anatomical organs (Bylaitė et al., 2000). Lovage oils as well as their main constituents were reported to demonstrate various bioactivities; however, the studies on antimicrobial properties of integral lovage oils against food-born microorganisms are rather scarce. Z-ligustilide as an important bioactive compound of *Radix Angelica sinensis* with different physiological effects on human health has been the focus of several studies. The reviews on lovage oils and their bioactivities have not been published until now and this chapter aims at surveying data published on lovage oils during last decades in order to highlight the possibilities of wider applications of this interesting and useful plant species.

BOTANICAL ASPECTS

Lovage (*L. officinale* Koch.) is a tall, stout perennial aromatic plant, the sole species in the genus *Levisticum* in the family Apiaceae, subfamily Apioideae, and tribe Apieae (Pimenov and Leonov, 1993; Downie et al., 2001). In English it has been also called Garden Lovage, Old English Lovage, Italian Lovage, Cornish Lovage, Maggi plant, and Smellage and has been thought to resemble Garden Angelica to some degree. In foliage and perennial habit of growth it does very closely resemble the Spanish *Angelica heterocarpa* (Grieve, 1984). The exact native range is disputed; some sources cite it as native to much of Europe and south-west Asia (Den virtuella floran: *Levisticum officinale*), others from only the eastern Mediterranean region in south-east Europe and south-west Asia (Huxley, 1992), and yet others only to south-west Asia in Iran and Afghanistan, citing European populations as naturalized (Blamey and Grey-Wilson, 1989; Germplasm Resources Information Network).

Lovage is an erect, herbaceous, perennial plant growing to 1.8–2.5 m tall, with a basal rosette of leaves and stems with further leaves, and flowers produced in umbels at the top of the stems. The stems and leaves are shiny glabrous green to yellow–green and smell of lime when crushed. The larger basal leaves are up to 70 cm long, tripinnate, with broad triangular to rhomboidal, acutely pointed leaflets with a few marginal teeth; the stem leaves are smaller and less divided, with few leaflets. The flowers are yellow to greenish–yellow, 2–3 mm in diameter, and produced in globose umbels up to 10–15 cm diameter. Lovage flowers in June–August depending on geographical growing zone. The fruits which mature in

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autumn (September) are small, elliptical in shape, and curved, with three prominent winged ribs, yellowish–brown in color and extremely aromatic, they are dry two-parted schizocarps 4–7 mm long (Blamey and Grey-Wilson, 1989; Germplasm Resources Information Network).

Cultivation of lovage is relatively easy; it is propagated by division of roots or by seeds. Rich, moist, but well-drained soil and a sunny situation are required. In late summer, when the seed ripens, it should be sown and the seedlings transplanted, either in the autumn or as early in spring as possible, to their permanent quarters, setting 30 cm apart each way. The seeds may also be sown in spring, but it is preferable to sow when just ripe. Root division is performed in early spring (Grieve, 1984).

USAGE AND APPLICATIONS

Lovage has been long cultivated in Europe, its leaves have been used as an herb, the roots as a vegetable, and the seeds as a spice. Lovage was particularly popular in southern European cuisine (Blamey and Grey-Wilson, 1989). The odor of the whole plant is very strong; specific flavor of lovage roots is characterized as medium aromatic (Heath and Reineccius, 1986) or as strong impact warmly aromatic with sweet, yeasty, musky, lemon-like, celery-like notes (Heath, 1981). Lovage taste is warm and aromatic, the plant contains abundant yellowish, gummy, resinous juice. It is sometimes grown in gardens for its ornamental foliage, as well as for its pleasant odor (Grieve, 1984). The EOs from leaves, seeds, and roots of lovage are used in food, beverage, perfumery, and tobacco industries (Cu et al., 1990). Fresh and dried lovage leaves and dried rhizomes or roots are suitable for flavoring meat soups, meat, fish, vegetable dishes, sauces, and various salads. Dried rhizomes and roots have also been used to flavor confectionery products, marinates, and liquors. The young stems of lovage are being treated like those of Angelica, to which, however, it is inferior, as its stems are neither so stout nor so succulent (Grieve, 1984). The leafstalks and stem bases were formerly blanched like celery, however their uses as a vegetable decreased. Herbal tea can be made from the dried leaves but the decoction possesses a very agreeable odor. The reported commercial uses of dried lovage preparations as well as extracts and oils include nonalcoholic and alcoholic beverages, baked goods, some frozen dairy produces, hard and soft candies, cheese, gelatin, pudding, meat products, chewing gum, sweet sauce, condiment, relish, gravies, and soups (Burdock, 2002). According to Flavor and Extract Manufacturers' Association (Burdock, 2002) lovage oil had been added to different products from 0.02 (hard candies) to 32.21 ppm (alcoholic beverages).

Lovage was much used as a drug plant in the fourteenth century; however, it was never an official remedy, nor were any extravagant claims made, as with angelica, for its efficacy in numberless complaints. Lovage roots have also been known as a medicine possessing carminative and spasmolytic activity (Segebrecht and Scilcher, 1989). The roots and fruit are aromatic and stimulant, and have diuretic action. In herbal medicine they have been used in disorders of the stomach and feverish attacks, especially for cases of colic and flatulence in children, its qualities being similar to those of angelica in expelling flatulence, exciting perspiration, and opening obstructions. The leaves eaten as salad, or infused dry as a tea, were also considered as a good emmenagogue. An infusion of the root was recommended for gravel, jaundice, and urinary troubles, and the cordial, sudorific nature of the roots and seeds caused their use to be extolled in "pestilential disorders" (Grieve, 1984). The old-fashioned cordial, "Lovage", although now not so popular, still occasionally may be found in public houses, and is brewed not only from the garden lovage but mainly from other species.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Lovage EOs have been studied by many authors in different countries. However, the majority of these studies were focused on chemical composition of oils and its variations depending on various factors, while antimicrobial and other biological activities of lovage EOs and their constituents were the subject of remarkably lesser number of investigations.

Isolation Methods of Lovage Essential Oil and Factors Affecting the Yield

All anatomical parts of lovage accumulate EO, which is traditionally isolated by steam or hydrodistillation. For instance, optimization of different hydrodistillation parameters (water content, pH, condensation temperature) affecting solid–liquid extraction, codistillation, and condensate separation enabled to increase root oil yield from 0.47% to 0.74% dry weight (dw) (Perineau et al., 1992). To improve the distillation process of different EO crops, pulsed electric fields were applied on dry seeds of lovage and an increase of oil yield by 67% was reported, whereas the quality and the composition of the treated EOs was not affected (Dobreva et al., 2013). Lovage may also be extracted with organic solvents such as ethanol, 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113), dichloromethane, benzene, hexane, cyclohexane, methyl furan (Cu et al., 1990),

methanol (Samiee et al., 2006), or carbon dioxide in its liquid or supercritical state (Daukšas et al., 1998, 1999, 2002; Menaker et al., 2004; Kemzūraitė et al., 2014). However, organic solvents, depending on their properties, together with EO constituents also extract other, less volatile substances. For instance, the yields of concretes isolated with solvents were from 0.53 (CFC-113) to 8.24% (ethanol); the content of steam distilled oils from these concretes was in the range of 0.24–0.39%, while direct distillation of plants yielded only 0.15% of EO constituted (Cu et al., 1990). The method of supercritical fluid extraction with carbon dioxide (SFE–CO_2) is particularly advantageous; it is performed at comparatively low temperatures, the extracts obtained are free from organic solvents, whereas a proper selection of extraction parameters (mainly pressure and temperature) enables to obtain the fractions enriched with desirable constituents. Optimization of SFE–CO_2 parameters enabled 2.26% and 2.29% of extracts to be obtained from roots and leaves, respectively, at 45 MPa pressure, 60 °C temperature, 90 min (roots) and 30 min (leaves) extraction time, whereas the yield of hydrodistilled EO was remarkably lower, 0.24% and 0.74%, respectively (Kemzūraitė et al., 2014). For analytical purposes simultaneous distillation and extraction procedure was also applied for the isolation of lovage volatile compounds (Santos et al., 2005; Raal et al., 2008), whereas for the characterization of odor-active compounds dynamic-headspace gas chromatography–olfactometry was used (Bylaitė et al., 1996, 2000).

EO yield depends on many factors such as anatomical plant part, propagation method, harvesting time, plant origin, isolation procedure, and others. Data, which have been selected from various articles and summarized in Table 1, show that EO yield isolated by distillation was from as low as 0.1% from lovage flowers (Mirjalili et al., 2010) to as high as 4.16 mL/100 g dw from the leaves grown by seedlings planting (Rosłon et al., 2013). Comparing different anatomical parts of lovage, the seeds in most cases yield the highest amounts of EO, followed by the herb and roots. It is of interest to note that lovage stems also accumulate large amounts of EO depending on harvesting time up to 1.16% (w/dw) (Bylaitė et al., 1998), which is not characteristic of many other EO-bearing plants. The concentration of volatile oil depends on harvesting time; for instance, in leaves and stems collected at vegetation phases from May 15 (shoot up) until September 21 (second shoot up of newly grown plants) it was 1.01–1.35% and 0.53–1.16%, respectively, whereas the flowers (July 7) yielded 1.53% of oil. It was also shown that EO yield depends on the plant age. Depending on harvesting time the content of oil in one-year-old fresh/dried leaves and roots was 1.1–1.84/0.9–1.72 and 0.15–0.3/0.13–0.26 mL/100 g, respectively, whereas in two-year-old plants it was higher, 1.5–2.78/0.9–2.22 and 0.42–0.45/0.22–0.47 mL/100 g (Novák and Németh, 2002). Harvesting leaves in the vegetation season had a negative impact on the accumulation of EO in the roots (Szebeni-Galambosi and Galambosi, 1992), while leaving the above-ground parts of lovage until autumn increased the active components in roots from 0.52% to 0.85% (Andruszczak, 2007). Independent of the date of leaf harvest, growing lovage from direct seeding was more profitable than cultivation from transplants, but the highest content of EO was achieved when the plants were propagated from direct sowing with leaf cutting in autumn. Higher content of EO was determined in leaves harvested from plantations established with seedlings in comparison to the one from late fall sowing, 0.890 and 0.755 mL/100 g fresh weight (fw), respectively (Rosłon et al., 2013). Cutting frequency did not have any systematic influence on yield and selected quality characteristics of lovage including the content of EO, which was in the range of 0.16–0.66 % (Rohricht, 2009). The effect of organic and chemical fertilizers on oil yield was studied in Egypt in 2008 and 2009 (Ezz El-Din and Hendawy, 2010). The yield from leaves based on dry weight was in the range of 0.13–0.27%, 0.15–0.29%, and 0.18–0.34% for the first, second, and third cuts, respectively, whereas for roots (second cut) it was 0.45–0.75%. In most cases application of fertilisers increased EO yield.

Chemical Composition of Lovage Essential Oils

EO content as well as its composition are the most important factors of lovage quality. Terpenes and phthalides are the main chemical classes of compounds dominating in lovage EOs; however, their concentrations in different anatomical parts of lovage may vary in a wide range (Table 1). For instance, remarkable variations in the oil composition were reported for *L. officinale* roots and leaves originated from different European countries (Raal et al., 2008). Early studies of lovage EOs were focused mainly on phthalides present in plant roots (Gijbels et al., 1980, 1982; Toulemonde et al., 1987; Segebrecht and Schilcher, 1989 and references herein). Application of powerful techniques of separation and identification of volatiles compounds, such as gas chromatography and mass spectrometry enabled to increase the number of identified compounds in lovage root, seed, and leaf oils up to 192 (Toulemonde and Noleau, 1988). Afterward the list of identified compounds was supplemented by some newly identified minor constituents and now exceeds 200 (Bylaitė et al., 1998; Raal et al., 2008). For instance, six odorants, namely 3-hydroxy-4,5-dimethyl-3(5H)-furanone (sotolon), *E*-β-damascenone, 2-ethyl-4-hydroxy-5methyl-3-(2H)-furanone, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-methylbutanoic acid, and acetic acid, having high sensory relevance were characterized in the commercial lovage extract (Blank and Schieberle, 1993; Blank et al., 1993). Sotolon was found to be responsible for the seasoning-like note of lovage extract.

TABLE 1 Essential Oil Content (%) and Percentage of Its Main Constituents in Different Anatomical Parts of Lovage

Reference	Plant Part	Essential Oil	α-Pinene	Myrcene	β-Pinene	β-Phellandrene	Limonene	*Z*-β-Ocimene	γ-Terpinene	α-Terpinyl acetate	*Z*-Ligustilide	*E*-Ligustilide
Toulemonde and Noleau (1988)	R		2.06	0.43	2.89	3.81	0.53	0.53	0.29	0.11	67.50	1.31
Toulemonde and Noleau (1998)	S		1.94		0.51	63.15	3.06	9.20	0.10	3.07	5.60	0.30
Perineau et al. (1992) (depending on solvent)	R	0.47–0.74	0.65	0.45	2.05	0.07	0.18	0.22		0.22	61.79	0.55
Cu et al. (1990)	R	0.15–0.39	0.18–2.67	0.03–0.85	1.08–4.32	0.11–1.33	0.13–1.03	0.08–0.22	0.07–0.87	t-3.76	40.15–63.87	0.22–0.67
Szebeni-Galambosi and Galambosi (1992) (October 10, 1985–September 2, 1986, (from different growing sites)	R	0.12–1.36	2.08–7.87	0.30–0.77	2.48–8.69	1.66–15.46	0.49–1.82	t–0.48			39.62–62.47	
Szebeni-Galambosi and Galambosi (1992) (October 10, 1985–September 2, 1986, (from different growing sites)	L	0.16–0.31	t–0.97	3.88–4.75	t–0.34	16.17–25.31	1.02–1.43	2.65–7.58	t-0.95	58.56–64.43	t–3.19	
Sertel et al. (2011)	L		1.52	5.07	0.41	13.16				48.15	3.86	
Samiee et al. (2006) (hydrodistilled oil and methanol extract)	A	3.2	0.6	2.3	0.5	16.7		4.5	7.8	40.5		
Samiee et al. (2006) (hydrodsitilled oil and methanol extract)	A		0.8	1.7	0.6	23.0		6.1	12.1	–		
Hogg et al. (2001)	R			0.3–0.9		1.7–15.5				0.1–0.2	37.0–67.5	
Ezz El-Din and Hendawy (2010)	L	0.13–0.34	0.8–1.2	2.3–5.02	1.1–1.8	9.6–18.3	0.0–1.8			59.8–66.5		
Ezz El-Din and Hendawy (2010)	R	0.45–0.75	0.3–1.7	0.4–1.3	0.5–4.3	6.4–16.5	1.4–2.3				22.8–33.6	0–0.8
Bylaitė et al. (1998) (May 15–September 21)	L	1.01–1.35	0.37–0.77	1.60–4.44	t–0.20	13.43–21.37	1.90–4.65	0.18–1.64	0.23–1.58	49.7–70.0	4.44–11.7	0.14–0.39
Bylaitė et al. (1998) (May 15–September 21)	St	0.53–1.16	0.76–1.25	1.3–3.34	0.16–0.77	10.85–19.21	2.04–5.38	0.13–1.28	0.16–0.83	48.2–68.97	4.8–13.95	0.13–0.47
Bylaitė et al. (1998) (May 15–September 21)	Sd	2.7	1.47	2.19	0.35	61.50	3.16	0.54	0.39	4.56	14.18	0.23
Bylaitė et al. (1998) (May 15–September 21)	F	1.53	1.01	7.12	0.31	40.82	2.25	2.50	1.87	16.27	16.01	0.27

Santos et al. (2005) (v/fw/dw)	R	0.16/0.60	7.8	1.3	9.3	4.6	1.4	0.4	0.4		36.8	1.2
Raal et al. (2008)	R		0–1.17	t–1.6	0–1.6	0.4–48.9			0–0.1	0–26.1	9.4–70.9	0–0.5
Raal et al. (2008)	L		0.6	1.4	0.1	11.3			0.2	55.8	17.0	2.5
Heidarpour et al. (2013)	H	~0.4[a]	0.1	1.1	–	12.9				52.4	29.7	0.4
Heidarpour et al. (2013)	F	~0.7[a]	0.1	2.7	Tr	26.6		1.6		10.4	53.4	3.5
Heidarpour et al. (2013)	Sd	~1.9[a]	0.5	1.5	Tr	47.8		0.1		3.6	38.5	4.2
Heidarpour et al. (2013)	Sd	~2.9[a]	2.9	3.3	0.9	34.4		0.5		4.2	35.1	6.7
Heidarpour et al. (2013)	R	~0.7[a]	0.9	0.1	1.3	2.3					79.7	0.3
Rosłon et al. (2013) (late fall sowing, seedlings planting, frozen, oven-dried, freeze-dried)	L	1.03–4.16	0.4–1.9	1.3–6.2	0.1–0.4	9.6–44.0	0.3–6.2	1.12–6.2	0.1–0.7	26.1–42.0	6.6–32.1	0.1–1.7
Moradalizadeh et al. (2012)	L		5.14			13.85		12.91	14.52	8.68		
Moradalizadeh et al. (2012)	St		5.30			7.21		10.42	12.86	8.52		
Moradalizadeh et al. (2012)	Sd		2.93			15.54		23.70	12.37	10.68		
Moradalizadeh et al. (2012)	R		3.78			6.95		8.89	12.56	6.15		
Dobreva et al. (2013) (control/pulse electric field treated)	S	0.60/1.00	1.45/1.91	1.16/1.33		79.32/83.21				2.11/1.27	7.95/5.81	
Mirjalili et al. (2010) (S: immature/mature/ripened)	F	0.1	5.3	1.3	17.7	11.7		1.9				
Mirjalili et al. (2010) (S: immature/mature/ripened)	S	1.5/1.0/0.6	4.6/4.3/2.9		11.5/4.1/2.9	56.4/62.4/60.5		0.8/0.8/0.8				
Kemzūraitė et al. (2014)	R	0.24	1.5		1.8	2.72				1.19	60.38	1.47
Kemzūraitė et al. (2014)	L	0.74		2.93		16.32				48.69	20.59	0.47
Menaker et al. (2004) (SFE CO_2)	H			2.3/0.1		12.4/0.1					7.6/5.9	
Daukšas et al. (2002) (SFE CO_2)	L					3.03				25.93	22.12	18.82
Daukšas et al. (2002) (SFE CO_2)	R					0.28				0.08	52.0	3.95
Daukšas et al. (2002) (SFE CO_2)	Sd					19.37				1.07	32.59	23.37

H, herb; L, leaves; St, stems; Sd, seeds; F, flowers; R, roots; A, Aerial parts; t, traces.
[a]*Determined approximately from the figure bars.*

Phthalides are the main chemical class of compounds in lovage root oil. To date 71 phthalides have been isolated from 40 species of the Apiaceae family, while 20 phthalides have been isolated from *L. officinale* roots and their content in the oil was 64–80% (European Medicines Agency, 2012 and references herein). Toulemonde et al. (1987) characterized 17 phthalides in commercial root EO; however, *Z*-ligustilide (3-[*Z*-butylidene]-4,5-dihydrophthalide, Figure 1), which constituted 67.5% was overhelmingly dominating compound. Other identified in this study phthalides were *Z*-3n-propylidenephthalide (0.22%), *Z*-3n-propylidene-4,5-dihydrophthalide (1.2%), 3n-butylphthalide (0.18%), 3n-butylidenephthalide (1.5%), sedanenolide (0.2%), *E*-3n-propylidenephthalide (0.67%), sedanolide (0.21%), 3n-valeryl-4,5-dihydrophthalide (0.15%), *E*-ligustilide (1.31%), *Z*-ternine (1.4%), *Z*-3n-validene-tetrahydrophthalide (<0.1%), and *E*-ternine (<0.10%). The content of *Z*-ligustilide in the until now published articles was found to vary from 37% to 83.9% (Table 1). The rest of the root oil is composed mainly of monoterpenes; however, the content of *Z*-3-butylidenephthalide may reach 29% as was found for the commercial samples from France (Raal et al., 2008). Hydrocarbon monoterpene β-phellandrene was reported in root oil up to >15% (Hogg et al., 2001; Szebeni-Galambosi and Galambosi, 1992); however, in the majority of studied oils its content was less than 5%. Other commonly found monoterpenes are α-pinene, myrcene, β-pinene, limonene, *cis*-β-ocimene, γ-terpinene, and α-terpinyl acetate. However, in some chemotypes of lovage phthalides were not detected neither in root nor in other anatomical parts; in this case monoterpenes were dominating constituents (Moradalizadeh et al., 2012). Several studies reported high amount of pentyl-cyclohexadiene in root oil, for example, 7.50% (Toulemonde and Noleau, 1988), 12.15–29.29 % (Szebeni-Galambosi and Galambosi, 1992), 9.1% (Santos et al., 2005), 0–12.3 % (Raal et al., 2008), 7.4–29.3 % (Hogg et al., 2001), and 7.9% (Heidarpour et al., 2013). In addition, 7% of *p*-allylanisole (Heidarpour et al., 2013) and 7.65% of methyl eugenol (Moradalizadeh et al., 2012) were reported in the oil from Iran, 17.2% of phenyl acetaldehyde in the oil from Scotland, 12.3% of *E*-sabinyl acetate in the oil from Holland (Raal et al., 2008), and 22.6–32.6 % of falcarinol in the oil from Egypt (Ezz El-Din and Hendawy, 2010).

Above-ground parts of lovage consist of leaves, stems, flowers, and seeds. In the majority reports on seed oil, β-phellandrene was reported as a major constituent, usually comprising more than 50% (Table 1) and in some cases reaching 83% (Dobreva et al., 2013). The content of *Z*-ligustilide in seed oil was reported in a wide range, from undetectable levels to 38.5%, which is remarkably lower than in root oil. However, the content of *Z*-ligustilide in SFE–CO_2 extract of seeds was reported higher than that of β-phellandrene (Daukšas et al., 2002). The seeds from lovage of Iranian origin contained an exceptionally low content of β-phellandrene (15.54%); instead it contained high percentages of *cis*-β-ocimene (23.7%), γ-terpinene (12.37%), and α-terpinyl acetate (10.68%) (Moradalizadeh et al., 2012). The composition of seed EO depends on the degree of seed ripeness (Heidarpour et al., 2013; Mirjalili et al., 2010).

EOs from lovage leaves, stems, and flowers were studied during plant vegetation period, from May 21 to September 21 (Bylaitė et al., 1998). Two major components were common for all studied lovage organs, namely β-phellandrene and α-terpinyl acetate (Figure 1). However, the differences in the ratio and seasonal variations in the content of these components in individual parts of the plant were quite considerable (Table 1). A monoterpene ester, α-terpinyl acetate, was the major constituent in the EOs from stems and leaves harvested in Lithuania at all harvesting periods: it constituted 48.20–68.97% and 49.70–70%, respectively. The highest content of this compound (70%) was determined in the leaves collected during the first harvesting in May 15; in the stems it was also high at this vegetation phase and exceeded 65% in the period from May 15 till June 9. Some reduction in the percentage and absolute concentration of α-terpinyl acetate in the leaves and stems can be observed during the flowering period of the plants. In the flowers it constituted only 16.27% (July 7).

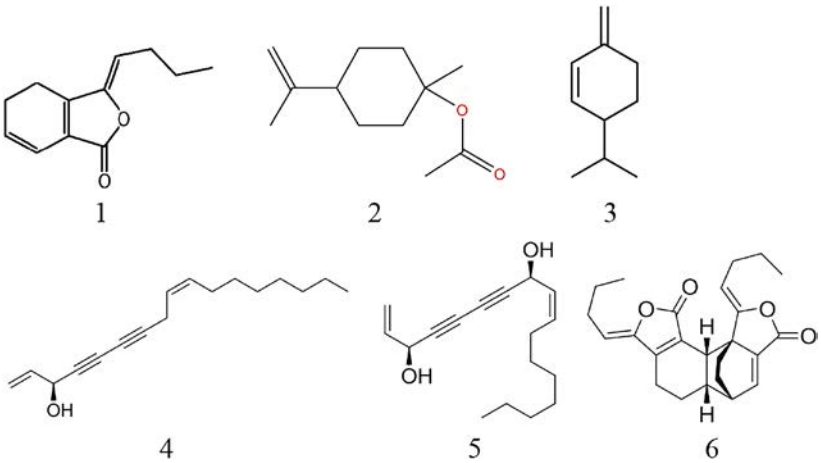

FIGURE 1 Structures of the main lovage essential oil constituents. *Z*-ligustilide (1), α-terpinyl acetate (2), β-phellandrene (3), pesticide falcarinol (4), and active antimicrobials falcarindiol (4) and levistolide A (5).

The content of the major monoterpene hydrocarbon β-phellandrene varied from 13.43% to 21.37% of the total leaf oil and from 10.85% to 19.21% in stem oil. It is worth noting that it increased during flowering both in leaves (5th harvesting, June 28) and stems (6th harvesting, July 7), while during this period the content of α-terpinyl acetate decreased. β-Phellandrene was also a major constituent in flowers (40.82%) and was described as an odor active compound of lovage possessing a grassy, chemical odor (Bylaitė et al., 1996). The contents of α-terpinyl acetate and β-phellandrene were almost similar in lovage leaf oil from Hungary; in fresh/dried one-year-old leaves β-phellandrene and α-terpinyl acetate constituted 30.7–47.2/32.2–37.5% and 33.8–47.2/32.5–41.9%, respectively, in two-year-old leaves 32.2–41.4/25.7–33.7% and 40.9–42.5/42.0–51.8%, respectively (Novák and Németh, 2002). Remarkable effects of plantation establishment and raw material stabilization on the EO composition of lovage leaves were determined. The content of β-phellandrene, α-terpinyl acetate, and *Z*-ligustilide depending on late fall sowing, seedlings planting, and stabilization (freezing, freeze- and oven drying) were in the ranges of 9.6–44.0%, 26.1–42.0% and 6.6–32.1%, respectively. It is interesting that other hydrodistilled EOs from lovage leaves contained lower amounts of *Z*-ligustilide; however, Heidarpour et al. (2013) reported the content of *Z*-ligustilide in lovage herb (full vegetative growth) and flowers (all inflorescence opened) was 29.7% and 53.4%, respectively. On the contrary, Moradalizadeh et al. (2012) reported comparatively even distribution of many monoterpenes and the absence of phthalides. For instance, besides commonly found major constituents in lovage leaves and stems (Table 1), they reported high content of γ-terpinene (14.52, 12.86%), α-terpinene (4.35, 3.11%), *p*-cymene (4.29, 5.02%), isopentyl-2-methyl butanoate (4.12, 4.81%), terpinen-4-ol (3.73, 4.60%), and α-terpineol (5.93, 10.81%). Rather controversial results were reported for aerial parts of lovage from the Hezar mountains in Iran: the contents of major hydrocarbon monoterpenes were comparable both for hydrodistilled oil and methanol extract, however, α-terpinyl acetate being major constituent in the oil (40.5%) was not detected in the extract. Instead, 20.6% of naphthalene was found in the extract (Samiee et al., 2006). The extracts isolated by SFE–CO_2 found, in addition to the components usually found in the distilled oils, less volatile compounds such as palmitic acid (2.81%), phytol (2.62%), linoleic acid (3.52%), stigmasterol (11%), and β-sitosterol (1.28%) (Daukšas et al., 2002).

Studies of the influence of cutting frequency on EO composition did not reveal any systematic effects although the contents of β-phellandrene (3.0–18.5%), α-terpinyl acetate (40.8–65.7%), *Z*- and *E*-butylidenphthalide (1.37–8.87%), and *Z*-und *E*-ligustilide (10.9–34.1%) were in a wide range; however, it was observed that as a result of frequent cuttings the ratio of α-terpinyl acetate decreases and the ratio of ligustilide increases (Rohricht, 2009).

There were attempts to produce lovage volatile compounds from hairy root cultures; however, this method resulted in products containing low amounts of volatile monoterpenes (trace(t)–0.9%), oxygenated monoterpenes (t–6.1%), and phthalides (t–17%). Polyacetilenes (1.4–75%), fatty acids (t–37%), and saturated and unsaturated aldehydes were dominating constituents depending on cultivation media and conditions (Costa et al., 2008). In another study, the main components of the oil samples from the hairy roots grown in four different types of liquid culture media were falcarinol (1.3–79.7%), *Z*-ligustilide (0.5–35.6%), *Z*-3-butylidenephthalide (0.7–19.2%), *E*-β-farnesene (t–15.4%), β-phellandrene (t–12.8%), *n*-octanal (0.5–10.4%), γ-elemene (t–5.8%), and *n*-heptanal (t–8.1%) in varying amounts depending on the culture media tested; however, EO yields from cultivated products were extremely low, from 0.006% to 0.018% (v/fw) (Santos et al., 2005).

Activities of Lovage Essential Oils and Their Individual Constituents

Bioactivities of herbal preparations depend on the composition and concentration of numerous compounds, mainly secondary metabolism phytochemicals biosynthesized by different pathways. The major lovage oil constituents, terpenes and phthalides, are also accumulated in many other plants and their activities were reported in numerous studies, which were surveyed in review by Lang and Buchbauer (2012). Therefore, although the studies on the activities of lovage oils and extracts are not so abundant compared with many other popular medicinal, aromatic, and spicy plants, their biological effects may be preliminary predicted from the available compositional information. For instance, in using the Thomson Reuters Web of Science database (accessed on April 09, 2014) keywords representing the main lovage oil constituents, "ligustilide" resulted in 222 records (eight of them with *L. officinale*), "terpinyl acetate" and "terpineol acetate" gave 422 records (eight of them with *L. officinale*), and β-phellandrene gave 524 records (seven of them with *L. officinale*). The medicinal bioactivities of lovage extracts and EOs as well as their components were tested both in vitro (inhibition of pancreatic lipase and 5-lipoxygenase product synthesis, TNF-α, spasmolytic, antiproliferative, and GABAergic activity, inhibition of human head and neck squamous carcinoma cells) and in vivo (diuretic, estrogenic, analgesic, neuroprotective, antiproliferative activity); these activities were reviewed elsewhere (European Medicines Agency, 2012). For instance, several studies reported positive health effects of *Z*-ligustilide such as antiinflammatory (Ma and Bai, 2012, 2013), antitumor (Long et al., 2012; Su et al., 2013), and antithrombotic (Zhang et al., 2009) activity and a positive effect against neurological disorders (Feng et al., 2012; Qi et al., 2012). However, these studies were performed with *Z*-ligustilide containing *Radix Angelica sinensis*. Lovage oil was also reported to cause contact dermatitis (Lapeere et al., 2013).

The reports on the evaluation of antimicrobial activity of hydrodistilled lovage oils are rather scarce. Commercial lovage EO was among the 10 oils that displayed the greatest inhibitory properties (Table 2) against 20 of the 25 tested Gram-positive and Gram-negative bacteria (Deans and Ritchie, 1987). Only *Alcaligenes faecalis* and *Clostridium sporogenes* were not inhibited by the pure oil nor by its dilutions. Unfortunately, the authors did not determine lovage oil composition used in antimicrobial tests. A study reported the antibacterial activity of *L. officinale* oils isolated from the seeds at different fruiting stages against seven pathogenic bacteria (Table 2). It was shown that the oils from mature and ripened fruits, consisting mainly of monoterpene hydrocarbons (79.2% and 75.2%, respectively), exhibited potent antibacterial activity

TABLE 2 Antimicrobial Activity of Lovage Essential Oil and its Dilutions Against Various Bacteria Species Expressed in Culture Inhibition Zones (mm)

	Dilution					
Bacteria	Undiluted	1:2	1:5	1:10	MIC[a]	Ampicilin
Acinetobacter calcoacetica	17.0	9.0	8.5	7.0		
Aerornonas hydrophila	13.0	10.5	9.0	8.0		
Alcaligenes faecalis	0	0	0	0		
Bacillus subtilis	8.0	8.5	0	0	0.9–3.8	14.0±0.7
Beneckea natriegens	0	0	6.0	5.0		
Brevibacterium linens	0	11.0	9.5	8.5		
Brocothrix thermosphacta	0	6.5	0	5.5		
Citrobacter freundii	0	7.0	5.0	6.0		
Clostridium sporogenes	0	0	0	0		
Enterobacter aerogenes	0	6.5	5.5	6.0		
Enterococcus faecalis					7.2–15.2	11.0±0.4
Erwinia carotovora	14.0	8.5	7.0	7.0		
Escherichia coli	9.0	0	5.0	0	0.9–1.9	12.0±0.6
Flavobacterium suaveolens	19.5	11.0	8.5	7.0		
Klebsiella pneumoniae	4.5	7.5	5.5	4.5	>14.4–>15.2	
Lactobacillus plantarum	0	6.0	6.0	4.5		
Leuconostoc cremoris	12.0	7.0	0	7.0		
Micrococcus luteus	8.5	9.5	0	6.0		
Moraxella sp	48.0	8.0	7.0	7.0		
Proteus vulgaris	5.0	7.5	7.5	6.0		
Pseudomonas aeruginosa	0	0	7.5	5.0	>14.4–>15.2	9.7±0.7
Salmonella pullorum	9.0	6.0	5.5	6.5		
Serratia marcescens	7.5	6.0	5.0	6.5		
Staphylococcus aureus	16.0	0	6.0	0	3.6–3.8	13.0±0.6
Staphylococcus epidermis	–	–	–	–	0.9–1.9	19.0±0.5
Streptococcus faecalis	6.0	7.0	5.0	0		
Yersinia enterocolitica	0	0	7.5	7.0		

[a]*Minimum inhibitory concentration, determined in agar by applying 10 μL of oil from immature, mature, and ripened seeds placed in culture wells.*
Adapted from Deans and Ritchie (1987) and Mirjalili et al. (2010).

against *Bacillus subtilis*, with minimum inhibitiory concentration (MIC) values of 0.90 mg/mL (Mirjalili et al., 2010). For comparison, MICs of EO of *Schinus molle* L. containing 35.8% and 29.3% of α- and β-phellandrenes, respectively, against *Escherichia coli* and *Enterococcus faecalis* were 9 mg/mL. Oil of *Elettaria cardamomum* containing 46% of α-terpinyl acetate possessed sporicidal activity (Lang and Buchbauer, 2012).

Several studies reported antimicrobial properties of lovage extracts isolated with organic solvents, which may also contain EO constituents. The lovage extract showed synergistic activity with antibiotics ciprofloxacin, tetracycline, chloramphenicol, erythromycin, and ethidium bromide against several *Salmonella typhimurium* isolates with innate efflux pump multidrug resistance AcrAB-TolC and was found the most active from 84 extracts isolated from 21 plants (Garvey et al., 2011). The extract was fractionated to active compounds falcarindiol, levistolide A (Figure 1), and oleic and linoleic acids. Bioassay screening with ciprofloxacin (0.5, 1, and 2 mg/L) in the absence or presence (100 mg/L) of lovage chloroform extract on agar plates demonstrated that a zone of inhibition of the ciprofloxacin plus plant extract larger than that of antibiotic alone was estimated as synergy. However, no synergism was observed with the fractions and purified substances, implying that a composition of active substances is needed for efflux inhibition. The dichloromethane extract of lovage root was tested in a microtiter plate dilution method against *Mycobacterium fortuitum* and *Mycobacterium aurum* with an MIC of 64 μg/mL. Further fractionation resulted in two active polyacetylenes: 3(R)-falcarinol and 3(R)-8(S)-falcarindiol with MICs against *M. fortuitum* of 30.4 and 16.4 μm, respectively, and against *M. aurum*, 60.8 and 16.4 μm, respectively. MICs of standard chemotherapeutics were as follows: ethambutol, 115.5 μm (*M. fortuitum*) and 14.6 μm (*M. aurum*); isoniazid, 3.4 μm (*M. fortuitum*) and 29.2 μm (*M. aurum*) (Schinkovitz et al., 2008).

Hadacek and Greger (2000) tested the activity of falcarindiol isolated from *Peucedanum cervaria* (L.) Lapeyr. by repeated chromatography on silica gel in various dilutions and diffusion bioassays against three selected plant filamentous microfungi, *Botrytis cinerea*, *Cladosporium herbarum*, and *Fusarium avenaceum*. MICs, defined as the lowest concentration/spot causing mycelium-free zones, were found as follows: for *Botrytis cinerea*, 25 μg/mL, for *Cladosporium herbarum*, 12 μg/mL, and for *F. avenaceum*, 50 μg/mL. Falcarindiol type polyacetylenes present in Apiaceae were identified as antifungal substances acting as prevention against infections (Garrod et al., 1979). They inhibited spore germination of different fungi at the concentration range of 20–200 μg/mL. It has also been shown that falcarindiol have antiinflammatory, antiplatelet, and cytotoxic activity (Christensen and Brandt, 2006). Acetylenes from Apiaceae were shown to be toxic to bacteria and fungi and play a role in resistance and protection of plants against infection. Kemp (1978) found a total inhibition of spore germination using falcarindiol against *Alternaria brassicicola* and *Septoria nodorum* at a concentration of 20 μg/mL; however, falcarinol even at a concentration of 200 μg/mL did not affect the fungi themselves. Falcarindiol has been identified as a phytoalexin in tomato fruits and leaves infected by fungi (Christensen and Brandt, 2006). Usually, falcarinols are found in distilled oils of lovage in minor concentrations; however, in the case of hydrodistilled oils of roots from Egypt the content of falcarinol was reported as high as 22.60–32.60% (Ezz El-Din and Hendawy, 2010). In SFE–CO_2 extracts, depending on the applied parameters it was 2.21–16.47% (Kemzūraitė et al., 2014). Falcarinol, which is also called a carotatoxin because it is found in carrots, is a natural pesticide protecting roots from fungal diseases, such as liquorice rot that causes black spots on the roots during storage.

CONCLUSION

Lovage has been used as a food, food flavoring, and medicinal plant since ancient times. Extensive studies of phytochemicals biosynthesized in different anatomical parts of lovage during last few decades resulted in the identification and quantification of several compounds possessing various bioactivities. However, the uses of lovage preparations are still restricted mainly to flavourings, while developments of broader applications require more systematic studies focused on antimicrobial, antifungal, and health beneficial activities of lovage oils and their valuable constituents. In conclusion, lovage may be considered as a promising plant in the future for development of functional ingredients for foods and other products.

SUMMARY POINTS

- Lovage has a long history and food and medicinal application.
- Lovage is used mainly as a food flavoring ingredient.
- Lovage oils contain several valuable phytochemicals.
- Lovage oils possess antimicrobial and other bioactivities.
- Lovage oil is a potential functional food ingredient.

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Chapter 63

Mexican Oregano (*Lippia berlandieri* and *Poliomintha longiflora*) Oils

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INTRODUCTION

The genus name *Origanum* comes from the Greek words *oros*, which means mountain, and *ganos*, meaning joy, referring to the beautiful aspect that this plant gives to the regions where it is cultivated (Fonnegra and Jiménez, 2007). The *Origanum* genus comprises different plant species, with flowers and leaves that have a characteristic odor and flavor. Based on morphological criteria, the genus *Origanum* has been classified into three groups, 10 sections, 38 species, 6 subspecies, and 17 hybrids. Four groups of oregano have been named, due to their common use and economic importance: Greek oregano (*Origanum vulgare* subsp *hirtus* (Link) letswaart), Spanish oregano (*Coridohymus capitatu* (L) Hoffmanns and Links), Turkish oregano (*Origanum onites* L), and Mexican oregano (*Lippia graveolens* Kunth or *Lippia berlandieri*) (Arcila-Lozano et al., 2004). These major types of oregano have commercial value because of their odor/flavor characteristics that are attributed to the presence of compounds such as thymol, carvacrol, and its precursor *p*-cymene. Table 1 displays three of the oreganos with the largest worldwide export figures (Turkish, Mexican, and Greek) and the relationship between the concentration of essential oil of oregano from their leaves and the proportion found of their major components. Furthermore, Table 1 presents the characteristics of another Mexican oregano, *Poliomintha longiflora*, which is also an aromatic species that grows in the wild and has been seldom studied. An important remark is that *P. longiflora* and two species of European oreganos belong to the family Lamiaceae and are oregano species containing higher proportion of thymol, while oreganos from the family Verbenaceae (*Lippia*) contain a higher percentage of carvacrol, which accounts for their differences in color, odor, and flavor. The European consumer prefers a mild fruity flavor with light-colored tones due to their oreganos major component thymol (white crystallizable component) while Mexican consumers tolerate strong flavors due to the higher concentration of carvacrol (yellow liquid component) in their oreganos.

BOTANICAL ASPECTS

Mexican oregano is recognized as the aromatic plants of three different species: *L. graveolens* (or *L. berlandieri*), *P. longiflora*, and *Monarda fistulosa* var. *menthifolia* (Brown et al., 2006). The genus *Lippia* is the major commercial cultivar (*L. graveolens* HBK or *L. berlandieri* Schauer: belonging to the family Verbenaceae and very different from of its European counterpart), which can be described as aromatic shrubs with a height of 2.5 and 1.2 m of foliage (Huerta, 1997; Dunford and Silva, 2005). It contains branched stems with a large number of oval leaves that measure 1–3 cm long with serrated edges and dark green color, rough texture, with villi and small white flowers that form inflorescences in clusters. The smell and taste of this plant is strong and robust, described as "wild" (Huerta, 1997). In Mexico, the cultivated *Lippia* species are *Lippia palmeri Wats* in the states of Baja California, Sonora, and Sinaloa, and *L. graveolens* most widely distributed in the rest of Mexico (González et al., 2007).

Lippia graveolens oregano is the most commercialized species in Mexico due to the high quality of the oregano leaves' essential oil (Mendoza and Mora, 2005). Mexico is the second largest exporter to international markets with 31%, only behind Turkish oregano (65%). Mexico in 2005 cultivated 368.05 tons (Villavicencio et al., 2007). The Mexican oregano *L. graveolens* is exported to the international market, and represents approximately 35–40% of oregano in the world,

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TABLE 1 Chemical Composition of the Major Species of Oregano

Oregano Species	Family	Essential Oil (%)	Thymol (%)	Carvacrol (%)	*p*-cymene (%)
Mexican oregano *Lippia graveolens*	Verbenaceae	2.0	10.4	43.7	6.4
Mexican oregano *Poliomintha longiflora*	Lamiaceae	1.2	37.2	24.2	8.1
Greek oregano *Origanum vulgare* subsp *Hirtus*	Lamiaceae	1.5	23.9	12.2	15.9
Turkish oregano, *Origanum vulgare* subsp *gracie*	Lamiaceae	1.5	15.1	9.9	8.1

Adapted from Huerta (1997).

FIGURE 1 Morphology of dried leaves from Mexican oreganos: *Poliomintha longiflora* (left) and *Lippia berlandieri* (right).

its high demand is due to its essential oil and leaf quality; however, consumption of Mexican oregano is focused worldwide mainly on two countries outside, the USA and Japan, that account for 90% of the total exports (Brown et al., 2006; González et al., 2007).

Poliomintha longiflora oregano is botanically known also with the synonyms *Poliomintha madereises* or *Poliomintha bustamante*, it has been designated just like *L. graveolens*, as Mexican oregano. The genus name derives from the Greek words *polies*, slightly hoary or gray, and *mintha*, mint. This oregano is a native plant from northern Mexico. In contrast to the genus *Lippia*, *Poliomintha* belongs to the family Lamiaceae. The native habitat is central Coahuila state where it grows wild in thickets with evergreen foliage with a height of 50–72 cm; glossy leaves with smooth edges; slightly light green oval stalks, and tubular flowers, with spines that sprout in the summer, almost pale white to lavender, and the color changes according to maturation. The flowers contain five petals in a long corolla with a strong fragrance, generally referred to as "garden smell." It is widely used as a spice for flavor and aromatic ornamental use on the edges of the plates. *Poliomintha longiflora* species is also used as a substitute for European oregano (*O. vulgare*) in culinary applications. In Mexico, based on its chemical and pharmacological characteristics, its use is different from its counterpart *L. graveolens* (Shimizu, 2002; Arnold, 2008; Rivero-Cruz et al., 2011).

Morphological differences between the leaves and stems of *Lippia* and *Poliomintha* oregano are exhibited in Figure 1. The leaves size, color, and edges are the most remarkable differences. Because of this botanic difference in leaves, *P. longiflora* has earned the nickname of "smooth oregano" because in contrast to *Monarda* and *Lippia*, *Poliomintha* has leaves without toothed edges.

Monarda fistulosa var. *menthifolia*, belongs to the family Lamiaceae, is a perennial aromatic plant with stems, 1.20 m tall, with toothed leaves and purple flowers with a tubular shaped corolla, the stems present a large number of flowers in bunches. This plant is marketed as a fragrance and for flavoring foods and beverages, as well as a meat additive.

USAGE AND APPLICATIONS

Oregano has two main uses, as a condiment and as raw material for the production of essential oils, which is extracted from the leaves (Sánchez et al., 2007). The dry leaves of *O. vulgare*, native to Europe and *L. graveolens* plant native to Mexico are commonly used in culinary applications (Arcila-Lozano et al., 2004). Oregano essential oil is generally recognized as safe by the Food and Drug Administration and it has been utilized as a natural antimicrobial agent (Bertelli et al., 2003; Arcila-Lozano et al., 2004; Silva and Dunford, 2005). The composition and quantity of secondary metabolites present in essential oils obtained from different types of oregano depend on geographical and climatic factors, altitude, harvest time, and growth status (Arcila-Lozano et al., 2004). Oregano leaves and essential oils are widely used as ingredients and/or additives in food, chemical pharmaceutical, and perfumery industries; reasons that have made them very important export products. Thymol and carvacrol are the most important constituents of oregano essential oils, and therefore its proportion determines the commercial value of the corresponding essential oil (Sánchez et al., 2007).

CHEMICAL COMPOSITION OF MEXICAN OREGANO EXTRACTS

Because Mexican oreganos are aromatic plants of important economic value, various extracts have been analyzed to determine their chemical composition and relate them to their biological activity and/or organoleptic characteristics when added, mainly to impart flavor, to various foods.

Oregano essential oils are the extracts with the most economic value for farmers and companies that engage in the cultivation/distribution of spices. Main compounds of oregano essential oils derived from mevalonic acid and classified as terpenoids, are derived from the condensation of isoprenoid units and categorized according to the number of units present in the skeleton (monoterpenes, sesquiterpenes, and diterpenes). However, a wide variety of low molecular weight molecules such as aliphatic hydrocarbons (linear, branched, saturated, and unsaturated), acids, alcohols, aldehydes, esters, and/or acyclic lactones are also present (Dorman and Deans, 2000).

Quantitative differences have been determined among the different species of oregano around the world, especially regarding the two isomeric phenols, carvacrol, and thymol (Ultee et al., 2000). Both volatile compounds exhibit an intense pungent odor, responsible for the characteristic fragrance and sensations of flavor of oregano extracts and essential oils (Mastelić et al., 2008). Carvacrol is a flavor agent; its use is limited to certain products, such as soft drinks (28.54 ppm), soft candy (21.43 ppm), chewing gum (8.42 ppm), and bakery products (15.75 ppm) (Ultee et al., 2000). However, due to its poor solubility in water and strong flavor that may impart, in some cases, the efficiency of these compounds is limited when used as antimicrobials, antioxidants, or preserving additives. These applications are limited due to the high concentrations required (Mastelić et al., 2008). The preponderance of carvacrol (phenol noncrystallizable) or thymol (crystallizable phenol) is responsible for the commercial classification of oregano essential oils.

Table 2 exhibits the 72 chemical compounds determined in Mexican oregano leaves. Volatile components were extracted by distillation (essential oils) or by micro-extraction techniques using microfibers in the headspace, and characterized by gas chromatography–mass spectrometry. For both oregano species (*Lippia* and *Poliomintha*) the determined terpenes are the precursors *p*-cymene, terpinene, and pinene; regarding the sesquiterpenes, oxygenated and nonoxygenated caryophyllene was determined, while regarding the position isomers the presence of carvacrol in both species was determined; however, in this single characterization of *Poliomintha*, thymol was not present or in a trace proportion. It is important to state that the chemical characterizations only show the percentage ratio (area under the curve) with respect to the total content of volatile components, it is only a qualitative chemical characterization that determines the chemical profile and the proportion in which each component might be found (regardless of molecular weight, mass, or charge). This qualitative characterization does not confirm the concentration of each of the compounds (quantitative characterization).

Other extracts, called hydrosols, also known as aromatic floral water, are generally obtained as a coproduct or byproduct of hydrodistillation or steam distillation. Hydrosols are mostly a mixture of compounds that contain traces of essential oil and various water-soluble compounds (Sagdic, 2003).

Various studies on the chemical composition of oregano using aqueous extracts and extracts with different polarity solvents, have identified flavonoids such as apigenin and luteolin, aglycones, aliphatic alcohols, terpene compounds, and phenylpropane derivatives (Arcila-Lozano et al., 2004). As displayed in Table 3, 29 chemical components were elucidated as nonvolatile extracts or essential oil free extracts of Mexican oregano by different researchers. Table 3 illustrates that not only volatile components, specifically essential oils, are directly responsible for the sensory or phytochemical characteristics of oregano leaves. When using the aromatic spice, dried or fresh, there is a loss and deterioration of a large number of chemical components, volatile, semivolatile, and nonvolatile, as discussed below.

TABLE 2 Volatile Chemical Components of Mexican Oregano *Lippia berlandier* (or *Lippia graveolens*) and *Poliomintha longiflora*

Compound Name	Oregano Species	Characterization Reported by
1-Acetoxy-4-ethylbenzene	2, 3	V
α-Alasken-8-ol	3	V
α-Bergamotene	2	II
Bicicloelemene	3	V
γ-Bisabolene	3	V
Borneol	1	I
2,3-Butanediol	1	I
α-Cadiene	3	V
α-Cadinol	2	V
α-Calacorene	3	V
Camphene	2, 3	V
Carvacrol	1, 2, 3	I, II, III, IV, and V
Carvacrol acetate	1, 2	V
β-Caryophyllene	1, 2, 3	I, IV, and V
β-Caryophyllene oxide	1, 2, 3	IV and V
1-8-Cineole	1	III and IV
Cyclohexenal,4-(1-methylethenyl)	3	V
cyclohexen,4-ethenyl-1,4-dimethyl	3	V
m-Cymene	2, 3	V
p-Cymene	1, 2, 3	I, II, III, IV, and V
β-Damascone	3	V
3,4-Dimethoxystirene	3	V
D-Elemene	3	V
Eucalyptol	2,3	V
Eugenol	2, 3	V
β-Farnesene	2, 3	V
α-Fenchol	3	V
Germacrene A	3	V
Germacrene D	2, 3	V
3-Heptanone	1	I
3-Tert-Butyl-4-hydroxyanisole	1	IV
α-Humulene	1, 2	II and IV
Humulene oxide	2	V
α-Ionene	3	V
p-Isopropylbenzylic alcohol	3	V
Jasmone	3	V
Limonene	1	I

TABLE 2 Volatile Chemical Components of Mexican Oregano *Lippia berlandier* (or *Lippia graveolens*) and *Poliomintha longiflora*—cont'd

Compound Name	Oregano Species	Characterization Reported by
Linalool	1	I
Methyldecailin-2,7-dione	3	V
6-Methyl-3,4-Xylenol	3	V
α-Myrecene	2, 3	V
β-Myrecene	1, 2	I, II, and IV
γ-Octanolide	2, 3	V
3-Octanone	1	I
1-Octen-3-ol	1	I
β-Ocymene	3	V
Opposita-4,15-(11)-diene	3	V
α-Phellandrene	2, 3	II and V
β-Phellandrene	1, 3	I and V
α-Pinene	1, 2,3	I, II, and V
β-Pinene	2, 3	V
Piperitenone	3	V
Piperitol	3	V
Sabinene	2, 3	V
Sabinene hydratade	3	V
Spathulenol	2	V
3-Tert-butyl-4-hydroxyanisole	1	IV
4-Tert-butylchatecol	1	IV
α-Terpinene	1, 2, 3	I, II, IV, and V
γ-Terpinene	1, 2, 3	I, III, IV, and V
α-Terpineol	1, 2	I, IV, and V
Terpinen-1-ol	1	I
α-Terpinolene	1	I
α-Thujene	2, 3	V
β-Thujene	1	I
Tricylene	3	V
3,3,6-Trimethyl-1,5-heptadien-2-one	3	V
Thymol	1, 2	I, II, III, IV, and V
Thymolmethylether	2, 3	V
m-Xylene	1	I
o-Xylene	1	I
p-Xylene	1	I

Oregano species:1, *Lippia berlandieri*; 2, *Lippia graveolens*; and 3, *Poliomintha longiflora*.
Characterization reported by: I, Yousif et al. (2000); II, Castillo-Herrera et al. (2007); III, Avila-Sosa et al. (2010); IV, Gomez-Sanchez et al. (2011); and V, Rivero-Cruz et al. (2011).

TABLE 3 Nonvolatile Chemical Components of Mexican Oregano *Lippia berlandieri* (or *Lippia graveolens*) and *Poliomintha longiflora*

Compound Name	Oregano Species	Characterization Reported by
Apigenin 7-O-glucoside	2	IV
Caffeic acid	3	I
Cirsimarin	2	III and IV
Eriodictyol	2	IV
Galangin	2	IV
Hispidulin	2, 3	I and IV
3-Hydroxyphloretin 6-O-hexoside	2	IV
6-Hydroxyluteolin	2	IV
6-Hydroxyluteolin 7-O-hexoside	2	IV
6-Hydroxyluteolin 7-O-rhamnoside	2	IV
Isokaempferol	2	III
Kaempferol	2	III
Luteolin	2	I and IV
Luteolin 7-O-glucoside	2	IV
Methylgalangin	2	IV
Naringenin	2	III, IV, and V
Pentaflavanone-B hexoside-1	2	IV
Pentaflavanone-B hexoside-2	2	IV
Pentahydroxyflavanone-A hexoside	2	IV
Phloridzin	2	IV
Pilosina	2	III
Pinocembrin	2	II and IV
Quercetin	2	IV
Rosmarinic acid	2, 3	I and V
Sakuranetin	2	IV
Scutallarein 7-O-hexoside	2	IV
Scutellarein	2	IV

Oregano Species: 1, *Lippia berlandieri*; 2, *Lippia graveolens*; and 3, *Poliomintha longiflora*.
Characterization by: I, Zheng and Wang (2001); II, Brown et al. (2006); III, González et al. (2007); IV, Lin et al. (2007); and V, Martinez-Rocha et al. (2008).

Rivero-Cruz et al. (2011) reported chemical differences in essential oils between the most important Mexican oregano species, *Lippia* and *Poliomintha*. In *Lippia*, 25 chemical components were determined among which carvacrol, eucalyptol, thymol, and *p*-cymene were predominant. While in *P. longiflora*, a greater number of chemical components were determined, being carvacrol, *p*-cymene, carvacrol acetate, 6-methyl-3, 4-xylenol, and β-caryophyllene oxide as major components. However, the authors did not detect the presence of thymol in oregano essential oil of *P. longiflora*. Another important difference was the extraction yield, *Lippia* exhibited a yield of 1.67%, higher than *Poliomintha* with 0.33% of essential oil yield from corresponding oregano leaves. Furthermore, volatile components were also extracted using microfibers in the headspace, for *Lippia* only 18 chemical compounds were determined, of which seven compounds were not determined in the profile of the essential oil (camphor, terpinen-4-ol, aromadendrene, carvacrol methyl ether, germacrene

A, longipinene, and sabinene hydrate). While in *Poliomintha*, 26 components were identified, six of which were not identified in the essential oil obtained by distillation (2-carene, limonene, terpinen-4-ol carvacrol methyl ether, bornyl acetate, and aromadendrene).

Yousif et al. (2000) were able to characterize 24 volatile components from fresh oregano leaves from *L. berlandieri* Schauer by the headspace technique, chemical composition main components were β–myrcene, α–terpinene, γ-terpinene, *p*-cymene, and thymol, while in a lower proportion carvacrol was also elucidated. They determined that the drying method is important when determining these plants' volatile compounds because removing water might decrease the concentration of some chemical components. This is the case of air drying that affected the composition and coloring of oregano leaves, followed by vacuum microwave drying, which affected only an amount of terpene precursors without affecting the presence of thymol as its concentration was similar to that found in fresh leaves. Freeze-drying was the best drying technique because reduction of the major compounds (β-myrcene, α–terpinene, γ-terpinene, and thymol) were minimal when compared with fresh oregano.

Lin et al. (2007) determined the presence of flavonoids (flavones, flavanones, flavonols, and chalcones) from a methanol–water extract of *L. graveolens* oregano obtaining glycosylated flavonoids and by subsequent acid hydrolysis determined 11 additional aglycones. Structural analysis was performed by liquid chromatography-diode array detection electrospray ionization/mass spectrometry elucidating galangin, pinocembrin, sakuranetin, naringenin, quercetin, luteolin, eriodictyol, scutellarein, phloridzin, taxifolin, hispidulin, cirsimarin, 6-hydroxyluteolin, glycosylated methylgalagin, and nine other compounds. Thus this Mexican oregano leaves are from an aromatic plant with a lot of flavonoids, compounds whose structure has been linked to antioxidant capacity.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Antimicrobial Activity of Mexican Oregano

The search for less processed or organic foods as well as natural additives and preservatives in the food industry has placed Mexican oregano as a plant that has called the attention of various research groups in these search for bioactive compounds. This is the case of the work performed by Paredes-Aguilar et al. (2007), whom determined the antimicrobial effect of *L. berlandieri* powdered oregano leaves and different fractions of essential oil (various concentrations of the major isomer), to inhibit the growth of five strains of *Vibrio* (*Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio mimicus*, *Vibrio parahaemolyticus*, or *Vibrio vulnificus*). *Lippia berlandieri* oregano leaves were able to inhibit and/or inactivate the studied strains of *Vibrio* (with minimum inhibitory concentrations, MICs, in the range of 1.5–2.0% and minimum bactericidal concentrations, MBCs, of 2.0–2.5%). While the essential oil fractions, inhibited and/or inactivated studied bacteria with lower concentrations (MIC of 100–200 mg/L and MBC of 100–200 mg/L). These results were corroborated with death kinetics performed at 5 and 35 °C, determining that this Mexican oregano is an effective antimicrobial agent. The authors reported that using the fraction with the higher percentage of thymol (64% with respect to carvacrol, 26%), tested strains were inactivated with a concentration of 100 mg/L, reducing 7 log cycles of the initial population in 30 min. Portillo-Ruiz et al. (2012) evaluated different fractions of *L. berlandieri* essential oil. Thymol (within the range of 3–64%) and carvacrol (ranging from 23 to 81%) concentration differences were related with antifungal activities against *Rhizopus* (MIC 150 mg/kg), *Aspergillus* (MIC 150 mg/kg), or *Penicillium* (MIC 200 mg/kg) in a wheat flour-based agar. Position of the hydroxyl group in the isomers (thymol and carvacrol) seemed not to influence their antimicrobial activity. A direct relationship between oregano essential oil concentration and antifungal activity was determined regardless of which of the two isomers was at higher concentration; antimicrobial activity increased with increasing concentration of essential oil in the culture medium. Tested in vitro wheat flour medium, could help describe the effect of tested essential oils in bakery products contaminated with studied molds (Portillo-Ruiz et al., 2012).

Aspergillus flavus growth response was evaluated by Gomez-Sanchez et al. (2011), exhibiting that *L. berlandieri* oregano essential oil applied in vapor phase at 25, 30, or 35 °C, was an effective antifungal agent to eliminate mold growth (minimum fungicidal concentration of 1.47 mL/L) on potato dextrose agar (a_w (water activity) 0.98 and pH 4). With higher concentrations of essential oil, greater inhibition of mold growth was observed. The mold lag phase was severely affected by decreasing the incubation temperature, the adaptation phase at 25 °C was almost a day (23.3 h) while adding 117.6 mg essential oil/L of air this lag phase was extended to 22.2 days to initiate mold growth. *Lippia berlandieri* Mexican oregano and especially its essential oil can be used as a biological agent, with antimicrobial and disinfectant properties. If utilized as a food additive, it not only reduces the growth of molds and bacteria, but may even improve or complement the flavor and color of many foods. However, it is important to emphasize that these foods should have to be compatible with the strong odor, color, and

also with water insolubility of oregano essential oils. It is widely recognized that the biological activity of Mexican oregano essential oils is attributable to the two major isomers due to its high partition coefficient and adherence of these components to the microbial lipid membranes, and various biochemical changes (change of gradient, pH, electrolyte changes, etc.) that the hydroxyl groups perform to enter the cell wall. The adaptation phase in the presence of these bioactive compounds is strongly affected, especially in the mold germination phase.

The use of natural additives in coatings or films has also been proposed, using essential oils an alternative for antimicrobial agents to be included during film formation. Avila-Sosa et al. (2010) reported the use of *L. berlandieri* essential oil as an antifungal agent in edible films from chitosan, amaranth, and starch, suggesting that the essential oil may increase the shelf life and inhibit the growth of *Aspergillus niger* and *Penicillium* spp, on the surface of various foods. The use of 0.50% of *L. berlandieri* Mexican oregano oil in starch edible films resulted in the inhibition of the two studied molds, while at the same concentration, in amaranth and chitosan films only inhibited one of the two molds. Amaranth and chitosan films needed 0.75% of tested essential oil to inhibit *Aspergillus* and *Penicillium*. Mexican oregano essential oils could help to increase product shelf life, reduce microbial load, and preserve natural, processed, and/or packaged foods.

Antioxidant Activity of Mexican Oregano

Pruneda et al. (2008) used nonpolar extracts from essential oil of *L. graveolens* Mexican oregano to show that extracts can act as antioxidants to capture free radicals. Several studies have determined that the free radical scavenging and metal reduction reactions are due to, in large measure, the major isomers present in Mexican oregano essential oils, and are directly linked to the use of essential oils and nonpolar extracts. However, the antioxidant activity also has a relationship with the chemical components, which are highly polar in Mexican oregano plants. Such is the case of methanol extracts; free of major components (thymol and carvacrol) of *L. graveolens* oregano from three regions of Mexico (Guanajuato, Puebla, and Queretaro states). The methanolic fractions displayed a high concentration of total phenols (211.81, 265.42, and 270.25 mg/g, respectively) and total flavonoids (136.1, 183.4, and 200 mg/g, respectively). Meanwhile, the chemical characterization determined that the antioxidant activity might be associated with the high concentration of rosmarinic acid and naringinin present in tested extracts (Martínez-Rocha et al., 2008). Similarly, *P. longiflora* extract characterization elucidated as a major component rosmarinic acid (124.8 mg/g), followed by luteolin (25.1 mg/g), hispidulin (10.8 mg/g), caffeic acid (8.1 mg/g), and vanillic acid (3.5 mg/g). In the aqueous extract a total phenolic compounds concentration of 17.51 mg/g was determined (Zhen and Wang, 2001). Rosmarinic and caffeic acids were determined in ethyl acetate and ethanol (free of essential oil) extracts with high total phenolic content (41.6 and 34.6 mg/g). Using polar solvents, a high concentration of total phenolics and/or flavonoids has been determined in fractions Mexican oregano extracts with a direct relation to displayed antioxidant activity. It is recognized that rosmarinic acid has a high antioxidant capacity that could be compared with antioxidants widely used by the food industry. Polar extracts of Mexican oreganos, not only are capable of capturing free radicals or reducing metals, but also because of its structure (hydroxyl group in the ortho position of the ring) may act as chelating agents.

Only leaves and flowers from oregano are used to obtain their essential oils by distillation. Therefore, there is a large amount of byproducts that are not utilized. For this reason, diverse researchers have taken the task of determining the existence of active compounds that can be found in unusable parts of the plant. González et al. (2007) determined the antioxidant activity of stems of *L. graveolens*. The ethyl acetate extract fraction presented components such as kaempferol, isokaempferide, pilosin, cirsimaritin, naringenin, and a catechin derivate. These chemical compounds were reported to have antioxidant activity. Lower antioxidant activity was reported for the extract fraction obtained with methanol, being a hexoside of quercetin and a large amount of glycones to whom this activity was directly attributed. Rocha-Guzmán et al. (2007) evaluated the antioxidant activity in "mother liquors" from *L. berlandieri* essential oil production. The liquor antioxidant activity was evaluated by capturing free radicals and by induced oxidation in low density lipoproteins with trapping hydroxyl radical by copper sulfate. A large amount of total phenols (150.5 gallic acid equivalents/mL) were reported, while the capture of free radicals showed that liquor in concentrations of 100 μg/mL had antioxidant activity, superior even to that of the essential oil of the same species of oregano, ascorbic acid, or butylated hydroxytoluene. Residues of Mexican oregano essential oils distillation contain chemical active compounds, different from the major oxygenated monoterpenes, which may have a large concentration of glycosylated components and a large amount of polyhydric phenols, with more antioxidant activity than other monohydroxilated compounds.

SUMMARY POINTS

- The plants known as Mexican oregano are *Lippia berlandieri* (or *Lippia graveolens*), *Poliomintha longiflora*, and *Monarda fistulosa*.
- Essential oils from Mexican oregano display quantitative differences regarding the two isomeric phenols, carvacrol, and thymol.
- Mexican oregano can be used as antimicrobial agents. Furthermore, Mexican oreganos enhance and complement the flavor and color of foods.
- Polar extracts from Mexican oreganos exhibit antioxidant activity. They are capable of capturing free radicals, reduced metals, and can act as chelating agents.
- Mexican oreganos leaves have bioactive components found in volatile (essential oils), semivolatile (hydrosols), and non-volatile (highly polar) fractions that can act as phytochemicals.

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Chapter 64

Mint (*Mentha spicata* L.) Oils

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List of Abbreviations

C_5H_8 Isoprene
CG/MS Gas chromatography/Mass spectrometry
CIPC chlorpropham isopropyl-*N*-chlorophenyl carbamate
DPPH Diphenylpicrylhydrazyl
FID Flame Ionization Detector
GC Gas chromatography
MGI Mycelial growth inhibition
t trace amount

INTRODUCTION

Natural products are related to the materials obtained from animal and plant kingdom, which are fine biochemical factories for the biosynthesis of primary and secondary metabolites (Figure 1). Essential oils include terpenoids, derived from isoprene (C_5H_8) units universally present in living organisms, biologically synthesized (Figure 2), and are classified according to the number of C_5H_8 units held together in various acyclic and cyclic forms. Essential oils find wide applications in the perfumery, pharmaceutical industry, food industries, etc. (Bhat et al., 2006).

The essential oils in the present scenario are used as natural food preservatives, antioxidants, for aroma, and are preferred over synthetic chemicals which have harmful effects. Essential oils also find their usage in protecting the food materials by inhibiting the growth of food-borne pathogens (Gupta et al., 2008). The present chapter describes various uses of mint essential oils as a natural food preservative, antioxidant, antifungal, and antibacterial agent.

BOTANICAL ASPECTS

Mint belong to the family Lamiaceae Lindl. (Labiatae Juss.) which is one of the highly diversified angiosperm family of the world. It contains 7852 species under 250 genera which are distributed worldwide but mostly in the Mediterranean, Irano–Turanian, and Eastern Asiatic regions (The Plant List, 2010; Takhtajan, 2009). Lamiaceae are characterized as herbs or shrubs, often aromatic with ethereal oils, with usually four-sided stems, opposite or whorled leaves, a verticillaster, or thyrse inflorescence (flower solitary and axillary in some), and zygomorphic (rarely actinomorphic), usually bilabiate flowers having a superior ovary, often deeply four-lobed by formation of false septum with a gynobasic style. The fruit is a schizocarp of usually four nutlets or a berry or drupe (Simpson, 2010). Lamiaceae is represented by 72 genera and 435 species in India and is ranked ninth among the largest families; with 60 genera and 225 species in West Himalaya; 42 genera and 110 species in Himachal Pradesh; 49 genera and 143 species in Uttarakhand state, and is ranked the seventh largest family (Karthikeyan, 2000; Rana et al., 1997; Uniyal et al., 2007). The family has clear affinity for cooler climate thus have higher diversity in the Himalaya in general and West Himalaya in particular. Uttarakhand state contains high diversity of Lamiaceae members as nearly one-third of the species of the whole of India are found in the state. The family Lamiaceae contains large number of economically important medicinal, culinary herbs, fragrance plants, food plants, and a large number of cultivated ornamental plants. Mint (*Mentha* L. species) is one of the important economic plants of the family which are aromatic and used variously. The genus *Mentha* L. contains around 38 accepted species worldwide (The Plant List, 2010) mainly concentrated in the Europe and West Asia (Table 1).

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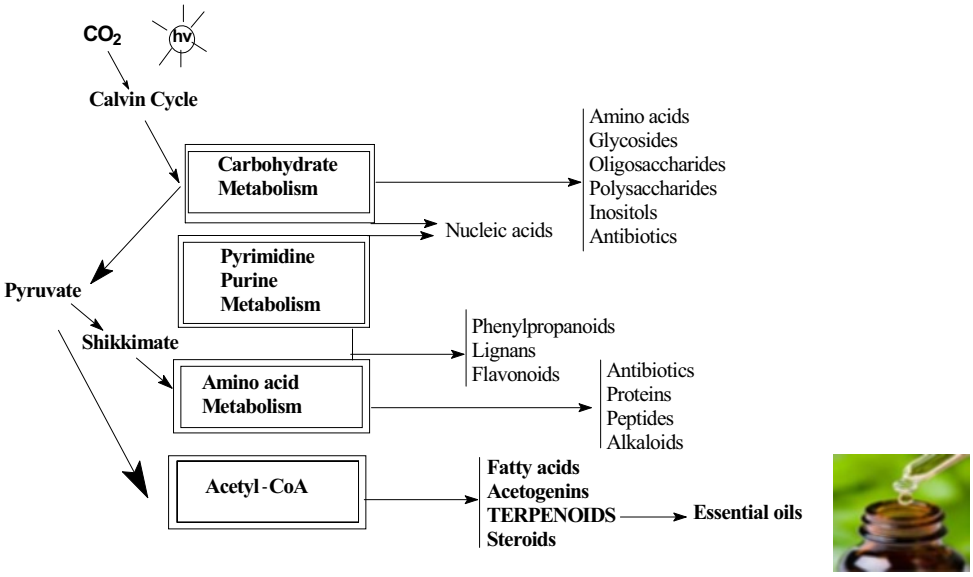

FIGURE 1 Outline of the biosynthesis of natural products.

Mentha species are strongly aromatic perennial, often rhizomatous or stoloniferous herbs. Upper leaves are sessile or subsessile; blade margin dentate, serrate, or crenate. Floral leaves are similar to stem leaves or reduced; bracts lanceolate to linear, more or less distinct. Flowers appear in many flowered (two to six) verticellasters in axillary or terminal spikes. Flowers are small, bisexual, variously shaped, and, unlike most other members of the family, are actinomorphic. Calyx are campanulate or tubular, five-toothed, regular or two-lipped, 10–13 nerved. Corolla four-lobed, funnelform, regular, or slightly irregular. There are four stamens, equal, divaricate, erect, exserted in bisexual flowers, filaments glabrous, two anther cells, parallel; pollen six-colpate, subprolate, tegillate. Style exserted, equally two-cleft at apex. Nutlets smooth or reticulate (Gaur, 1999).

The genus *Mentha* is represented by six species in India of which four species, *Mentha arvensis* L., *Mentha longifolia* (L.) Huds., *Mentha piperita* L., and *Mentha spicata* L. are common in Uttarakhand (Uniyal et al., 2007). Among these *M. longifolia* (Horse mint) is exclusively temperate wild specie with considerable variation in morphology and three varieties of it *M. longifolia* (L.) Huds. var *longifolia*, *M. longifolia* var. *incana* (Willd.) Dinsm., *M. longifolia* var. *royleana* (Benth.) Hook.f. are known between 1200 and 3200 m altitude range in Uttarakhand. *Mentha arvensis* (corn mint), *M. piperita* (Peppermint), and *M. spicata* (Spearmint) are commonly cultivated up to 2500 m altitude as pot herbs, or sometimes occur in a semiwild state. All *Mentha* species have been known to the natives for a long time and are called *Podina* in Uttarakhand. These species are commonly used to provide flavoring and coolant, in sauces, and are medicinally used in indigestion, vomiting, malarial fever, or as culinary herbs (Gaur, 1999).

USAGE AND APPLICATIONS

The mint species have great importance. The leaves, flowers, and stems of *Mentha* spp. are frequently used traditionally in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor (Kothari and Singh, 1995; Moreno et al., 2002). In addition, *Mentha* spp. are also used in folk remedy for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints due to its antinflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharrhal activities (Iscan et al., 2002; Moreno et al., 2002). Peppermint oil is one of the popular and widely used essential oils, because of menthol and menthone as the main constituents of the oil (Mimica-Dukic et al., 2003). Carvone-rich mint plants (spearmint) are used as spices and cultivated in most of the countries (Kokkini et al., 1997). The essential oil from *M. spicata* and its main constituent *p*-menthane exhibits good antibacterial activity against *Escherichia coli*, *Pseudomonas aruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Rhizobium leguminosarum*, and *Bacillus subtilis* (Sivropoulou et al., 1995). The herbage of mint (*M. spicata and M. piperita*) contain an acceptable limit of mineral content in addition to its anti bacterial and antioxidant activity. Piperitone oxide, R-carvone, linalool, menthol, and menthone present in the essential oil of mint possess antibacterial and antifungal activity against the disease causing agents and hence protect the products from spoilage and contamination (Edris and Farrag, 2003; Regnier et al., 2010; Rasooli and Rezaei, 2002; Sokovic and van-Griensven, 2006; Ziedan and Farrag, 2008).

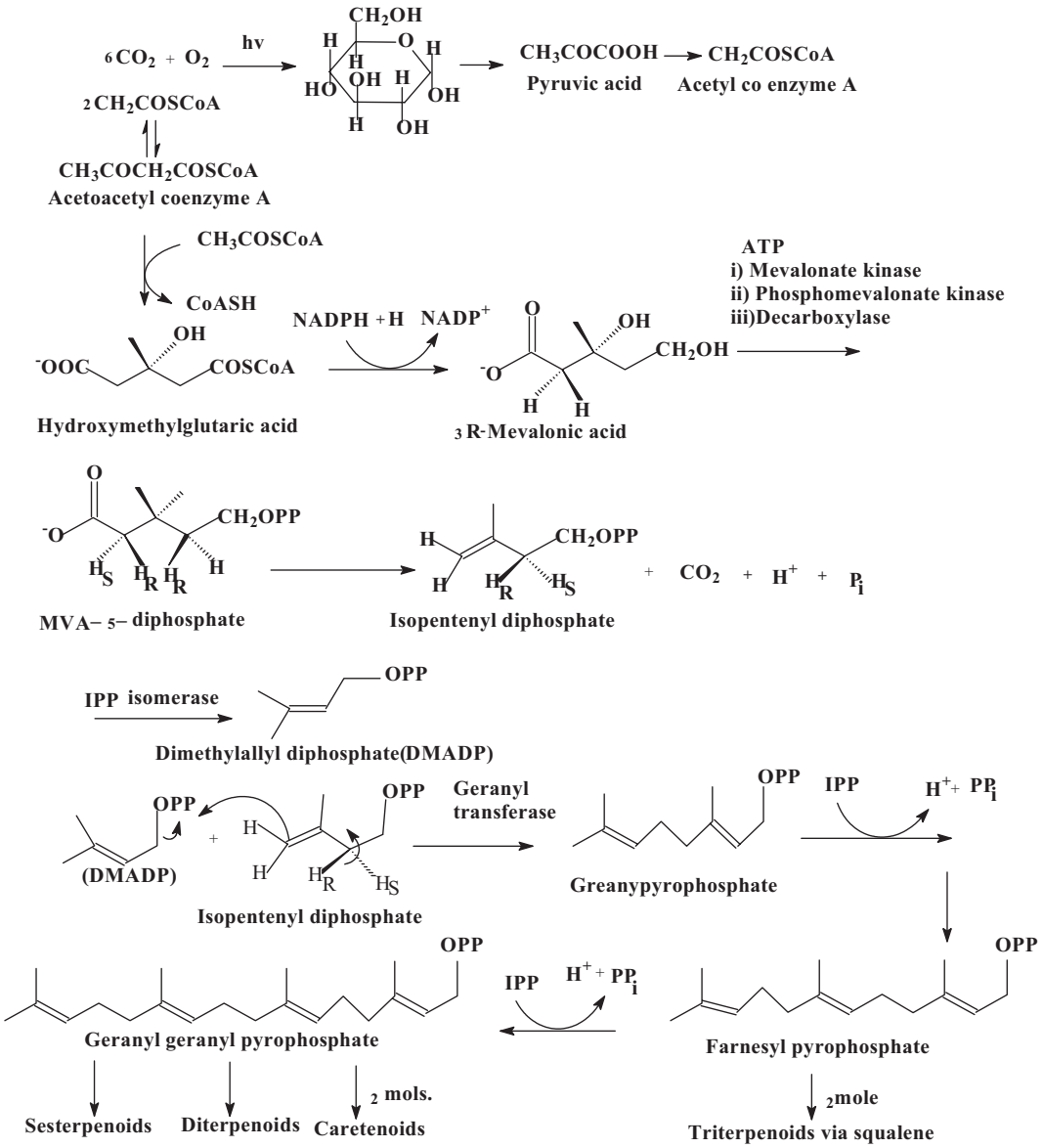

FIGURE 2 Biosynthesis of terpenoids.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Mint essential oil is a complex mixture of mono and sesquiterpenoides. The main constituents of the essential oils of *Mentha* spp. are menthol, menthone, menthyl acetate, isomenthone, menthofuran, piperitone, piperitenone oxide, carvone, and linalool carvone, etc. Many of these compounds find a wide usage in food and flavor (Mathela et al., 2005). Because of its wide applications in food, preservative, cosmetics, aromatherapy, we investigated 10 different accessions (I–X in Table 2) of *M. spicata* for variation in composition of their essential oils. The essential oil yield of this plant ranged from 0.5% to 1.0% of fresh weight. As this plant is traditionally used as flavoring agent in various types of foods hence in vitro activity of all the 10 oils were studied by different methods: reducing power activity, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, and Fe^{2+} chelating activity. Potato is an important crop and after long-term storage the tubers sprout, causing weight loss, poor tuber quality, and impediment of air passage into the piles which cause malformation, making them unacceptable for food, marketing, and seed purposes. To overcome this problem, sprouting inhibition techniques like fumigation of stored potato with chlorpropham isopropyl-*N*-chlorophenyl (CIPC) is carried out. Sprout inhibition by CIPC is not environmentally benign as it controls sprouting by

TABLE 1 List of *Mentha* Species in the World

S. N.	*Mentha* Species	S. N.	*Mentha* Species	S. N.	*Mentha* Species
1.	*Mentha alaica* Boriss.	2.	*Mentha aquatica* L.	3.	*Mentha arvensis* L.
4.	*Mentha australis* R. Br.	5.	*Mentha canadensis* L.	6.	*Mentha carinthiaca* Host
7.	*Mentha cervina* L.	8.	*Mentha cunninghamii* (Benth.) Benth.	9.	*Mentha dahurica* Fisch. ex Benth.
10.	*Mentha dalmatica* Tausch	11.	*Mentha darvasica* Boriss.	12.	*Mentha diemenica* Spreng.
13.	*Mentha dumetorum* Schult.	14.	*Mentha gattefossei* Maire	15.	*Mentha gayeri* Trautm.
16.	*Mentha gentilis* L.	17.	*Mentha grandiflora* Benth.	18.	*Mentha japonica* (Miq.) Makino
19.	*Mentha kuemmerlei* Trautm.	20.	*Mentha laxiflora* Benth.	21.	*Mentha locyana* Borbás
22.	*Mentha longifolia* L.	23.	*Mentha maximilianea* F.W. Schultz	24.	*Mentha micrantha* (Fisch. ex Benth.) Heinr.Braun
25.	*Mentha pamiroalaica* Boriss.	26.	*Mentha piperita* L.	27.	*Mentha pulegium* L.
28.	*Mentha pyramidalis* ten.	29.	*Mentha requienii* Benth.	30.	*Mentha rotundifolia* (L.) Huds.
31.	*Mentha royleana* Wall. ex Benth.	32.	*Mentha saturejoides* R. Br.	33.	*Mentha smithiana* R.A. Graham
34.	*Mentha spicata* L.	35.	*Mentha suaveolens* Ehrh.	36.	*Mentha verticillata* L.
37.	*Mentha villosa* Huds.	38.	*Mentha villosa-nervata* Opiz		

After The Plant List (2010).

inhibiting the cell division and may be detrimental to other life forms which comes in its contact. In view of this, we studied two accessions with different chemovariants (carvone and piperetenone oxide types) for sprout inhibition. Various amounts of essential oils (100, 200, and 300 µL, in triplicate) in glass vials without a lid were placed inside covered clay pots for fumigation. Experimental tubers were fumigated with test essential oils as vapors, for 5 weeks (135 days) at room temperature and sprouting growth inhibition activity was monitored after seven days by counting the number of sprouts and weight loss of the tubers per clay pot. CIPC and iodine were used as standards, and the clay pot without essential oil was used as control (Chauhan et al., 2011). On the basis of its different chemical makeup of essential oils, *M. spicata* has been classified into carvone (Kokkini et al., 1997), piperitenone oxide (Misra et al., 1989), linalool (Melnikov et al., 1984) menthone/isomenthone (Kokkini et al., 1997), dihydrocarvone (Gora et al., 1975), and menthyl acetate types (Pracha et al., 2007). However, in our study we could find only the carvone and piperetenone oxide types, along with two mixed populations (collections I and II), which contain both carvone and piperetenone oxide as their major constituents. This may be possible due to the hybrid nature of the plant (Chauhan et al., 2011). The chemical compositions of all the 10 chemovariants are given in Table 2 and structures of main constituents are illustrated in Figures 4 and 5. The essential oils showed good in vitro antioxidant activity and good sprout inhibition activity compared with CIPC and iodine, the standard sprout suppressants (Figure 3). It has been reported that essential oils posses good food preservative quality for dairy products, fruits, meat, etc. The bioactivity associated with essential oils is because of their volatile nature which makes them useful as possible fumigants for the protection of stored commodities. Shelf life of food products can also be extended by inhibiting or retarding the growth of pathogens in packed food and packing materials (Bhat et al., 2012). It has been reported that mint essential oil exhibited good antibacterial activity against *Salmonella enteritids* and *Phytobacterium phosphoreum* in low-fat foods (Tassou et al., 1995). Mint oil has been reported to be effective against the pathogens which have developed resistance against various antibiotics (Tables 3 and 4). It has also been reported that the oil is effective against fungi, bacteria, and viruses (Jeyakumar et al., 2011).

It has been stated that *M. spicata* essential oil exhibited fungitoxic activity against *Aspergillus ochraceus*, *Penicillium digitatum*, and *Pyricularia oryzae*. The oil has been found to retain its activity up to 80 °C and remained active up to 24 months when stored at room temperature. It killed *Aspergillus ochraceues* within 30 min, *Penicillium oryzae* within

TABLE 2 Comparative Essential Oil Composition of *Mentha spicata* from Different Locations of Uttarakhand, India

S. N.	Compounds	Flame Ionization Detector Percent of Different Collection Sites by Us (Chauhan et al., 2011)									
		I	II	III	IV	V	VI	VII	VIII	IX	X
1	α-Pinene	0.2	–	0.3	0.5	0.3	0.4	0.2	0.3	0.3	0.3
2	Camphene	0.1	–	–	0.4	t	t	t	t	t	t
3	Sabinene **(1)**	0.4	–	0.5	1.0	0.1	1.1	0.2	0.3	t	t
4	β-Pinene **(2)**	0.2	–	0.2	0.7	0.5	1.2	0.7	0.6	0.2	0.2
5	Myrcene	0.8	–	–	t	–	t	t	0.4	0.4	0.4
6	α-Phellandrene	–	–	–	0.3	–	–	2	1.4	0.4	0.4
7	α-Terpinene	–	–	–	t	–	–	–	–	1.8	1.7
8	*p*-Cymene	0.2	0.1	0.7	t	0.5	0.4	0.2	0.3	0.2	0.2
9	Limonene **(3)**	2.7	0.3	0.5	0.8	1.5	10.8	**14.0**	**11.9**	8.4	8.6
10	1,8-Cineole **(4)**	5.8	1.3	t	1.3	1.7	0.5	0.7	0.4	0.3	0.8
11	(Z)-β-Ocimene	0.1	–	–	0.1	0.2	0.1	0.2	t	0.2	–
12	(E)-β-Ocimene **(5)**	0.1	–	0.4	0.1	0.5	t	–	–	t	0.2
13	Linalool **(6)**	0.5	0.3	1.8	0.4	0.2	0.3	0.4	0.4	0.5	0.4
14	*Cis*-*p*-mentha-2-en-1-ol	–	–	t	0.1	0.5	t	–	0.4	t	–
15	*Trans*-*p*-mentha-2- en-1-ol	0.9	–	–	0.2	0.2	t	t	t	0.4	t
16	Borneol	0.5	0.8	1.6	t	0.9	0.3	0.4	t	t	0.4
17	Trpin-4-ol **(7)**	2.4	4.0	6.4	1.8	6.1	5.2	1.6	2.0	6.8	7
18	α-Terpineol	0.8	t	–	t	0.3	–	0.2	t	0.2	0.1
19	*Trans*-carveol	t	0.6	–	t	–	0.2	t	2.4	0.9	t
20	*Cis*-carveol	t	–	–	0.1	0.8	3.2	0.6	0.4	t	0.6
21	**Carvone (8)**	**15.3**	**15.7**	**–**	**0.1**	**t**	**67.2**	**68.5**	**66.4**	**64.0**	**66.8**
22	Bornyl acetate **(9)**	t	0.7	8.2	0.3	0.3	t	t	t	t	0.2
23	δ-Elemene **(10)**	0.2	2.6	2.2	t	0.5	0.1	t	0.4	0.1	0.1
24	α-Cubebene **(11)**	–	–	2.3	t	1.0	0.3	0.2	0.1	0.1	0.2
25	Eugenol **(12)**	–	–	1.1	1.0	2.2	0.7	0.1	1.3	0.2	1.3
26	**Piperetenone oxide (13)**	**49.4**	**63.0**	**24.4**	**79.2**	**60.6**	**t**	**t**	**t**	**t**	**t**
27	β-Bourbonene	2.1	2.3	–	1.3	–	–	1.2	–	1.3	0.3
28	β-Elemene	0.2	–	–	t	–	t	0.2	0.3	0.3	0.1
29	α-Gurzunene	1.0	–	–	0.1	0.1	t	0.2	0.3	0.2	0.2
30	β-Caryophyllene **(14)**	2.6	1.7	**11.1**	4.2	9.8	1.4	1.6	0.4	3.2	2.0
31	β-Garjunene	–	–	–	t	2.4	0.2	0.1	–	0.1	0.1
32	*Cis*-β-Farnesene	0.4	–	–	0.3	–	0.2	0.2	0.1	0.3	0.1
33	α-humulene **(15)**	1.7	1.0	**29.9**	0.1	0.2	0.8	1.2	0.3	0.2	0.3

Continued

TABLE 2 Comparative Essential Oil Composition of *Mentha spicata* from Different Locations of Uttarakhand, India—cont'd

S. N.	Compounds	Flame Ionization Detector Percent of Different Collection Sites by Us (Chauhan et al., 2011)									
		I	II	III	IV	V	VI	VII	VIII	IX	X
34	(*E*)-β-Farnesene	t	–	–	0.5	0.6	0.2	t	2.0	1.0	1.3
35	Aromadendrene	t	–	–	t	–	t	0.1	–	0.1	t
36	γ-Muurolene **(16)**	t	–	–	–	–	1.5	2.3	3.8	2.7	3.4
37	Germacrene D **(17)**	5.0	3.2	t	1.7	3.2	0.1	0.2	0.4	0.3	0.1
38	*epi*-Cubebol	0.9	–	–	0.3	t	0.4	0.1	0.2	0.2	0.2
39	γ-cadinene	t	–	–	t	0.4	t	0.5	–	0.6	–
40	*trans*-Calamenene	0.7	–	–	t	–	t	t	0.2	0.1	–
41	α-Cadinene	t	–	–	t	–	–	t	–	t	–
42	Germacrene D-4-ol	t	–	–	t	–	–	–	–	–	–
43	Spathulenol	t	–	–	–	0.2	t	t	0.2	0.3	t
44	Caryophyllene oxide	t	0.1	–	–	–	t	t	–	0.1	t
45	*Epi*-α-cadinol **(18)**	0.9	–	5.8	0.2	0.1	0.2	0.3	0.5	0.6	0.3
46	Cubenol	0.4	–	–	0.1	–	0.2	0.2	0.3	0.2	0.1
47	α-Cadinol	1.0	0.1	0.2	0.3	0.2	0.1	0.3	0.5	0.1	0.3
48	α-Bisabolol	t	–	–	0.5	–	t	0.3	0.5	0.2	0.4
	Total	**97.5**	**97.8**	**97.6**	**98.0**	**96.1**	**97.3**	**99.2**	**99.4**	**97.5**	**99.1**

Mode of identification: linear retention index (based on homologous series of *n*-alkanes; C_8–C_{24}), coinjection with standards/peak enrichment with known oil constituents, gas chromatography–mass spectrometry; t, trace (<0.1%); –, absent; I, Khatima (Uttarakhand,India); II, Rishikesh (-do); III: Almora (-do); IV: Tanakpur (-do); V,Kashipur (-do); VI, Nainital (-do); VII, Champawat (-do); VII, Pithoragarh (-do); IX, Haldwani (-do); X: Harinagar (-do).

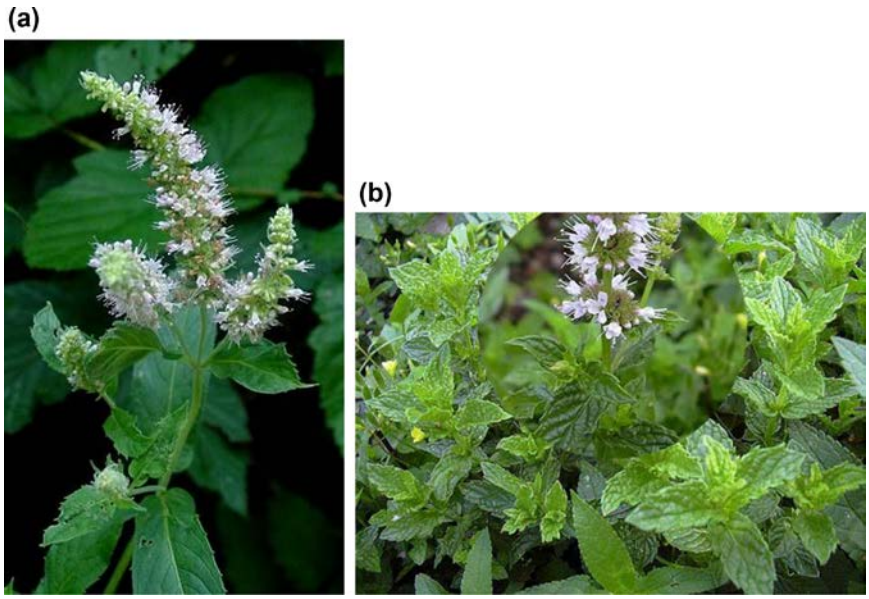

FIGURE 3 Aerial part of (a) *Mentha spicata*, and (b) *Mentha longifolia*.

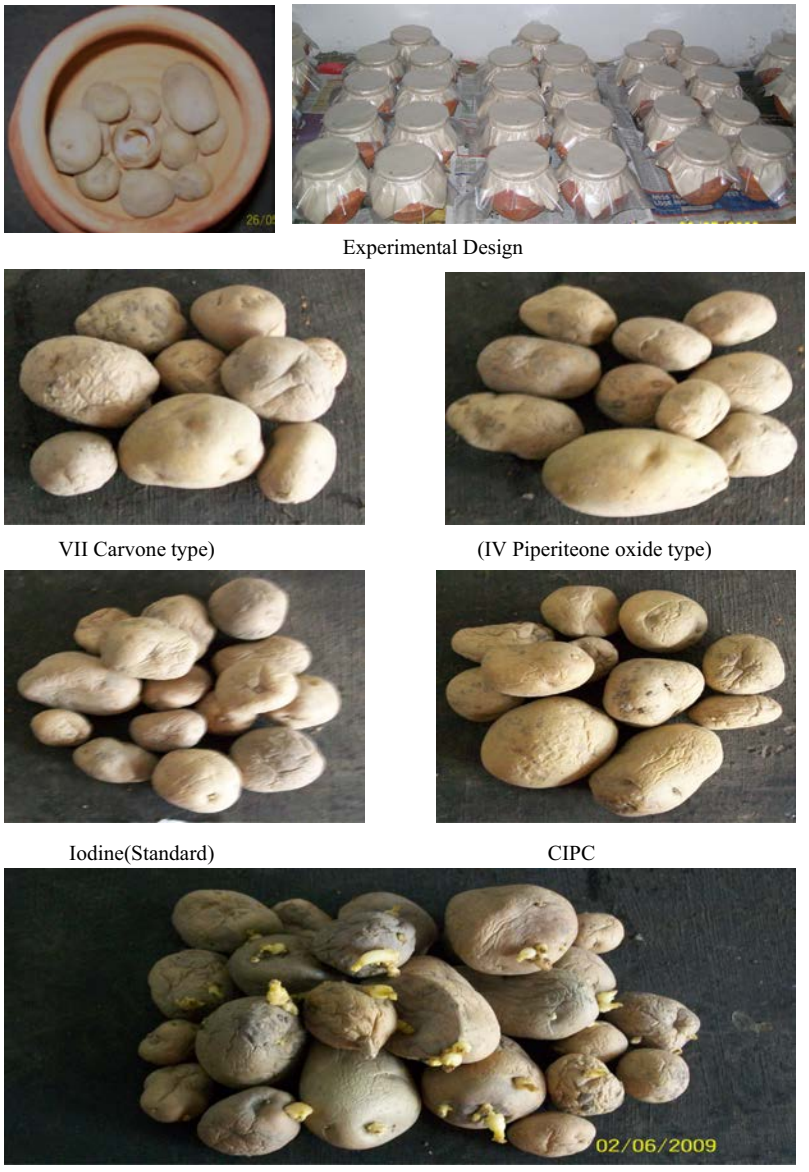

FIGURE 4 Fumigation effect of essential oils of *Mentha spicata* on sprouting behavior of potato.

15 min, *Penicillium digitatum*, and *Aspergillus alternata* within 10 min and possessed maximum fungi toxicity between pH levels 4.5–7.5. A volatile vapor emitted from the oil was also fungitoxic. Perusal of results obtained by Yadav et al. (2006) in Table 5 also revealed that *M. spicata* has a wide range of fungi toxicity activity at doses of 1100 ppm and 2200 ppm.

Several *Mentha* species are industrial crops and are extensively cultivated for their oils and terpenoid components of the essential oil, such as menthol, carvone, linalyl acetate, and linalool for their use in pharmaceuticals, cosmetics, food, flavor, beverage, and allied industries. Menthofuron, menthol, carvone, limonene, dihydrocarveol, menthyl acetate, menthone, linalool, linalyl acetate, piperitenone, diosphenol, pulegone, dihydrocarveole acetate, carvone, etc. are the major compounds in the essential oils of *Mentha aquatica*, *M. spicata*, *M. arvensis*, *Mentha citrate*, *M. longifolia*, *M. piperita*, *Mentha pulegium*, and *Mentha rotuntifolia* with their usage in flavoring foods, liquors, medicine, beverages, soaps, perfumes, folk medicine, cosmetic industry, mouthwashes, nervous disorders, insect repellent, confectionery etc., have been reported (Bhat et al., 2002).

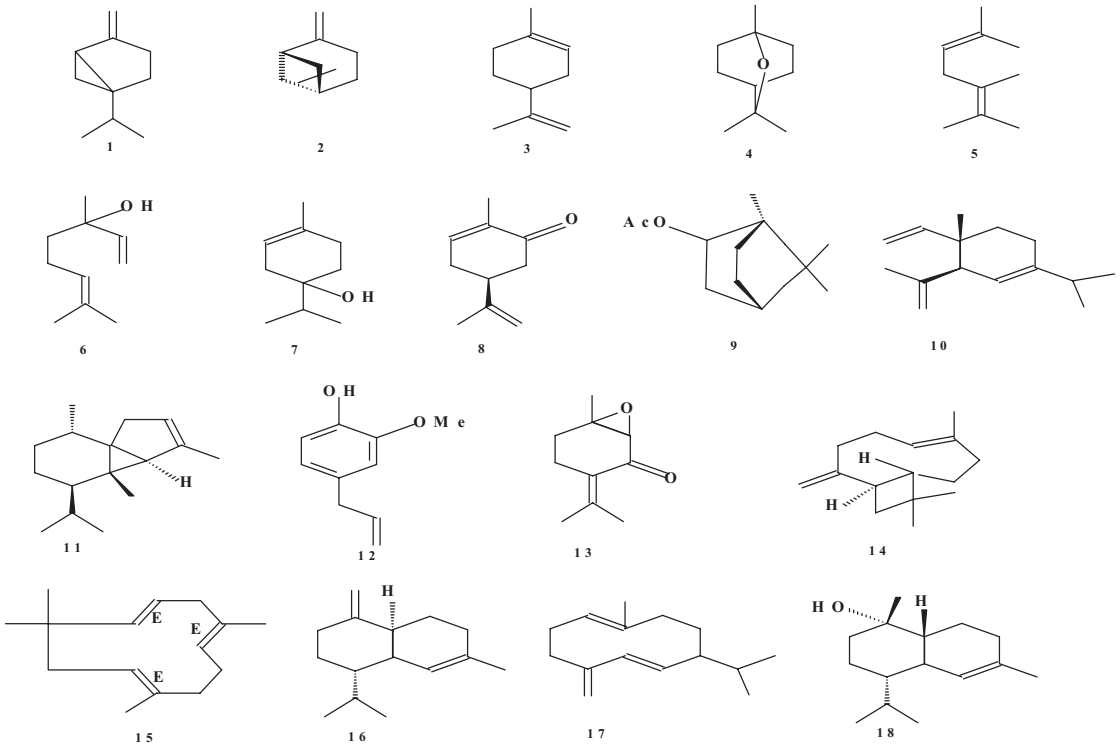

FIGURE 5 Structures of some major terpenoides present in the essential oil of *M. spicata*.

One of the disadvantages with volatile oil is their thermolabile nature; as a result their effectiveness to protect and flavor the food components decreases at higher temperature and with time. To overcome this problem and to increase the long-term effectiveness and reduce the evaporation, food industry essential oils and their active ingredients are being encapsulated in presence of matrices like modified starch to entrap or lock the essential oil droplets or active ingredients, which reduce the evaporation and control the release of flavor during storage and applications. To entrap the oil or active ingredient the carriers, emulsifiers, etc. are also being used. The essential oil of peppermint (*M. spicata*) has been studied and reported as encapsulated product with modified starch matrices which increased the leakage of aroma from encapsulated product during storage and applications (Baranauskiene et al., 2007).

SUMMARY POINTS

- As well as medicinal and perfumery usage, mint oil has wide application in food preservation by inhibiting the growth of microorganisms like bacteria and fungi and preventing it from spoilage and contamination.
- Mint as a whole herb is used in the form of spice in indigenous system of Indian traditional foods.
- The presence of phenolics in plant and its essential oil posses antioxidant activity so the oil as well as the extract can be used as source of natural antioxidant to preserve the food material from oxidative decay in place of synthetic antioxidants like butylated hydroxytoluene, which has been reported to posses carcinogenic effects.
- Mint essential oil could also be utilized as an ecofriendly, nontoxic sprout suppressant fumigants in the future, so that the food quality of the stored material is sustained.
- The major constituents of mint oil, like linalool, piperitone, carvone, menthofuran, menthol, limonene, dihydrocarveole acetate etc., can be used as natural flavoring agents.
- Pure mint essential oil and its emulsion and encapsulation with matrices can be used as a flavoring agent during spray drying and storage of food.

TABLE 3 Inhibitory Activity of Peppermint Oil Against Selected Pathogenic Bacteria (Mean ± S.D) mm

S. N.	Zone of Inhibition (%)	Test Organism						Results	Reported by
		Gram-Positive			Gram-Negative				
		S. aureus	*B. subtilis*	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*		
1	5	12.00 ± 0.81	11.00 ± 0.81	9.66 ± 0.46	9.33 ± 0.46	7.33 ± 0.47	6.00 ± 0.00	Sensitive	Jeyakumar et al. (2011)
2	10	12.66 ± 0.46	12.33 ± 0.46	11.33 ± 0.46	11.00 ± 0.81	9.00 ± 0.81	6.33 ± 0.46	Sensitive	
3	15	15.33 ± 0.46	14.33 ± 1.24	13.66 ± 0.94	13.00 ± 0.00	10.00 ± 0.81	8.00 ± 0.00	Sensitive	
4	29	17.66 ± 0.46	16.66 ± 0.47	16.33 ± 2.05	13.66 ± 0.47	11.00 ± 0.81	8.66 ± 0.46	Sensitive	
5	25	19.00 ± 0.81	18.66 ± 0.46	18.33 ± 1.24	14.33 ± 0.46	12.00 ± 0.81	9.66 ± 0.46	Sensitive	
6	30	24.33 ± 1.70	21.66 ± 0.46	20.66 ± 0.46	16.00 ± 0.81	14.66 ± 0.46	10.33 ± 0.47	Sensitive	

S. aureus, Staphylococcus aureus; B. Subtilis, Bacillus subtilis; B. cereus, Bacillus cereus; E. coli, Eschericia coli; K. pneumonia, Klebsiella pneumonia; P. aeruginosa, Pseudomonas aeruginosa.

TABLE 4 Effect of Peppermint Oil Compared with Antibiotics Against Selected Pathogenic Bacteria

S. N.	Antibiotics	Organisms						Reported by
		S. aureus	*B. subtilis*	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	
1	Azihro-mycin	++	–	–	–	–	–	Jeyakumar et al. (2011)
2	Ceftria-xone	++	++	++	++	–	–	
3	Cipro-loxacin	++	++	++	++	++	++	
4	Chlora-mphenicol	++	++	–	++	++	–	
5	Clinda-mycin	++	++	++	–	–	–	
6	Cotriumo-xazole	++	++	++	–	+	–	
7	Erythro-mycin	++	–	++	–	–	–	
8	Genta-mycin	++	++	–	++	–	+	
9	Ofloxacin	++	++	++	++	++	++	
10	Penicillin	–	+	+	–	–	–	
11	*Mentha piperita* oil	++	+	++	++	++	+	

++, sensetive; +, intermediate, –, resistant; *S. aureus*, *Staphylococcus aureus*; *B. Subtilis*, *Bacillus subtilis*; *B. cereus*, *Bacillus cereus*; *E. coli*, *Eschericia coli*; *K. pneumonia*, *Klebsiella pneumonia*; *P. aeruginosa*, *Pseudomonas aeruginosa*.

TABLE 5 Fungitoxic Spectrum of the *Mentha spicata* Essential Oil

S. N.	Fungi Tested	MGI (%) of the Oil at Different Concentrations			Reference
		Sublethal 550 ppm	Lethal 1100 ppm	Hyperlethal 2200 ppm	
1	*Curvularia lunata* (Wakker) Boedijn[b]	70	Static activity (100)	Cidal activity (100)	Yadav et al. (2006)
2	*Helminthosporium oryzae* Breda de Haan[b]	80			
3	*Botrytis cinerea* Pers[a]	Static			
4	*Penicillium brevicompactum* Dierckx[a]	70			
5	*Aspergillus niger* Van Tieghem[b]	85			
6	*Penicillium funiculosum* Thom[a]	75			
7	*Penicillum chrysogenum* Thom[a]	77			
8	*Fusarium moniliforme* Sheld	86			
9	*Colletotrium capsici* (Syd.) Butler & Bisby[b]	65			
10	*Colletotricum falcatum* Went[b]	63			
11	*Fusarium oxysporum* Schlecht; fr[a]	82			
12	*Helminthosporium maydis* Nesikado & Miyakel[b]	74			
13	*Penicillium imlicatum* Bioarge[b]	70			
14	*Penicillium italicum* Wwhmeg[b]	76			
15	*Aspergillus parasiticus* Speare[b]	75			
16	*Aspergillus versicolor* (Vull) Terboschi[b]	71			
17	*Penicillium expansum* Link[b]	76			
18	*Aspergillus candidus* Fres[b]	85			
19	*Aspergillus fumigatus* Fres[b]	74			
20	*Fusarium proliferatum* (Matsushima) Nirenberg[b]	87			
21	*Penicillium citrinum* Thom[b]	73			
22	*Aspergillus wentii* Wehmer[b]	80			
23	*Aspergillus tamarii* Kita[b]	84			
24	*Cladosporium cladosporioides* (Fres) devries[b]	82			
25	*Fusarium graminaream* Schwabe	72			

MGI, mycelial growth inhibition.
[a]*Fungi obtained from Indian Agricultural Research Institute (IARI) New Delhi.*
[b]*Fungi obtained from Institute of Microbial Technology Chandigarh (IMTECH) Chandigar.*

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Chapter 65

Mugwort (*Artemisia vulgaris*) Oils

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INTRODUCTION

Mugwort (*Artemisia vulgaris* L.), belonging to the genus *Artemisia,* is very much popular due to its characteristic odor and unique medicinal and food flavoring applications. There are around 500 species of *Artemisia* distributed across Asia, Europe, and North America that are frequently employed in folk medicine and food preparations (Willcox, 2009). Chemical studies on these plants indicate that several classes of bioactive compounds, in particular, terpenoids and flavonoids are present in this genus. The presence of a significant amount of essential oils and other terpenoids in the plants of this species can be linked with their potential uses as flavoring agents in foods and as ingredients of pharmaceutical products (Wright, 2002).

Mugwort, due to its aromatic nature and distinctive scent, has considerable essential oil potential which can be explored as ingredient of several functional foods, cosmeceuticals, and pharmaceutics. Indeed, research on essential oils has been revived due to their potential antioxidant and antimicrobial activities against aging, inflammation, and infectious diseases as well as their applications in food science (Hussain et al., 2008). This chapter focuses on the botanical and functional food aspects, medicinal uses, and applications of mugwort essential oil in food science.

BOTANICAL ASPECTS

Artemisia vulgaris L., commonly known as mugwort, belongs to family Compositae, and is native to Europe, Asia, and northern Africa. It is grown wildly in semiarid or arid areas throughout the Mediterranean basin and extending into the north-west Himalayas (Asta and Juste, 2006). Mugwort has a pleasant tangy taste. The root is sweet and pungent and the herb is aromatic and bitter in nature. The branched tips are gathered during the flowering season and carefully dried. Other fresh above and under ground parts of the plant are harvested at the beginning of winter, primarily from the wild (Gruenwald et al., 2008).

The plant is a long-stemmed, 70–150 cm high shrub with a branched, many headed and creeping rhizome without runners or rosette, and the medicinal parts are roots and aerial parts, particularly dried branched tips (Gruenwald et al., 2008). A typical photograph of mugwort plant is shown in Figure 1.

Flower and Fruit: Flower heads are ovoid, 3–4 mm long by 2 mm wide. The numerous flowers are short and stemmed erect or slightly drooping. They are dense, heavily branched panicles with numerous lanceolate bracts. The flowers are yellowish or red brown and almost glabrous. The fruit has an indistinct margin (Gruenwald et al., 2008).

Leaves and Stems: The leaves are 5–10 cm long, coriaceous, and the margins are often rolled back. The upper surface is usually dark green and glabrous, occasionally pubescent, and the lower surface is tomentose. The basal leaves are short petioled and lobbed with an end section and one to two pairs of small side leaflets. The rest of the leaves are sessile or almost sessile with a slit base. The shoots are slightly pubescent, often red-tinged, and have week unpleasant smell. The erect or ascending edged, and coriaceous long stems die off each year. They are in branched panicles and downy (Gruenwald et al., 2008).

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FIGURE 1 Mugwort (*Artemisia vulgaris*) (Tobyn et al., 2011).

USES AND APPLICATIONS

The plant is known to possess multiple medicinal uses and application in folk medicine, which include, gastrointestinal tract such as stomach ulcers, and indigestion and liver disorders (Gilani et al., 2005; Gruenwald et al., 2008). The plant is also used for worm infestations, epilepsy, vomiting, to promote circulation, as a sedative, and for delayed or irregular menstruation. The root is used for asthenic states as a tonic and in combination with other remedies also for psychoneuroses, neurasthenia, depression and hypochondria, autonomic neuroses, general irritability and restlessness, insomnia, and anxiety states. Mugwort is not used in pregnancy (Gruenwald et al., 2008).

Essential oils are known to make a major contribution to the plant's biological activity and generally have a broad spectrum of bioactivity, owing to the presence of several active ingredients or secondary metabolites, which work through various modes of action. The essential oil exhibits many other biological activities useful in food science such as antiseptic, antioxidant, larvicidal, nematicide, pesticide, antibacterial, antifungal, and antiviral, and is also used in the flavor and perfumery industry (Silva, 2004).

Mugwort essential oil has been employed for the treatment of diabetes and epilepsy, and in combination for psychoneurosis, depression, irritability, insomnia, and anxiety stress (Walter and Memory, 2003). The essential oil has a warming effect on the body which can be used to counter the effects of cold and moisture in the air; it also acts as a tonic for the nerves.

Mugwort is used to flavor tea and rice dishes in Asia and as a culinary herb for poultry and meat in Western cultures. It has also been utilized as an analgesic agent and in conjunction with acupuncture therapy (Yoshikawa et al., 1996). The leaves and buds are used as a bitter flavoring agent to season fat, meat, and fish. In China, it is used mostly for moxibustion (Tang and Eisenbrand, 2011). A paste or powder of its leaves is applied over skin diseases. It is used as an inferior substitute for cinchona for treating fever (Silva, 2004; Judzentiene and Buzelyte, 2006; Haider et al., 2003).

USES AND APPLICATIONS IN FOOD SCIENCES

There has been a growing interest in research concerning the possible use of plants in their natural form for pest and disease control in agriculture, that are less damaging to the human health and environment (Wang et al., 2005; Hussain

et al., 2008). The essential oil exhibits many other biological activities useful in food science such as antiseptic, antioxidant, larvicidal, nematicide, pesticide, antibacterial, antifungal, and antiviral as well as being used in the flavor and perfumery industry (Silva, 2004). Mugwort plant is valued for its medicinal food value. The essential oil of mugwort has been reported to slow down or inhibit the growth of different kinds of insects, microbes, and parasites and can be employed to protect foods from related deteriorations as a part of natural management practise. Repellent and fumigant activity of mugwort essential oil against *Musca domestica* L. and the stored-product insect pest *Tribolium castaneum* (Herbst) have also been reported (Judzentiene and Buzelyte, 2006). Erel et al. (2012) investigated in vitro antimicrobial activity of mugwort's essential oil against different microbial strains including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhimurium*, and *Candida albicans* using the disk diffusion method compared with positive control (ceftazidime and ketoconazole). The essential oil produced inhibition zones greater than or equal to that of the standard antibiotic suggesting its uses as a natural antimicrobial agent. The strong antimicrobial activity of mugwort essential oil is attributed to the presence of 1,8-cineole, α-thujone, and camphene (Silva, 2004; Blagojevica et al., 2006). The antitumoral activity has also been linked to artemisic acid and artemisinin B extracted from mugwort (Sun et al., 1992).

Interestingly, mugwort's essential oil has 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity similar to that of the standard antioxidant (butylated hydroxyl toluene, BHT) indicating its appreciable potential toward preservation of foods using natural additives (Erel et al., 2012). In another investigation, the essential oils of *A. vulgaris* also showed antioxidant activity in terms of free radical scavenging activity similar to those of the synthetic antioxidant BHT (Erel et al., 2012). It is well known that there is a relationship between antioxidant activity and the phenolic content of the plant extracts (Albayrak et al., 2010). Therefore, antioxidant properties of this potential oil can be attributed to the phenolic and flavonoid contents of the *A. vulgaris* (Wright, 2002).

The essential oil extracted from mugwort stem is a potential larvicide against *Aedes aegypti* in low concentrations of the oil solution and thus could be explored as a natural larvicidal agent against certain vector borne diseases and pest control in food science (Govindaraj and Kumari, 2013). Sharifian et al. (2013) evaluated insecticidal activity of mugwort essential oil against *Tribolium castaneum* (Herbst), *Callosobruchus maculatus* (F.), and *Rhyzopertha dominica* (F.). The results revealed that mortality of the insects increased as the doses of essential oil and exposure time increased thus prompting the need to explore this oil as natural insecticidal agent for insect control in food crops. The insecticidal and larvicidal properties of mugwort essential oil can be attributed to camphene and its chloro derivative and α-Thujone (Silva, 2004). Camphor, an active component of mugwort essential oil, has moth repellent properties and is used as preservatives in pharmaceuticals and cosmetics. Extracts of *A. vulgaris* L. appear to possess blood pressure lowering actions which can be linked with the presence of yomogin (Tigno et al., 2000). The prescribed health food functions coupled with bioactive profile support the potential uses of mugwort essential oil as a medicinal food.

ESSENTIAL OIL PRODUCTION

The extraction of essential oils from plant material can be achieved by various methods such as supercritical fluid extraction, solvent extraction, hydrodistillation, steam distillation, cold or hot pressing, effleurage, and phytonic process (Surburg and Panten, 2006). Various reports have shown that the essential oil yield from mugwort may vary from 0.1 to 1.4% depending on the plant part used and the origin of harvest (Table 1). Mugwort essential oil is clear to pale yellow in color with characteristic fragrance (slightly licorice-like), and can be blended with several other essential oils (Thao et al., 2004; Alizadeh et al., 2012).

CHEMICAL COMPOSITION OF MUGWORT ESSENTIAL OIL

The strong and aromatic smell of *A. vulgaris* is mainly due to high concentrations of volatile terpenes, constituents of their essential oil (Abad et al., 2012). The active components of *A. vulgaris* identified include flavonoids, coumarins, sesquiterpene lactones, volatile oils, inulin, and traces of alkaloids. The chief compounds of volatile oils include camphor, camphene, α-thujone, germacrene D, 1,8-cineole, and β-caryophyllene (Silva, 2004; Judzentiene and Buzelyte, 2006; Haider et al., 2003). The contents and composition of Mugwort (*A. vulgaris*) essential oil appear to be dependent on biochemical and geographical variability as well as on the method of extraction as shown in Table 2. It has been demonstrated that mugwort grown in different countries possessed different composition of essential oils. The oils from mugwort plants harvested in Turkey were found to be rich in α-thujone (56.13%), β-thujone (12.02%), caryophyllene oxide

TABLE 1 Extraction of Essential Oil From Different Parts of Mugwort (*Artemisia vulgaris*)

Origin/Parts	Technique	Apparatus	Yield (%)	References
China/leaves and stems	Hydrodistillation	Clevenger	-	Wang et al. (2005)
Serbia/aerial parts	Hydrodistillation	Clevenger	-	Blagojevic et al. (2006)
Turkey/aerial parts	Hydrodistillation	Clevenger	0.40	Erel et al. (2012)
North Lithuania/aerial parts	Hydrodistillation using hexane and diethyl ether mixture	---------	0.2–0.4	Judzentiene and Buzelyte (2006)
Iran/aerial parts	Steam distillation	Clevenger	1.4	Alizedah et al. (2012)
Vietnam/aerial parts	Hydrodistillation	Clevenger	0.32–1.14	Thao et al. (2004)
Italy/aerial parts	Hydrodistillation	Likens-Nickerson apparatus	-	Mucciarelli et al. (1995)
Cuba/aerial parts	Hydrodistillation	Clevenger	0.1	Pino et al. (1999)
India/aerial parts	Hydrodistillation	Clevenger	0.16–0.5	Haider et al. (2003)
Nepal/leaves	Steam distillation	Clevenger	-	Bhatt et al. (2007)
Iran/aerial parts	Hydrodistillation	Clevenger	0.25	Bamoniri et al. (2010)
India/stem	Hydrodistillation	Clevenger	-	Govindaraj and kumari (2013)

TABLE 2 Chemical Composition of Mugwort (*Artemisia vulgaris*) Essential Oil

Origin/Parts	Major Components	Yield (%)	References
Turkey (aerial parts)	α-Thujone	56.13	Erel et al. (2012)
	β-Thujone	12.02	
	Caryophyllene oxide	10.19	
	1,8-Cineole	8.47	
North Lithuania (aerial parts)	Germacrene D	5.3–15.1	Judzentiene and Buzelyt (2006)
	1,8-Cineole	2.6–17.6	
	β-Pinene	0.1–12.9	
	cis-Thujone	0–12.9	
	trans-Thujone	0–20.2	
	Chrysanthenyl acetate	0–23.6	
	Caryophyllene	2.5–12.2	
Iran (aerial parts)	α-Pinene	23.56	Alizadeh et al. (2012)
	Menthol	9.71	
	β-Eudesmol	8.297	
	Spathulenol	4.582	
	Trans-Caryophyllene	24.76	Bamoniri et al. (2010)
	1, 8-Cineol	18.64	
	Trans-Salvene	14.87	
	β-Cubebene	11.82	

TABLE 2 Chemical Composition of Mugwort (*Artemisia vulgaris*) Essential Oil—cont'd

Origin/Parts	Major Components	Yield (%)	References
Cuba (aerial parts)	caryophyllene oxide	31.1	Pino et al. (1999)
	Hexadecanoic acid	6.3	
	Isobornyl 2-methylbutyrate	5.3	
	2-Heptadecanone	5.1	
Vietnam (aerial Part)	β-Anole	21.7	Thao et al. (2004)
	Camphor	10.9	
	β-Pinene	10.2	
	α-Pinene	9.1	
India (seeds)	Camphor	17.3	Govindaraj and Kumari (2013)
	α-Thujone	10.7	
	γ-Muurolene	9.0	
	Camphene	6.0	

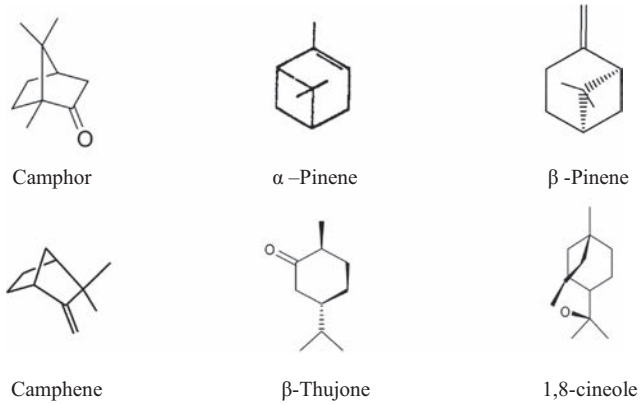

FIGURE 2 Chemical structures of important constituents of mugwort essential oil (Barney et al., 2005; Abad et al., 2012).

(10.19), and 1,8-cineole (8.47%) (Erel et al., 2012). Furthermore, 1,8-cineole (28.9%), sabinene (13.7%), β-thujone (13.5%), and caryophyllene oxide (6.5%) were reported as the principal components in leaf essential oils of Egyptian mugwort plants (Blagojevic et al., 2006), whereas in Croatia, the chief components reported were β-thujone (20.8%), α-pinene (15.1%), and 1,8-cineole (11.7%) (Jerkovic et al., 2003). The oils isolated from North Lithuanian Mugwort (*A. vulgaris*) plants were high in sabinene, β-pinene, 1,8-cineole, artemisia ketone, *cis*- and *trans*-thujone, chrysanthenyl acetate, germacrene D, and β-caryophyllene (Judzentiene and Buzelyt, 2006). The oils extracted from the plants from Iran were mainly composed of *trans*-caryophyllene (24.76%), 1,8-cineol (18.64%), *trans*-salvene (14.87%), and β–cubebene (11.82%) (Bamoniri et al., 2010). Whereas the oils isolated from Indian grown plants were characterized by large amounts of camphor (38.7%), isoborneol (8.2%), and artemisia alcohol (4.5%) (Haider et al., 2003). The components of mugwort essential oil vary depending on where they are cultivated, for example, USA (10 compounds), Serbia (94 compounds), Pakistan (24 compounds), and Turkey (14 compounds). Table 2 describes the content and chemical composition of essential oil from different parts of mugwort. The chemical structures of some important constituents of mugwort essential oil are given in Figure 2.

TABLE 3 Antimicrobial Activity of Mugwort (*Artemisia vulgaris*)

Microorganisms	Bacteria/Fungus		Inhibition Zone (mm)	References
Eschericia coli	Gram-negative bacterium	Plant extract	8–20	Harimath et al. (2011)
Staphylococcus aureus	Gram-positive bacterium	Plant extract	4–20	
Pseudomonas aeruginosa	Gram-negative bacterium	Plant extract	23	Erel et al. (2012)
Staphylococcus aureus	Gram-positive bacterium	Essential oil	23	
Staphylococcus epidermidis	Gram-positive bacterium	Essential oil	13	
Salmonella typhimurium	Gram-negative bacterium	Essential oil	10	
Enterococcus faecalis	Gram-positive bacterium	Essential oil	14	
Enterobacter cloacae	Gram-negative bacterium	Essential oil	8	
Escherichia coli	Gram-negative bacterium	Essential oil	11	
Candida albicans	Fungus	Essential oil	20	
Salmonella Enteritidis	Gram-negative bacterium	Essential oil	25–37	Blagojevic et al. (2006)
Pseudomonas aeruginosa	Gram-negative bacterium	Essential oil	13.5–31	
Klebsiella pneumonia	Gram-negative bacterium	Essential oil	13.5–34	
Aspergillus niger	Fungus	Essential oil	24–31	
Staphylococcus aureus	Gram-positive bacterium	Essential oil	13.5–29	

SUMMARY POINTS

- Mugwort (*Artemisia vulgaris* L.) is an aromatic plant from the family Compositae.
- Mugwort has a pleasant tangy taste with a characteristic scent.
- Mugwort plant is known to possess multiple medicinal uses and applications, which include stomach ulcers, indigestion, liver disorders, emmenagogue, nervine, digestive, diuretic, and diaphoretic, as well as having food flavoring properties.
- Mugwort has also antitumoral activity which is mainly linked to artemisic acid and artemisinin B.
- The active components of mugwort include flavonoids, coumarins, sesquiterpene lactones, volatile oils, inulin, and traces of alkaloids.
- The essential oil yield from mugwort may vary from 0.1% to 1.4% depending on the plant part used and the origin of harvest.
- Mugwort essential oil is mainly composed of β-pinene, α-pinene, camphor, and 1,8-cineole.
- The essential oil exhibits many biological activities useful in food science such as antiseptic, antioxidant, larvicidal, nematicide, pesticide, antibacterial, antifungal, and antiviral properties, as well as being used in the flavor and perfumery industry.
- Mugwort essential oil has high potential as a natural antiinflammatory agent to impart functional food and physiological benefits if incorporated into some food or cosmetic products.
- Mugwort (*Artemisia vulgaris*) plant essential oil and extract have appreciable antimicrobial potential (Table 3).

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Chapter 66

Myrtle (*Myrtus communis* L.) Oils

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List of Abbreviations

m Meter
mm Millimeter
cm Centi-meter
LC Lethal concentration
MIC Minimum inhibitory concentration
MBC Minimum bactericidal concentration
ZGI Zone of growth inhibition
MFC Minimum fungicidal concentration

INTRODUCTION

Plants or oils extracted from aromatic plants have been extensively used for the protection of cereals (Bertoli et al., 2012). *Myrtus communis* is one of the important aromatic and medicinal species belonging to the Myrtaceae family, comprising about 50 species native to the Mediterranean basin. It is a typically Mediterranean sub-shrub with white flowers growing wild in Corsica, widely used in herbal medicine in Mediterranean countries, and containing very high amounts of hydrolysable tannins and flavonoid glycosides (Traveset et al., 2001).

The development of different methods to preserve food is a major interest in the food industry worldwide. Combined technologies are required for food safety and maintenance of the organoleptic qualities of foods, while at the same time decreasing the intensity of the hurdle caused by an antimicrobial dose. Several studies have reported the efficiency of combined processes, including traditional and emerging technologies with essential oils or their constituents. For example, thermal treatments are commonly used for the preservation of food. However, at the intensities needed to achieve required levels of safety, undesirable changes in nutritional and sensory properties of food could occur. From this point of view, the use of milder heat treatments in combination with essential oils appears to be an adequate solution (Cherrat et al., 2013).

Essential oil obtained from various parts of myrtle is chemically composed of mainly three categories: terpenes (monoterpene hydrocarbons and sesquiterpene hydrocarbons), terpenoids (oxygenated monoterpenes and oxygenated sesquiterpenes), and phenylpropanoids. Compounds that have been found in myrtle oil include E–2-hexenal, Z-3-hexenol, tricyclene, α-thujene, α -pinene, sabinene, β-pinene, myrcene, δ-3-carene, α-terpinene, p-cymene, limonene, 1,8-cineole, E-β-ocimene, linalool E-oxide, terpinolene, linalool, α-terpineol, myrtenol, and eugenol (Andrade et al., 2011). Traditionally, essential oil and crude extracts obtained from the myrtle plant are used in the pharmaceutical, cosmetic, and food industries in various preparations. These applications are possible due to presence of characteristic volatile constituents.

BOTANICAL ASPECTS

Myrtus is a small genus belonging to the Myrtaceae family. It is native to Europe but is widespread throughout the Mediterranean region and the Middle East, having an important cultural significance. *Myrtus* genus includes approximately 100 genera and 3000 species growing in temperate, tropical, and subtropical regions. *Myrtus communis*, commonly known as myrtle, is the only species of the genus found in the Northern Hemisphere (Traveset et al., 2001).

This evergreen shrub grows to a height of about 1–5 m, with white flowers (blossoming time: June to July), growing wild at altitudes of 0–600 m. The oppositely arranged leaves are ovate-lanceolate, 2–5 cm long, coriaceous, glabrous,

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punctuate-glandular, and entire. When crushed, they have a delicate aromatic odor. White, star-like flowers, which have five petals, five sepals, and a mass of tufted stamens, appear from June to September. After the summer, subglobose to ellipsoid berries, which are bluish-black (or rarely yellowish-white) on ripening, appear around November. The flowers are pollinated by insects and have a specialized strategy of seed dispersal by birds, mammals, and ants. Seeds have no apparent dormancy. As a consequence, germination can occur soon after dispersal and their strategy of rapid establishment might allow seedlings to take advantage of autumn and winter rains during the crucial first stages of growth. These features can be considered advantageous in order to maximize reproductive success, with seedling establishment and survival in Mediterranean environments (Ozkan et al., 2009).

Myrtus species have been reported as being very rich in volatile oils phenolic acids, flavonoids, tannins, anthocyanin pigments, and fatty acids (Kanoun et al., 2014). *Myrtus communis* contains polyphenols and essential oils. Researchers have revealed the presence of several specific chemical compounds in aerial parts; for example, the dried leaves of this herb contain 1,8-cineole (13.5–19.6%), linalool (7.7–15.8%), linalyl acetate (2.5–6%) terpineole, terpinolene, tannins, and flavonoid compounds (Chryssavgi et al., 2008). The leaf and flowers contain essential oils, phenolic acids, flavonoids, and tannins. Berries are composed of tannins, anthocyanins (0.2–54%), fatty and organic acids (9–52%), and its content depends on the extraction solvent used and/or ripening period. It is evident that the content of these compounds also differs depending on the plant part used, but generally the most common compounds found in myrtle leaves, stems, and flowers are α-pinene (~10–60%) and 1,8-cineole (~12–34%) (Messaoud et al., 2012).

USAGE AND APPLICATIONS

Myrtle has been used since ancient times as a spice and medicinal agent, as well as for food preparation purposes. Myrtle as a spice finds no wide application because of its bitterness, despite its pleasant odor. The taste is very intense, quite unpleasant, and strongly bitter, so its culinary application is limited to the regions of origin, such as Italy (Gortzi et al., 2008). In Italy, especially in Sardinia, berries and leaves are used to produce two well-known liquors (Mirto Rosso and Mirto Bianco, respectively) (Messaoud et al., 2012). Foods flavored with the smoke of myrtle are common in rural areas of Italy (Gortzi et al., 2008). Some parts of the plant are used in the food industry for flavoring meat and sauces (Chalchat et al., 1998), and its berries and leaves are mostly employed for the industrial formulation of sweet liquors with advertised digestive properties (Clark, 1996).

The leaves are very fragrant and have been extensively used in the perfume and cosmetic industries, particularly in Portugal and Turkey (Clark, 1996). It is traditionally used as an antiseptic, disinfectant, and hypoglycemic agent. In Turkey, myrtle leaves and fruits have been used as an antiseptic medicine in villages. Similarly, in Italian folk medicine, the fruit of this plant is used in the treatment of many types of infectious disease, including diarrhea and dysentery; the leaves are used as antiseptic and anti-inflammatory agent, as well as a mouthwash, for the treatment of candidiasis (Gortzi et al., 2008). The essential oil obtained from myrtle leaves has been used in the treatment of lung disorders. Myrtle is frequently consumed as an infusion and decoction (Clark, 1996). Infusions made from the leaves and young branches are used as a stimulant, antiseptic, astringent, and hypoglycemic agent and considered to be a health remedy for asthma, eczema, psoriasis, diarrhea, gastrointestinal disorders, and urinary infections. The leaf decoction is used for vaginal washing, enemas, and against respiratory diseases; decoction from the fruits is used as an antidiarrheal and antihemorrhoidal agent and in the treatment of mouth and eye diseases (Ziyyat et al., 1997).

USAGE AND APPLICATIONS IN FOOD SCIENCES

Myrtle Oil and Antibacterial Activity for Food Preservation

Earlier studies show that 1,8-cineole, linalool, eugenol, α-terpineol, and γ-terpinene, as myrtle essential oils components, have a good bactericidal action against many Gram-positive and Gram-negative bacteria (Oyedemi et al., 2009). The linalool is found to possess antibacterial activity, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values in the range of 0.18–5.88 mg/mL and 0.18–11.75 mg/mL, respectively; these values for 1,8-cineole range from 0.37 to 11.75 mg/mL and 0.73–11.75 mg/mL, respectively (Randrianarivelo et al., 2009). Moreover, myrtle single compounds such as limonene (0.17–2%), 1,8-cineole (2–16%), and α-pinene (1–16%) have significant activity against *Mycobacterium tuberculosis* strains (Zanetti et al., 2010). These biological effects of myrtle essential oils and extracts are result of activity of compounds belonging to various chemical classes. For example, in the mid-1970s, researchers reported the isolation of phloroglucinol antibiotics from the myrtle leaves—myrtucommulone A and semimyrtucommulone. These myrtle compounds have been shown to possess significant activity against *Staphylococcus aureus* strains, with MIC values in range of 0.5–2 g/mL for myrtucommulone A, and 32–64 g/mL for

semimyrtucommulone (Appendino et al., 2002). Another study indicated that essential oil obtained from leaves of myrtle by steam distillation contains monoterpenes 95.17% (α-pinene: 16.52%, 1,8-mineole: 26.93%, myrtenyl acetate: 47.29%, camphene: 2.45%, methyl eugenol: 4.8%) and others 4.8%. The antibacterial activity of essential oil was studied against five Gram-positive bacteria and four Gram-negative bacteria. Oil exhibited moderate-to-strong antibacterial activity (Cherrat et al., 2013).

A comparative study on the chemical composition of essential oils isolated by hydro-distillation from leaves of Tunisian and Algerian *Myrtus communis* was performed and led to identification of 23 compounds, representing 93.73% of total oil, which was found to be rich in monoterpenes hydrocarbons (53.38%), particularly α-pinene (35.30%) and α-limonene (14.76%). The study of antibacterial activity revealed that 10 μL of essential oil significantly inhibited the growth of five tested bacteria, especially *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella* sp., and *Listeria* sp. (Table 1; Ghnaya et al., 2013).

The essential oil of myrtle leaves collected from Khuzestan and Lorestan provinces of Southwest Iran exhibited valuable antibacterial activity against *Erysipelothrix rhusiopathiae*. The inhibition zones and MIC values for bacteria that were sensitive to the essential oil were in the range of 14.7–27.0 mm and 0.031–0.25 mg/mL, respectively (Pirbalouti et al., 2014). Moreover, combination of *Myrtus communis* and *Thymus vulgaris* essential oils displayed a partial synergistic antibacterial effect against *S. aureus* ATCC 25922 and *E. coli* O128B12. These findings suggest that the mixture of these essential oils at suitably low concentrations could be a promising alternative to replace synthetic antimicrobial agents and lead to new research about natural products to enhance its antibacterial properties (Moulay et al., 2014). Antibacterial activities of myrtle essential oil are summarized in Table 1. These studies strongly suggest the usefulness of oil in food and food products.

Myrtle Oil and Antifungal Activity for Food Preservation

The management of fungal infections has many problems, such as the limited number of antifungal agents, toxicity, resistance to commonly used antifungal agents, relapse of infection, and high cost. It is therefore necessary to discover new antifungal agents to combat the strains expressing resistance to the available antifungal drugs. One natural product used as a therapeutic agent against fungi is myrtle, particularly its essential oils and extracts. An antifungal activity of myrtle essential oil was tested against different *Candida* species, and MIC values were in range of 2–4 μl/mL (Cannas et al., 2013). Results are outlined in Table 2. The antifungal effect exhibited by myrtle is attributed to essential oil and phenolic compounds that are known to cause cell membranes damage, causing leakage of cellular materials and ultimately the microorganism's death. The antifungal property of myrtle is suspected to be associated with its high content of polyphenols and oxygenated monoterpenes. The mechanism of antifungal actions is quite similar to that described for bacteria, concerning irreversible damage of the cell membrane and coagulation of the cell content.

Ergoda and coworkers revealed that methanol, acetone, ethanol, and ethyl acetate extracts of myrtle possessed good activity against *Candida albicans*, with MIC ranging from 0.187 to 1.5 mg/mL and minimum fungicidal concentration (MFC) ranging from 0.375 to 3 mg/mL (Erdoga et al., 2014). Furthermore, hydroalcoholic extracts of myrtle were assessed for antifungal activity on dermatophytes against *Microsporum canis*, *Microsporum gypseum*, and *Trichophyton mentagrophytes* species. Ethyl acetate and total methanol extracts had promising antifungal effects against three genera of dermatophytes, as tested by Mitra et al. (2013). The antifungal properties of myrtle essential oil are summarized in Table 2.

Myrtle Oil and Antioxidant Activity for Food Preservation

To prevent oxidation, the addition of either synthetic or natural antioxidants to fats, fatty foods, and cosmetics is a common practice. Because of the carcinogenicity of synthetic antioxidants, their use in products for human application is being restricted, thereby increasing interest in antioxidants of natural origin. Aromatic and medicinal plants, such as myrtle, are a source of natural antioxidants because of the activity of secondary metabolites, such as phenylpropanoids and essential oils. These plant essential oils and extracts have been used for many thousands of years in food preservation. It is well established that the antioxidant capacity of plant extracts is strongly related to phenolic content. The antioxidant activities of the phenolic compounds were attributed to redox properties allowing them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal chelators.

Several reports describe the antioxidant activities of different extracts and compounds obtained from myrtle leaves as well as other parts of the plant (Chryssavgi et al., 2008). Myrtle berries are used to make liqueurs, jams, and other food products. Ethanol extracts obtained from berries were studied according to their antioxidant properties. Moronto

TABLE 1 Antibacterial Activity of *Myrtus communis* Extract and Essential Oil

Source	Antibacterial Activity		References
M. communis essential oil	Gram-positive bacteria	Gram-negative bacteria	Cherrat et al. (2013)
	Staphylococcus aureus CECT 976 • ZGI 24.2 ± 2.1 • MIC 8 • MBC >56.0 *Listeria monocytogenes* EGD-e • ZGI 21.5 ± 1.5 • MIC 2 • MBC >56.0 *Listeria monocytogenes* CECT 4031 • ZGI 11.5 ± 2.5 • MIC 1.0 • MBC >56.0 *Enterococcus faecium* CECT 4932 • ZGI 22.0 ± 2.0 • MIC 4 • MBC >56.0 *Bacillus subtilis* CECT 4071 • ZGI 21.1 ± 0.7 • MIC 14 • MBC 56.0	*Escherichia coli* O157:H7 • ZGI 7.4 ± 0.4 • MIC 56.0 • MBC >56.0 *E. coli* CECT 471 • ZGI 10.8 ± 2.6 • MIC 56.0 • MBC >56.0 *Yersinia enterocolitica* CECT 4315 • ZGI 7.2 ± 0.2 • MIC 56.0 • MBC >56.0 *Salmonella Senftenberg* CECT 4563 • ZGI 11.7 ± 2.3 • MIC 56.0 • MBC >56.0	

M. communis essential oil		10 µL of oil (ZGI mm)		Ghnaya et al. (2013)
		Algerian origin	Tunisian origin	
	Gram-negative			
	Escherichia coli (ATCC10536)	15	20	
	Salmonella	14	12	
	Gram-positive			
	Staphylococcus aureus (ATCC 6538)	10	23	
	Bacillus subtilis (ATCC 6633)	9	12	
	Listéria	10	15	

M. communis essential oil		MIC (µg/mL)	Gursoy et al. (2009)
	Aggregatibacter actinomycetemcomitans NTCC 9710	2	
	A. actinomycetemcomitans AHN 24195	1	
	Porphyromonas gingivalis ATCC 33277	2	
	P. gingivalis AHN 24155	0.25	
	P. gingivalis AHN 24135	0.25	
	Parvimonas micra ATCC 33270	2	
	P. micra AHC 15107	1	
	P. micra AHC 15154	0.5	
	Tannerella forsythia AHN 24212	0.25	
	Fusobacterium nucleatum ATCC 25586	0.25	
	F. nucleatum AHN 9508	0.5	
	Prevotella intermedia ATCC 25611	2	
	P. intermedia AHN 8290	2	
	Prevotella nigrescens ATCC 33563	2	
	P. nigrescens AHN 8293	1	

Continued

TABLE 1 Antibacterial Activity of *Myrtus communis* Extract and Essential Oil—cont'd

Source	Antibacterial Activity				References
M. communis essential oil	*E. rhusiopathiae*		MIC (μg/mL) 31.5	MBC μg/mL) 62.5	Pirbalouti et al. (2014)
M. communis leaves extract	*Morganella morgani*		MIC (mg/mL) 2.5	MBC (mg/mL) 5	Mohammad et al. (2013)
Hyrdoalcoholic extract of *M. communis* leaves		Extract concentration (80 mg/mL) ZGI (mm)	MIC (mg/mL)	MBC (mg/mL)	Ali et al. (2013)
	S. aureus	20.4 ± 0.3	0.2	2	
	P. aeruginosa	–	–	–	
	E. coli	2	8	40	
	Vibrio cholerae	12.4 ± 0.3	2	20	

ZGI, zone of growth inhibition (mm); MIC, minimal inhibitory concentration (μL/L); MBC, minimal bactericidal concentration (μL/L).

and coworkers have revealed that there is a very high correlation between antiradical/antioxidant capacity and total phenol amount in myrtle berries (Montoro et al., 2006). Methanol extracts of myrtle obtained from leaves, stems, and flowers have shown better antioxidant activity when compared with other essential oils using 2,2-dihenyl-1-picry-hydrazyl (DPPH) radical scavenging, β-carotene-linoleic acid bleaching, reducing power, and metal chelating activity assays (Aidi Wannes et al., 2010). The crude extracts, ethyl acetate fraction, butanol fraction, tannins, and anthocyanins from the leaf, stem, and berry of myrtle were also studied for their antioxidant capacity by Kanoun et al. The results are illustrated in Table 3 (Kanoun et al., 2014). Furthermore, the essential oil of myrtle has been demonstrated to exhibit moderate DPPH scavenging activity with IC_{50} values of 6.24 and 5.99 mg/mL (Mimica-Dukić et al., 2010). Essential oil of myrtle prepared by infusion was evaluated by using various antioxidant assays. It was determined that essential oil had considerable antioxidant properties that varied according to the infusion time (Messaoud et al., 2012). These data showed that myrtle essential oil obtained from extract is a potential source of natural antioxidants and therefore could be used as a food stabilizer in food products and natural health products. The antioxidant activity of myrtle is detailed in Table 3.

Myrtle Oil and Food Spoilage

Myrtle oil is being used to prevent the growth of molds on food products. Bertoli and coworkers studied six essential oils, including myrtle essential oil, for toxic and repellent activity against *Sitophilus zeamais* Motsch (*Coleoptera dryophthoridae*). *Sitophilus zeamais* Motsch attacks both standing crops and stored cereal products, including wheat, rice, sorghum, oats, barley, rye, buckwheat, peas, and cottonseed. The maize weevil also infests other types of stored, processed cereal products such as pasta, cassava, and various coarse, milled grains. It has been even known to attack fruit while in storage, such as apples.

Results from topical applications on insects showed that all essential oils have variable and significant insecticidal activity. The results of repellency tests for myrtle essential oil indicated high repellent activity against *S. zeamais* adults (Bertoli et al., 2012). Also, khapra beetle is one of the world's most destructive pests of grain products and seeds. It is considered one of the 100 worst invasive species in the world. Infestations are difficult to control because of the insect's ability to survive without food for long periods, its preference for dry conditions and low-moisture food, and its resistance to many insecticides. The fumigant toxicity of myrtle leaf essential oil was assessed against khapra beetle *Trogoderma granarium* Everts. Oil was active against different life stages of the khapra beetle. The adult stage was the most sensitive of all developmental stages to essential oil vapors. Larvae were the most tolerant, with 94% and 100% mortality obtained after exposure of larvae to 562.5 μL/L air for 24 and 48 h, respectively. For a 48-h exposure period, LC_{50} and LC_{90} (lethal concentrations) were 221 μL/L air and 487 μL/L air, respectively. These results suggest the possibility of using the leaf essential oil of the myrtle as an insecticide against *T. granarium* Everts in grain stores (Ghaleb et al., 2012).

Salih et al. studied the fumigant toxicity of myrtle oil against the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), both as eggs and adults. The mortality and longevity of *E. kuehniella* adults were estimated

TABLE 2 Antifungal Activity of *Myrtus communis* Extract and Essential Oil

Source	Antifungal Activity							References
M. communis Essential Oil			MIC – 24 h (μg/mL)			MIC – 48 h (ug/mL)		Cannas et al. (2013)
	Candida albicans		0.5–2			1–2		
	Candida glabrata		0.5–4			1–4		
	Candida krusei		1–4			2–4		
	Candida tropicalis		0.25–1			0.5–2		
	Candida parapsilosis		0.5–2			0.5–4		
M. communis Extracts	Against *Candida albicans* • Methanolic extract MIC:1.5 mg/mL & MFC: 3 mg/mL • Ethanolic extract MIC: 0.75 mg/mL & MFC: 1.5 mg/mL • Ethy acetate extract MIC: 0.375 mg/mL & MFC: 0.75 mg/mL • Acetone extract MIC: 0.187 mg/mL & MFC: 0.375 mg/mL							Erdoga et al. (2014)
M. communis Extracts	MIC							Mitra et al. (2013)
	Fungal species	TA	PE	DM	EA	HA	GF	
	Microsporumcanis	11.2	6.0	6.0	19.3	8.1	22.1	
	Microsporumgypseum	9.2	6.0	6.0	16.2	7.1	25.3	
	Tricophytonmentagro-phytes	10.3	6.0a	6.0	17.2	8.1	26.2	
	Growth Inhibition Zones, mm							
	Extracts of Myrtle Leaves, 100μg/disk	Griseofulvin, 6 μg/disk						
	Extracts of Myrtle Leaf, mg/mL	Griseofulvin, μg/mL						
	Fungal SPECIES	TA	PE	DM	EA	HA	GF	
	Microsporumcanis	1.25	5.00<	5.00<	0.187	5.00	1.00	
	Microsporumgypseum	1.25	5.00<	5.00<	0.375	5.00	5.00	
	Tricophyton mentagro-phytes	1.25	5.00<	5.0<	0.37	500	4.00	

TA, total methanolic extract; PE, petroleum ether fraction; DM, dichloromethane fraction; EA, ethyl acetate fraction; HA, hydroalcoholic fraction; GF, griseofulvin; MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration.

TABLE 3 Antioxidant Activity of *Myrtus communis* Extract and Essential Oil

Source	Constituents			References
M. communis essential oils	DPPH assay (IC_{50})			Mimica-Dukić et al. (2010)
	• Myrtle essential oil		6.24 (μg/mL) × 10^3	
Standard antioxidants	• Butylated hydroxytoluene • Butylated hydroxyanisole • Propyl galate		8.62 (μg/mL) 3.09 (μg/mL) 0.42 (μg/mL)	
Leaf and berry extracts of *M. communis* at 2000 μg/mL	**Leaf Extract Type**	**FRAP (absorbance)**	**PRAP (absorbance)**	Tumen et al. (2012)
	Dichloromethane	0.284 ± 0.030	0.316 ± 0.001	
	Acetone	1.352 ± 0.057	0.379 ± 0.012	
	Ethyl acetate	1.876 ± 0.062	0.525 ± 0.018	
	Methanol	3.254 ± 0.115	0.439 ± 0.008	
	Berry Extract Type			
	Dichloromethane	0.732 ± 0.028	0.261 ± 0.007	
	Acetone	3.369 ± 0.046	0.368 ± 0.018	
	Ethyl acetate	3.372 ± 0.158	0.395 ± 0.011	
	Methanol	3.346 ± 0.032	0.460 ± 0.002	
	Chlorogenic acid (reference for FRAP)	3.932 ± 0.005		
	Quercetin (reference for PRAP)		0.989 ± 0.003	
Myrtle liquor	In vitro antioxidant assays			Montoro et al. (2006)
	• FRAP (mmol Fe^{2+}/L)		26.7 ± 4.7	
	• DPPH (mmol TEAC/L)		9.3 ± 0.6	
	• ABTS+ (mmol TEAC/L)		11.5 ± 0.9	
	• EC50 (%)		4.97	

Continued

TABLE 3 Antioxidant Activity of *Myrtus communis* Extract and Essential Oil—cont'd

Source	Constituents		References
Myrtle infusion 5 min infusion time	• Total phenols (mg GAE/g DM)	146.74 ± 1.32	Messaoud et al. (2012)
	• Flavonoids (mg RE/g DM)	6.07 ± 0.73	
	• DPPH scavenging capacity (IC_{50}, μg DM/mL)	356.14 ± 1.15	
	• β-Carotene bleaching capacity (IC_{50}, μg DM/mL)	247.91 ± 2.08	
	• Chelating ability capacity (IC_{50}, μg DM/mL)	223.56 ± 4.1	
	• FRAP (mmol Fe^{2+}/mL)	31.26 ± 0.92	
M. communis leaves extract (microwave-assisted extraction; MAE)	• TPC (mg GAE/g)	162.49 ± 16.95	Dahmoune et al. (2015)
	• ABTS scavenging (IC_{50}(lg GAE/mL))	38.20 ± 1.08	
	• DPPH scavenging (IC_{50}(lg GAE/mL))	16.80 ± 0.29	
	• ORAC value (μmol TE/g)	757.77 ± 31.18	
M. communis leaves extract (ultrasound-assisted extraction; UAE)	• TPC (mg GAE/g)	144.77 ± 30.23a	
	• ABTS Scavenging (IC_{50}(lg GAE/mL))	71.81 ± 2.19	
	• DPPH scavenging (IC_{50}(lg GAE/mL))	20.61 ± 1.66a	
	• ORAC value (μmol TE/g)	428.48 ± 28.00b	
M. communis leaves extract (conventional solvent extraction; CSE)	• TPC (mg GAE/g)	128.00 ± 18.07	
	• ABTS scavenging (IC_{50}(lg GAE/mL))	39.85 ± 1.13	
	• DPPH scavenging (IC_{50}(lg GAE/mL))	21.56 ± 0.10	
	• ORAC value (μmol TE/g)	459.00 ± 53.49	
M. communis methanolic extract	Reducing power (EC_{50} mg/mL)		
	• Leaf	0.45 ± 0.007	
	• Stem	0.49 ± 0.013	
	• Berry extract	3.41 ± 0.247	
	DPPH scavenging assay (EC_{50} mg/mL)		
	• Leaf	0.47 ± 0.021	
	• Stem	0.25 ± 0.005	
	• Berry extract	1.09 ± 0.02	

M. communis ethyl acetate fraction extract	Reducing power (EC_{50} mg/mL)		Kanoun et al. (2014)
	• Leaf	0.26 ± 0.004	
	• Stem	9.87 ± 2.213	
	• Berry extract	12.63 ± 1.312	
	DPPH scavenging assay (EC_{50} mg/mL)		
	• Leaf	0.09 ± 0.002	
	• Stem	0.87 ± 0.015	
	• Berry extract	0.60 ± 0.054	
M. communis Butanolic fraction extract	Reducing power (EC_{50} mg/mL)		
	• Leaf	0.26 ± 0.004	
	• Stem	9.87 ± 2.213	
	• Berry extract	12.63 ± 1.312	
	DPPH scavenging assay (EC_{50} mg/mL)		
	• Leaf	0.21 ± 0.007	
	• Stem	0.58 ± 0.018	
	• Berry extract	0.51 ± 0.037	
M. communis tannins	Reducing power (EC_{50} mg/mL)		
	• Leaf	0.41 ± 0.016	
	• Stem	1.64 ± 0.026	
	• Berry extract	0.89 ± 0.046	
	DPPH scavenging assay (EC_{50} mg/mL)		
	• Leaf	0.22 ± 0.004	
	• Stem	0.32 ± 0.011	
	• Berry extract	0.35 ± 0.006	

Continued

TABLE 3 Antioxidant Activity of *Myrtus communis* Extract and Essential Oil—cont'd

Source	Constituents		References
Anthocyanins	Reducing power (EC_{50} mg/mL)		
	• Leaf	–	
	• Stem	–	
	• Berry extract	0.21 ± 0.000	
	DPPH scavenging assay (EC_{50} mg/mL)		
	• Leaf	–	
	• Stem	–	
	• Berry extract	0.20 ± 0.008	
Ascorbic acid	Reducing power (EC_{50} mg/mL)		
	• Leaf	–	
	• Stem	0.06 ± 0.00	
	• Berry extract	–	
	DPPH scavenging assay (EC_{50} mg/mL)		
	• Leaf	–	
	• Stem	0.12 ± 0.000	
	• Berry extract	–	

FRAP: Ferric Reducing Ability of Plasma; TPC: Total Phenolic Content; EC_{50}: Effective Concentration; ORAC: Oxygen Radical Absorbance Capacity.

after essential oil treatments. The LC_{50} of 19.75 and LC_{99} of 33.79 μL/L air after 9-h exposure, whereas 90.00 ± 5.00% larvae emergence; 48.00 ± 2.00% adult; 44% of decrease in egg production; after 24 h exposure at dose of 90 μL/L air was observed. These results suggest that myrtle essential oil can be used as fumigant for stored product pests (Salih et al., 2011).

SUMMARY POINTS

- *Myrtus communis* Linn is an aromatic evergreen perennial shrub or small tree.
- It is native to Southern Europe, North Africa, and West Asia.
- Myrtle essential oil is composed of terpenes (monoterpene and sesquiterpene hydrocarbons), terpenoids (oxygenated monoterpenes and sesquiterpenes), and phenylpropanoids.
- Myrtle essential oil and extracts have antibacterial, antifungal, and antioxidant properties.
- Essential oil has been proved to be fumigant and insecticidal against various food pests.
- Myrtle can be used in food preservation, but more specific research has to be done on different food products.

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Chapter 67

Neem (*Azadirachta indica*) Oils

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List of Abbreviations

% percentage
°C degree centigrade
AFM Atomic force microscopy
cm centimeter
DPPH 1,1-diphenyl-2-picryl hydrazyl
EC Effective concentration
FTIR Fourier transform infrared spectroscopy
g gram
m meter
MBC minimum bactericidal concentration
MIC minimum inhibitory concentration
mL milliliter
mm millimeter
ppm parts per million
SEM Scanning electron microscopy
TBARS Thiobarbituric acid reactive substances
TEM Transmission electron microscopy
µg micro gram
µL microliter

INTRODUCTION

Neem (*Azadirachta indica*) has been used for centuries in Asia as an insecticide, fungicide, and anticonceptional in popular medicine. Almost every part of the neem tree—bark, branches, leaves, roots, seeds, and the trunk—has a biological application. Neem oil is extracted from the seeds of *A. indica* and has versatile medicinal and biological properties, including antibacterial, antifungal, antifertility, immunostimulant, antipyretic, and acaricidal activities. These applications are a result of the active phyochemicals present in neem: azadirachtin, gedunin, isomargolonone, margolone, margolonone, nimbidin, nimbin, nimbolide, salannin, etc. The antimicrobial and antioxidant properties of neem oil suggest its potential for food preservation against microbial spoilage.

BOTANICAL ASPECTS

Neem (*A. indica*) is a large tree growing about 15–20 m in height. The trunk of the tree is semi-straight to straight, with an average diameter of 3 m; it spreads its branches to form a broad, crown-like structure. Generally, a neem tree becomes productive after 3–5 years and starts fruiting.

The fruit shape varies from oval to round; ripened fruit has a size of 1.4–2.8 cm length and 1.0–1.5 cm diameter. The fruit morphology can be divided into an outer thin layer of skin (exocarp), middle pulpy layer (mesocarp), and an inner layer composed of seeds (endocarp) covered with a seed coat. Table 1 shows the taxonomic classification of neem (*A. indica*).

The structure of a neem leaf is simple, having a single leaf blade. The leaf blade is attached to the stem by a small petiole. The neem leaf arrangement is opposite: two neem leaves grow opposite to each other at a single node. Each neem leaf has 10–12 dark green elliptic leaflets with serrated margins and pinnate venation (i.e., the leaflets are arranged along the mid-vein), approximately 7 cm in length and 2.5 cm in width.

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TABLE 1 Taxonomic classification of neem (*Azadirachta indica*)

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	*A. indica*

Neem plants are angiosperms that bear flowers and belong to the magnoliophyta division. Flowers are white in color. The flower inflorescence bears almost 150–200 flowers, of approximately 5 mm length and 8 mm width.

The neem tree can acclimatize to a wide range of climatic, geographic, and topographic factors. The tree requires sunlight in abundance but does not need much water for growth. Hence, it cannot withstand water-logged areas or areas with poorly draining soil. The neem tree can survive in areas with temperature up to 49 °C. The neem tree grows in almost all soil types, including alkaline, clay, and saline.

The neem tree is distributed widely all over the world. It grows in India and other southeast countries, such as Bangladesh, Burma, Indonesia, Malaysia, Pakistan, Sri Lanka, and Thailand. Neem trees also can be seen growing in 72 different countries worldwide in Asia, Africa, America, and Australia (Koul et al., 1990).

USAGE AND APPLICATIONS

Neem has wide-ranging use in Ayurveda, homeopathic, and Unani medicine. Different parts of neem plants are used for the treatment of an assortment of diseases, including acne, chronic wounds, diabetic foot, eczema, gangrene, and ringworm. Boiled neem extract is used to clean wounds and also for blood purification and toxin removal. Neem plant parts are reported to possess extensive biological activities such as acaricidal, antibacterial, anticarcinogenic, antifungal, antihyperglycaemic, anti-inflammatory, antimalarial, antimutagenic, antiprotozoan, antioxidant, antiulcer, antiviral, immunomodulatory, insect-repellent, and spermicidal properties (Figure 1).

The biological properties of neem are attributed to the more than 135 active phytochemical constituents—including alkaloids, carotenoids, flavonoids, ketones, phenolic compounds, steroids, and triterpenoids—that have been isolated from different parts of neem plant. These compounds include azadirachtin, gedunin, isomargolonone, margolone, margolonone, nimbidin, nimbin, nimbolide, and salannin. The biological activities of few phytochemicals are listed in Table 2.

Neem plant parts are also reported to possess insect-repellent and larvicidal properties. Anjali et al. (2011) reported the larvicidal effects of neem oil based nanoemulsion against *Aedes aegypti* larva. The possible mode of action of insecticidal or larvicidal activity of neem oil is explained in Figure 2. Azadirachtin in neem oil is reported to block the release of prothoracicotropic hormones, thereby downregulating of hemolymph ecdysteroid levels and disruption of endocrine events. This phenomenon consequently leads to molting disruption and growth inhibition of mosquito larva.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Neem essential oil is used in the preservation of food against microbial spoilage because of the antimicrobial properties of its active principles. The active principles in the neem oil are sparingly soluble in water but are lipophilic and very much soluble in organic solvents such as methanol, ether, and alcohols.

A report on neem tree morphology and its correlation with oil content says that the content of neem seed oil and the oil yield (%) corroborates with the morphological parameters of seeds but not with the age of the neem tree. Hence, it is required to improve neem populations by afforestation so that better neem fruits will produce oils of higher quality and better yield (%). The report also states that kernal weight of fruit ranging from 31.9% to 21.1% correlated with oil content of 23.8% and 15.4%, respectively (Muñoz-Valenzuela et al., 2007).

FIGURE 1 Various applications of neem.

TABLE 2 Applications of different bioactive compounds in neem

Plant part	Bioactive compound	Activity
Oil	Azadirachtin, gedunin, nimbidin, nimbin, nimbolide	Antiarthritic, antibacterial, antifungal, antigastric ulcer, antiinflammatory, antimalarial, antipyretic, diuretic, hypoglycaemic, spermicidal
Leaf	Cyclic tetrasulphide, cyclic trisulphide	Antifungal
Bark	Margolone, margolonone, isomargolonone, polysacharrides	Antibacterial, antitumor, anti-inflammatory

Antibacterial Activity

Hoque et al. (2007) studied the antibacterial activity of neem essential against six different foodborne pathogens, such as *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococcus aureus,* and *Vibrio parahaemolyticus*; and four different food-spoilage bacteria such as *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*.

Upadhyay et al. (2010) studied the antibacterial activity of neem oil against five Gram-positive bacteria such as *B. cereus*, *Lactobacillus acidophilus*, *Micrococcus luteus*, *S. aureus*, and *Streptococcus pneumoniae*; and two Gram-negative bacteria such as *E. coli* and *Klebsiella pneumoniae*. The lowest minimum inhibitory concentration (MIC) value was obtained against *L. acidophilus* (0.125 μL/mL) and the lowest minimum bactericidal concentration (MBC) value was

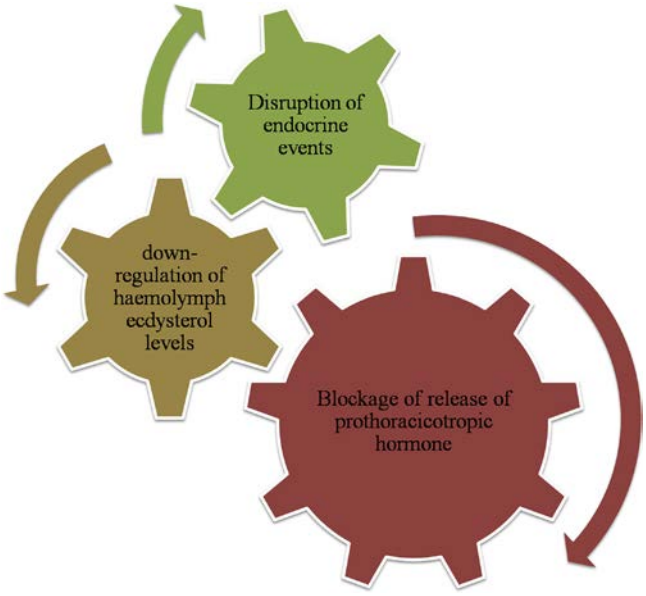

FIGURE 2 Azadirachtin disrupts molting and inhibits growth of mosquito larva.

obtained against *L. acidophilus* and *S. pneumonia* (0.25 µL/mL). The MIC value of neem oil against *L. acidophilus* is lower than that of antibiotics tetracycline (0.892 µL/mL), ampicillin (0.223 µL/mL), and ciprofloxacin (0.223 µL/mL). Also, the MBC value of neem oil against *L. acidophilus* is lower than that of the antibiotics tetracycline (0.915 µL/mL), ampicillin (0.915 µL/mL), and ciprofloxacin (3.66 µL/mL).

The quantification of the antibacterial activity of neem essential oil was also determined by an agar disc diffusion method and expressed as zone of growth inhibition around the filter paper disc. The highest growth inhibition zone was obtained against *B. cereus* (45.63 ± 0.23). A comparatively smaller growth inhibition zone was obtained using antibiotics such as tetracycline (19.06 ± 0.08), ampicillin (14.73 ± 0.29), and ciprofloxacin (23.03 ± 0.12) against *B. cereus*. The present work shows considerably higher antibacterial activity for neem essential oil than for antibiotics such as tetracycline, ampicillin, and ciprofloxacin.

Imran et al. (2010) studied the phytochemical analysis of neem (*A. indica*) leaves by using different organic solvents, such as chloroform, methanol, and petroleum ether, and confirmed the presence of fatty acids, glycosides and triterpenes. Antibacterial activity of *A. indica* demonstrated the significant activity of chloroform extract of leaves compared with methanol extracts and petroleum ether extracts. Prior to this, there were reports of ethanol being the most efficient solvent for extraction of antibacterial bioactive compounds from plants.

Neem extract also demonstrated antimicrobial activity against human pathogenic bacteria, such as *Bacillus pumilus*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *Salmonella typhimurium* in concentrations of 25 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm, respectively, by a disc diffusion method (Maragathavalli et al., 2012). Neem extract demonstrated better antibacterial activity against *B. pumillus*, *P. aeruginosa*, and *S. aureus,* in increasing order of efficiency. Also, neem extract exhibited comparatively better antibacterial activity than the standard antibiotic gentamycin.

Antibacterial activity of neem essential oil can be assessed by checking morphological and functional alterations (Figure 3). Functional alterations in bacteria upon treatment with essential oil change membrane permeability. This can be investigated by crystal violet staining and acridine orange/ethidium bromide staining. Acridine orange is permeable to all cells and stains green, whereas ethidium bromide is permeable to cells with compromised membranes and stains red. Hence, staining with acridine orange/ethidium bromide will result in green staining of live cells and red staining of damaged cells. Morphological alterations can be visualized by different microscopic techniques such as scanning electron microscopy, transmission electron microscopy, and atomic force microscopy. Changes in functional groups on the bacterial surface can be confirmed by Fourier transform infrared spectroscopy. Hydrophobic compounds in neem oil fuse with lipid components of bacteria cell walls, thereby damaging cell walls and causing leakage of intracellular components.

Plant essential oil-based nanoemulsion has been reported to have antibacterial and larvicidal activity (Ghosh et al., 2012, 2013a,b,c,d; 2014). Similarly, antibacterial neem oil formulations can be used for food preservation.

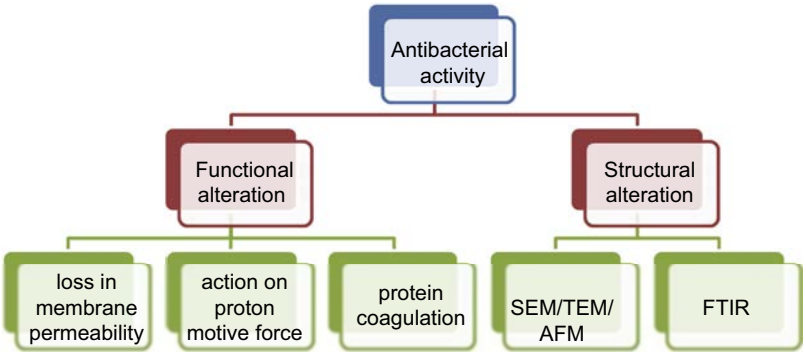

FIGURE 3 Possible mode of antibacterial action of neem oil.

Antibacterial Activity In Vivo

Serrone and Nicoletti (2013) worked on the antimicrobial activity of neem (*A. indica*) cake against bacteria spoiling the quality of retail fresh meat in a broth model meat system. Antibacterial activity was investigated against *Campylobacter jejuni*, *Carnobacterium* spp., *Lactobacillus curvatus*, *Lactobacillus sakei*, *Leuconostoc* spp., *E. coli*, *Enterococcus faecalis*, and *S. aureus*. Greater growth reduction was observed against *S. aureus* (91.80 ± 3.21%) followed by *L. curvatus* (90.73 ± 1.53%), *E. faecalis* (90.61 ± 1.15%), *L. sakei* (89.81 ± 1.00%), *E. coli* (89.75 ± 1.53%), *Leuconostoc* spp. (89.71 1 ± 0.29%), *Carnobacterium* spp., (88.90 ± 1.00%), and *C. jejuni* (88.88 ± 0.61%) after being treated with 10 µg of neem cake for 24 h of incubation time.

Antifungal Activity

The antimicrobial components in neem oil are reported to be potent inhibitors of the biosynthesis of aflatoxin, a naturally occurring mycotoxin that is extracted from the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Cross-sections and longitudinal sections of fungal mycelia and vesicles demonstrated alterations in the morphology of mycelium, vacuoles, and vesicles. The intracytoplasmic components were bulging out, causing a deformation of the mycelium shape. These results also corroborated with microscopic observations that the biosynthesis of Aflotoxin is inhibited by neem extract by mycelium deformation and damage (Abyaneh et al., 2005).

Sitara et al. (2008) reported the antifungal activity of neem essential oil against pathogenic fungi, such as *Aspergillus niger*, *A. flavus*, *Drechslera hawiinesis*, *Fusarium moniliform*, *Fusarium oxysporum*, *Fusarium nivali*, and *Fusarium semitectum*. Of these, better antifungal activity occurred against *F. moniliform* (zone of inhibition = 0.33 ± 0.577 at 0.5% concentration of neem essential oil). Neem oil showed better antifungal activity than a standard fungicide (i.e., Ridomyl Gold; zone of inhibition = 3.6 ± 0.1 at 0.5% concentration of fungicide) against *F. moniliform*.

Growth medium plays an important role in determining the antifungal activity of neem oil (Mohanty et al., 2008). The growth inhibition of *Lagenidium giganteum* mycelium by neem essential oil was lower than that of *Metarhizium anisopliae* in both PYG agar medium (peptone:yeast extract:glucose:agar:distilled water = 1.25:1.25:3.0:20.0:11.0) and Emerson's YpSs agar media (yeast extract:soluble starch:dipotassium hydrogen phosphate:magnesium sulfate:agar:distilled water = 4:15:1:0.5:20:11). However, when grown in sunflower seed extract agar medium, neem essential oil did not inhibit mycelia growth of *L. giganteum* but inhibited the *M. anisopliae* mycelial growth. The MIC value of neem essential oil for *L. giganteum* was higher than that for *M. anisopliae* in all three growth media: 413.7 ± 16.74 ppm/104.94 ± 3.65 ppm, 187 ± 4.72 ppm/78.43 ± 4.91 ppm, and 0 ppm/72 ± 2.48 ppm for *L. giganteum/M. anisopliae* in PYG Emerson's agar medium, YpS's agar medium, and sunflower seed extract medium, respectively.

Neem formulations such as Achook (0.15% E.C.), Bioneem (0.03% E.C.), Neemark (0.03% E.C.), and Nimbicidine (0.03% E.C.), which are commercially available, possess antifungal activity against pathogenic fungi such as *Alternaria solani*, *Curvularia lunata*, *F. oxysporum*, *Helminthosporium* sp., and *Sclerotium rolfsii* (Bhonde et al., 1999). The antifungal activity of neem seed kernel extract was also reported against postharvest diseases (Wang et al., 2010). Pathogens such as *Alternaria alternate*, *Monilinia fructicola*, *Penicillium expansum*, and *Trichothecium roseum* were isolated from the infected fruits and were incubated with neem extract. Growth of all the four fungal pathogens was considerably reduced after being treated with neem extract. Furthermore, the neem extract significantly prevented disease in fruits such as plum (*Prunus salicina*) and Yali pear (*Pyrus bertschneideri*) inoculated with the fungal pathogens.

Antioxidant Activity

Different parts of the neem plant, such as leaves, fruits, flowers, and stem bark extracts, have been reported to possess antioxidant activity. Sithisarn et al. (2005) assessed antioxidant activity in vitro by using various methods: 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay, total antioxidant activity, and inhibition of lipid peroxidation in Chago K1 cancer cell culture by the thiobarbituric acid reactive substances (TBARS) method. Outcomes of the study showed 50% DPPH scavenging activity for leaf, flower, and stem bark extracts at 26.5 g/mL, 27.9 g/mL, and 30.6 g/mL, respectively.

Agricultural Use

Approximately one-third of world's agricultural production gets damaged by more than 20,000 species of field and storage insect and pest species. In India, pest infection results in a huge loss of about Rs. 50,000 million worth of agricultural productivity per year. Synthetic pesticides are applied to prevent this loss, which destroy the target pests. However, these pesticides also affect nontarget organisms, contaminate ecosystems, result in resistant development in pests, and lead to outbreaks of secondary pests. Hence, there is a need for cost-effective and ecofriendly bio-based pesticides. Plant-based essential oils are the best solution to these problems. Among different plant essential oils, neem oil is reported to have significant insectide and insect-repellent properties. Hence, neem oil can be used in integrated pest management to increase the shelf life of storage food against pest spoilage.

SUMMARY POINTS

- Neem (*A. indica*) oil is extracted from neem seed.
- Neem oil yield depends on the morphological parameters of seeds but not the age of the neem tree.
- Bioactive compounds can be extracted from different plant parts, such as the bark, leaf, seed, and branches.
- Neem oil contains various phytochemicals, such as azadirachtin, gedunin, somargolonone, margolone, margolonone, nimbidin, nimbin, nimbolide, and salannin.
- Neem oil exhibits a wide range of biological activities, including antiarthritic, antibacterial, antifungal, antigastric ulcer, anti-inflammatory, antimalarial, antipyretic, diuretic, hypoglycaemic, and spermicidal activities.
- Azadirachtin in neem oil disrupts molting and inhibits growth of mosquito larva.
- Growth medium plays an important role in determining the antifungal activity of neem oil.
- Neem oil has higher antibacterial activity than antibiotics such as tetracycline, ampicillin, and ciprofloxacin.
- Neem (*A. indica*) cake is reported to have antimicrobial activity against bacteria that spoil the quality of retail fresh meat in a broth model meat system.

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Chapter 68

Negundo Chastetree (*Vitex negundo*) Oils

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List of Abbreviations

AChE Acetylcholinesterase
CarE Carboxylesterase
CAT Catalase
GC/MS Gas chromatography/mass spectroscopy
L Linnaeus
LC_{50} Lethal concentration 50
VN *Vitex negundo*

INTRODUCTION

The five-leaved chaste tree (*Vitex negundo* L.) is an aromatic medicinal shrub commonly found in tropical, subtropical, and warm temperate regions throughout the world, being native to South Asia, China, Japan, Indonesia, East Africa, and South America (Zheng et al., 2009a). All parts of this plant have been used as folk medicine for a long time in China and other Asian countries. *Vitex negundo* (VN) leaves and seeds are two medicinal parts frequently used for the treatment of various disorders, mainly exhibiting analgesic, anti-inflammatory, antirheumatic (Zheng et al., 2009a,b), antitumor (Zhou et al., 2009), and antioxidant activities (Zheng et al., 1999). Phytochemical investigations of VN revealed the presence of bioactive lignans (Zheng et al., 2009a; Ono et al., 2004), terpenoids (Chawla et al., 1992; Zheng et al., 2010; Ono et al., 2004), flavonoids (Bhargava, 1989), and volatile oil (Singh et al., 2010; Lu et al., 2009).

Modern pharmacological research on VN constituents mainly focuses on its flavonoids, lignans, and terpenoids, with little medicinal interest cast on VN volatile oils. Despite the lack of medicinal benefits reported, volatile oils from VN leaves and seeds found their merits in food science, possessing significant insecticidal (Liu et al., 2013; Lu et al., 2011) and antimicrobial (Singh et al., 2010; Khokra et al., 2008) effects for food protection. Both VN leaves and seeds have also been claimed to have edible purposes, being occasionally used as a condiment and tea, and even as a famine food when all else fails (Kunkel, 1984). The edible uses of VN leaves and seeds further confirm the safety of VN volatile oil and make it an optimal selection for food protection against infestation and bacteria contamination.

BOTANICAL DESCRIPTIONS

VN is an erect, branched, and shedding shrub or small tree that is 2–5 m high (Figure 1). Its bark surface is slightly rough, peeling off in papery flakes of pale reddish-brown. Leaves are opposite, with digitately 3–5 foliolate pattern (Figure 1). Leaflets are lanceolate and of the entire margin, 3–10 cm long with green above and gray pubescent beneath. Panicles are terminal, slightly hairy and many-flowered, being 10–20 cm long. Additional axillary ones are often present. It flowers from May to August. The scented flowers are hermaphrodite (i.e., they have both male and female organs) and are pollinated by insects. Flowers are blue to lavender, 5–7 mm long. Fruits are globose to broadly egg-shaped, black when mature in October, 3–6 mm long, and 2–3 mm in diameter.

VN is an easily grown plant; it prefers a light, well-drained loamy soil in a warm sunny position, and succeeds in poor dry soils. VN is always found in humid places or along watercourses, in thickets, waste places, and mixed open forest. It can be propagated by use of vegetative cuttings, viable seeds, and root suckers, and it has been grown for a long time in China, India, Japan, Philippines, and other Asian countries for various medicinal purposes (Figure 2). In addition to the above-mentioned conventional propagation methods, in vitro culture techniques have been established for micropropagation of VN through high-frequency axillary shoot proliferation (Sahoo and Chand, 1998).

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FIGURE 1 *Vitex negundo* plant. The five-leaved chaste tree is a small aromatic plant with typical five-foliolate leaf pattern that flourishes abundantly in wastelands and is widely distributed in tropical to temperate regions.

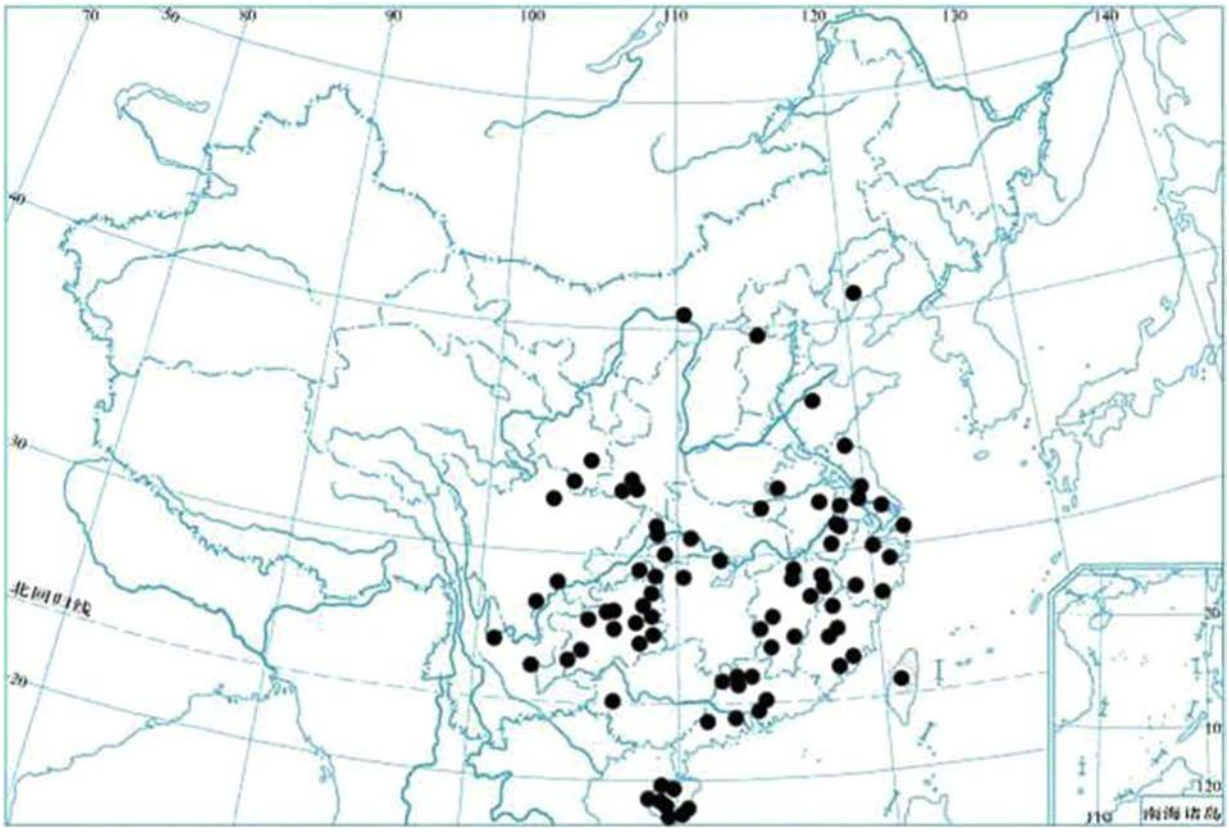

FIGURE 2 Geographical distribution of *Vitex negundo* in China. The five-leaved chaste tree is widely distributed in southern China, up to an altitude of 1500 m.

USAGE AND APPLICATIONS

All parts of VN, including leaves, flowers, twigs, roots, and seeds, have been commonly used for various applications in folk medicine for a long time in China and other Asian countries. VN leaves and seeds are the most frequently used medicinal parts. VN leaves are used in folk medicine as a cure for arthritis and vermifuge (Singh et al., 2010), showing anti-inflammatory, analgesic, antioxidant, and antihyperglycemic activity (Vimal et al., 2011), while VN seeds have been claimed to possess analgesic, anti-inflammatory, antirheumatic, antitumor, antioxidant, sedative, and hepatopreventive

TABLE 1 Antimicrobial Spectrum of the Essential Oil of *Vitex negundo*

Activity	Medicinal Part	Microorganisms Involved	Action Mode	References
Insecticidal effects	Leaves	*Sitophilus zearnais* adults	Fumigation effect; contact toxicity; repellency; population restriction	Lu et al. (2005) Lu et al. (2009)
	Leaves	*Rhizopertha dominica* *Tribolium castaneum* *Oryzaephilus surinamensis* *Callosobruchus chinensis*	Contact toxicity; repellent effect; comprehensive toxicity; sustainable population restriction	Lu et al. (2011)
	Leaves	*Sitotroga cerealella* *Culex quinquefasciatus*	Repellent effect	David et al. (1988)
	Seeds	*Sitophilus zearnais* adults	Comprehensive toxicity	Lu et al. (2011)
Antimicrobia effects	Leaves and seeds	*Bacillus subtilis* *Escherichia coli* *Staphylococcus aureus*	Inhibition	Khokra et al. (2008)
	Leaves	*Klebsiella pneumoniae* *Micrococcus luteus* *Candida albicans*	Inhibition	Singh et al. (2010)

properties (Zheng et al., 2009a,b). In addition, both VN leaves and seeds have also been claimed to have edible purposes, being occasionally used as a condiment and tea, and even as a famine food when all else fails (Kunkel, 1984).

As an aromatic plant, VN leaves and seeds are a rich source of volatile oil (Singh et al., 2010; Lu et al., 2009), in addition to the abundance of other secondary metabolites such as flavonoids, lignans, and terpenoids. Essential oil present in the leaves can be used as bathing oil and for sloughing wounds and ulcers (Singh et al., 2010). Despite limited reports of medical benefits, both VN leaves and seed oils revealed significant insecticidal (Liu et al., 2013; Lu et al., 2011) and antimicrobial (Singh et al., 2010; Khokra et al., 2008) activities, which makes VN a natural and green food preservative against various insect pest and microorganism species, with low poison and even no pollution to food and environment (Table 1).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Many plants have been traditionally used to extend the shelf life of foods, exhibiting inhibition against various bacteria, fungi, yeasts, and pest (Kao et al., 1999). These properties can be mostly attributed to the essential oils produced by plant secondary metabolism (Lu et al., 2011). Essential oils from many reported plant species showed strong ability to control pests and microorganisms related to food infestation and spoilage (Adam et al., 1998). Actually, many aromatic plant leaves have been used as spices and teas after drying; in particular, the essential oil is used in cosmetics, pharmaceuticals, and food preservation (Guillen et al., 1996).

Chemistry of Volatile Oils

VN essential oils ae extracted from air-dried leaves or seeds, mainly by steam distillation or supercritical fluid extraction with CO_2, which is then dried over anhydrous sodium sulfate and analyzed by gas chromatography/mass spectroscopy (GC/MS).

Singh et al. (2010) reported that dried VN leaves (collected from Ganeshpur village, Saharanpur) extracted by steam distillation using a Clevenger apparatus yielded 1.6% v/w essential oil, characterized by a high amount of sesquiterpenes (47.14%), monoterpenes (45.50%), and fatty acids (7.36%), with 10 components identified, comprising 100% of the total volatile constituents. α-Copaene (25.26%), β-elemene (19.16%), and camphene (21.10%) were the predominant characterized constituents. The other seven identified compounds were t-caryophyllene (2.72%), linalool (3.69%), α-thujene (9.86%), α-pinene (7.74%), sebinene (3.11%), stearic acid (5.84%), and behenic acid (n-docosanoic acid) (1.52%), respectively (Figure 3).

A total of 31 constituents were identified in the essential oil extracted by steam distillation from VN leaves collected in Tai mountain, Shandong Province, accounting for 91.6% of the total volatile fraction. Caryophyllene (35.97%) and 1, 8-cineole (8.21%) were found to be the major compounds. A total of 88.8% of the identified volatile constituents were terpenoids (Lu et al., 2009). These results were in part consistent with previous data published by Chen et al. (1999).

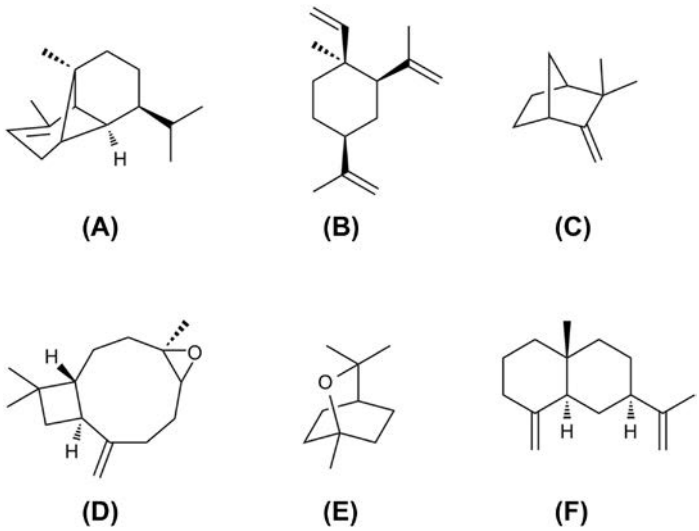

FIGURE 3 Main constituents reported in the essential oil of *Vitex negundo*. (A) α-copaene; (B) β-elemene; (C) camphene; (D) caryophyllene oxide; (E) 1,8-cineole; (F) β-selinene.

Lu et al. (2011) evaluated the extraction efficiency of different extraction methods for obtaining essential oils from VN leaves and seeds; they confirmed that the extraction efficiency of supercritical fluid extraction with CO_2 was 5.39 and 322.5 times higher than that of steam distillation as for leaves and seeds, respectively.

Khokra et al. (2008) compared the essential oils from fresh leaves and dried seeds of VN (collected in Kurukshetra, Haryana, India) obtained by hydrodistillation. The chemical constituents of essential oil of leaves and dried seeds were analyzed using a GC/MS technique. The main constituents identified in leaves oil were δ-guaiene, caryophyllene oxide, and ethyl-hexadecenoate, while fruit oil showed β-selinene, α-cedrene, germacrene D, and hexadecanoic acid as the main constituents.

Both the compositions and their percentage of above-mentioned volatile oils varied considerably with that in other reports, which might be due to the results of different origins of VN samples, different collected seasons, different living environment conditions, or different chromatographic conditions in GC–MS analysis.

Insecticidal Bioactivity

Plant essential oils have been extensively utilized in the control of insect pests for their advantage of high volatility, safety, environmental friendliness, and very low residue, which are therefore a valuable source for the development of fumigant and repellent for food storage.

VN essential oils have been proven to possess significant insecticidal activities against many stored-product insect pests, with broad insecticidal scope. Lu et al. (2005) demonstrated the significant fumigation effect, contact insecticide effect, repellency, and population restriction of VN leaves oils against *Sitophilus zearnais* adults. At doses of 50 and 100 $\mu L.L^{-1}$, VN leaves volatile oil caused 92.1% and 100% mortality of *Sitophilus zeamais* adults after 72 h exposure, while its contact insecticide LC_{50} value is 0.3377 $mg.L^{-1}$. Its repellency rate reached 84.9% after 48 h treatment at dose of 0.2% (w/w), while the toxicity effect amounted to 100% at 4 g/kg after 72 h and at the same dose, the rate of inhibition of F1 was 100% after 55 days with perfect persistence control (Lu et al., 2005). All these data suggest essential oil from VN leaves has immense potential to be exploited as grain protectant. In 2009, Lu et al. continued to investigate the insecticidal bioactive components and toxicity of VN leaves essential oil against *S. zeamais* and their action mechanisms Lu et al. (2009). 1,8-Cineole (8.21%) and α-pinene (0.69%) were proven to be the main constituents responsible for the insecticidal activity of VN leaf oil, which showed high overall toxicity to the adults of *S. zeamais* after 72 h of treatment and the highest one was 1,8-cineole with LC_{50} of 0.7171 $g.kg^{-1}$. At dose of 2.0 $g.kg^{-1}$, 1,8-cineole, α-pinene, and VN oil could control F1 population effectively, with inhibitory rates of 100%, 85.45%,and 89.73%, respectively; at dose of 4 $g.kg^{-1}$, they all exhibited distinctly repellent effects with repellency rates of 96.49%, 84.26%, and 90.61%, respectively. The contact toxicity LC_{50} values of 1,8-cineole, α-pinene, and VN oil to *S. zeamais* were 0.2690, 0.7529 and 0.2969 $mg.cm^2$, respectively, while their fumigation LC_{50} values were 14.053, 28. 648, and 21.429 $\mu L.L^{-1}$, respectively, after 72 h of treatment. Furthermore, VN leaf oil and its main bioactive components of 1,8-cineole and α-pinene could all affect the breath of *S. zeamais* and distinctly inhibited the activities

of acetylcholinesterase, catalase, and carboxylesterase of *S. zeamais* in vitro, suggesting the diverse characteristics of action mechanisms of VN essential oil and its main components. Besides the insecticidal effect against *S. zeamais*, VN leaf and seed essential oils are also claimed to possess high contact toxicity, repellent effect, comprehensive toxicity, and sustainable population control effect to *Rhizopertha dominica*, *Tribolium castaneum*, *Oryzaephilus surinamensis*, and *Callosobruchus chinensis* (Lu et al., 2011). Lu et al. (2011) also compared the comprehensive toxicity of leaf and seed oil against the above-mentioned insect pests and confirmed VN leaf oil obtained by steam distillation showed the strongest activity.

VN leaf essential oil also exhibited a potential repellent effect against *Sitotroga cerealella* and *Culex quinquefasciatus* (David et al., 1988). In addition, VN leaf and seed liposoluble extracts could also enhance the insecticidal effects of imidacloprid and other insecticides (Liu et al., 2013).

Antimicrobial Activity

Essential oils from VN leaves and seeds exhibit broad-spectrum antimicrobial activity (including Gram-negative/positive bacteria, coccus and bacillus, and fungi). Leaf and seed oils demonstrated promising results against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* (Khokra et al., 2008). Leaf oil was also found to be active against the following pathogenic microorganisms: *Klebsiella pneumoniae*, *Micrococcus luteus*, and *Candida albicans* (Singh et al., 2010). In addition, VN leaf extract showed convincing inhibitory effects against three soybean fungal pathogens (*Colletotrichum truncatum*, *Fusarium oxysporum*, and *Macrophomina phaseolina*, which cause the pod blight, wilt/root rot, and charcoal rot diseases of soyabean, respectively) (Arora and Kaushik, 2003); it also suppressed mycelium growth and sclerotia initiation of *F. oxysporum*, a charcoal rot and wilt pathogen of cowpea (Ushamalin et al., 1997).

Safety

Both VN leaves and seeds are reported to be edible (Kunkel, 1984). Edible uses of VN leaves and seeds further confirm the safety of VN volatile oil and make it an optimal selection for food protection against infestation and bacteria contamination.

SUMMARY POINTS

- The essential oil of *Vitex negundo* leaves demonstrate strong insecticidal properties against many stored-product insect pests.
- The essential oil of *V. negundo* leaves exhibited broad-spectrum antimicrobial activities.
- 1,8-Cineole and α-pinene were recognized as the major bioactive constituents in the essential oil of *V. negundo* leaves.
- The essential oil of *V. negundo* leaves can be an ideal food protectant to facilitate food preservation.

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Chapter 69

Nutmeg (*Myristica fragrans* Houtt.) Oils

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INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.), belonging to the family Myristicaceae, is a spice seed from the fruit of a tropical, dioecious evergreen tree called *M. fragrans* Houtt. (Gils and Cox, 1994), which is native to the Moluccas, or Spice Islands, of Indonesia. Nutmeg has a distinctive, pungent fragrance and a warm, slightly sweet taste; it is used to flavor many kinds of baked goods, confections, puddings, meats, sausages, sauces, vegetables, and some beverages such as eggnog. Grated nutmeg has been used as a sachet; the Romans used it as incense.

Nutmeg has been reported to have aphrodisiac, stomachic, carminative, tonic, nervous stimulant, aromatic, narcotic, astringent, hypolipidemic, antithrombotic, antifungal, antidysentric, and anti-inflammatory (Tajuddin et al., 2005) properties. It is said to be antihelminthic and is also used against skin diseases such as eczema and scabies.

Phytochemical studies indicate that nutmeg contain a volatile oil, a fixed oil, proteins, fats, starch, and mucilage. The fixed oil contains myristin and myristic acid. Nutmeg yields 5–15% of volatile oil, which contain pine, sabincene, camphene, myristin, elemicin, isoelemicin, eugenol, isoeugenol, methoxyeugenol, safrole, diametric phenylpropanoids, lignas, and neolignas (Isogai et al., 1973; Janssen and Laeckman, 1990). Nutmeg is reported to be useful in paralysis and increases blood circulation. It is also demonstrated to have antioxidant properties (Murcia et al., 2004; Olaleye et al., 2006) and antidiarrheal properties (Parle et al., 2004). This chapter focuses on the botanical aspects, uses, and applications of nutmeg in food sciences, production of essential oil, and its composition.

BOTANICAL ASPECTS

The nutmeg tree has several botanical names, as *Myristica officinalis*, Linné, *Myristica moschata*, Thunberg, *Myristica aromatica*, Lamarck, and *M. fragrans*, Houttuyn; the latter is now generally adopted by botanists. The nutmeg tree is indigenous to the Molucca Isles and is raised in Sumatra, the Mauritius, French Guiana, and various places in the West Indian islands. Commercially, the nutmeg tree is grown on the Caribbean islands of Grenada and Trinidad as well as in Central and East Java.

This tree is 20–25 feet high, having a greyish-brown, smooth bark, and abounding in a yellow juice; the branches are spreading, in whorls. The leaves are alternate on petioles from 0.5 to 0.75 in long, plane above, or oblong, approaching to elliptical, subbifarious, glabrous, rather obtuse at the base, acuminate, quite entire, aromatic, dark-green, glossy above, paler beneath, and from 3 to 6 in long.

The flowers are dioecious and small, in axillary, subumbellate racemes, sometimes forked, or compound. The peduncles and pedicels are glabrous, with the latter having a quickly deciduous, ovate bract at its summit, often pressed close to the flower. Male flowers, three to five or more, are on a peduncle. The female flowers are scarcely different from the male, except that the pedicel is very frequently solitary.

The fruit is a fleshy pericarp and nearly spherical; it is the size and somewhat of the shape of a small pear. The flesh is astringent, yellowish, almost white within; four or five lines thick; and opening into two, nearly equal, longitudinal valves. The arillus (mace) is thick, between horny and fleshy, much laciniated, folded and anastomosing toward the extremity, almost enveloping the nut—so tightly as to form inequalities on its surface. When fresh, it is brilliant scarlet; when dry, it is much more horny, of a yellowish-brown color, and very brittle (Wallis, 1985; Kolammal, 1979).

The nut is oval or broadly ovate, with a hard, rugged, dark-brown, glossy shell, pale, smooth within, about half a line thick. The seed, or nutmeg, is oval, pale-brown, and quite smooth when fresh, but it soon becomes shriveled; it has irregular, vertical lines or furrows on its surface (Figure 1).

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FIGURE 1 Nutmeg (*Myristica fragrans*) (http://www.celkau.in/Crops/Spices/Nutmeg/nutmeg.aspx).

USES AND APPLICATIONS

Nutmeg (*M. fragrans*) is traditionally used in the preparation of sweets (Sanghai-Vaijwade et al., 2011). The seed part of nutmeg fruit has many applications, particularly in the culinary, pharmaceutical, and cosmetic industries (Abdullah et al., 2010). The characteristic pleasant fragrance and slightly warm taste of nutmeg is used to flavor many kinds of baked goods, confections, puddings, meats, sausages, saucers, vegetables, beverages; and as component of curry powder, teas, and soft drinks; or is added to milk and alcohol (Olaleye et al., 2006).

Many countries use nutmeg as a spice. In India and in the Middle East, nutmeg is used in sweet dishes. Europeans use it in most dishes to flavor potatoes, eggs, meats, and even spinach, along soups and sauces. Nutmeg is used as a drug of traditional medicine by Arabs of Israel and Jewish communities, especially Yemennities; there, it is also used as spice and as an important ingredient in "love potions." It is used to manage vomiting and to control the movements of the bowels; to treat tuberculosis, colds, and fever; and in general for respiratory problems. It is reported to have antihelminthic effects and is also used to alleviate skin diseases such as eczema and scabies (Bamidele et al., 2011). In the Unani system of medicine, it is used as alternative medicine in the management of male sexual disorders (Tajuddin et al., 2005).

Moreover, nutmeg is traditionally used for inflammatory conditions and to treat joint and muscle pain, as well as liver disease. Nutmeg oil is helpful to dissolve kidney stones and alleviate infections of the kidney, as well as for the treatment of diarrhea (Sanghai-Vaijwade et al., 2011; Al-Jumaily and Al-Amiry, 2012), rheumatism, and cholera. In addition, it is reported that nutmeg and nutmeg oil have been used for illnesses related to the nervous and digestive systems, such as psychosis, stomach cramps, nausea, vomiting, flatulence, and anxiety, in addition to its use as an aphrodisiac and abortifacient (Al-Jumaily and Al-Amiry, 2012; Saxena and Patil, 2012).

Nutmeg is used in both Western and Chinese herbal medicine, as a muscle relaxant, to eliminate gas from the digestive system, to sedate the body, to alleviate digestion problems, and to reduce abdominal pain. In Indochina, powdered seeds in boiled rice are used as a remedy against dysentery, anorexia, colic, and treat malarial debility. In Indonesia, the mace part of nutmeg is reported to have been used for reducing pain and to treat rheumatism (Al-Jumaily and Al-Amiry, 2012).

USES AND APPLICATIONS IN FOOD SCIENCES

Nutmeg (*M. fragrans* Houtt, Myriticaceae) is an important commodity. It is widely used in the food and medical industries and is one of the oldest traded commodities in the world. It is used extensively in the form of essential oil, powdered, crushed, and whole in food, medicine, and cosmetics. Nutmeg has a characteristic pungent and pleasant fragrance, with a slightly warm and sweet taste. It is used to flavor many kinds of baked goods, breads, confections, puddings, dairy products, meats, sausages, saucers, vegetables, and beverages. It is also used as a component of candies, chewing gum, syrups, curry powder, teas, and soft drinks, or is mixed in milk and alcohol (Olaleye et al., 2006; Shafiei et al., 2012; CAC, 2014; Nutmeg, 2014).

TABLE 1 Nutmeg Products and Their Uses

Nutmeg Product	Uses
Dried whole, ground nutmeg	Flavoring in the food industry: meat and dairy products (sausages, soups, spice mixes, baked products, eggnog, ice cream etc.), both domestic and industrial use
Nutmeg oil	Flavoring of processed foods and beverages
Mace: dried, whole, ground	Domestic and industrial culinary uses as flavorings for sweet foods, cakes, doughnuts, dairy products, and cigarettes
Mace oil	Flavorings in processed foods and baked products; extract used in perfumes, scented soaps, denture creams, and chewing gum

Nutmeg has numerous uses and applications in food sciences; some of different products of nutmeg along with their corresponding uses are presented in Table 1 (CAC, 2014).

Nutmeg is one of the most popular spices, known since olden days for its aromatic, aphrodisiac, and curative properties. Nutritionally, nutmeg is rich source of energy, carbohydrates, proteins, and dietary fiber. It is mainly rich in vitamins A, C, and E, but it also contains electrolytes (sodium and potassium), minerals (magnesium, calcium, copper, iron, zinc, manganese, and phosphorus), and phytonutrients including carotene-B and crypo-xanthin B (Agbogidi and Azagbaekwe, 2013). Because of its aroma, nutmeg's essential oil has been used as a natural flavoring extract and as a perfume in the cosmetic industries. In particular, the oil has been used as a flavoring agent, replacing ground nutmeg in order to avoid leaving particles in foods and beverages. In addition to being nutritionally rich and having widespread use in flavorings, current research seems to focus on the potential use of nutmeg and its essential oil as food preservatives.

Owing to its demonstrated antimicrobial and antioxidant properties, nutmeg essential oil is considered to be a promising biopreservative. Recently, the interest in essential oils (EOs) and their application in food preservation has been amplified because (1) it is desired to reduce the potential health risk and increasing negative consumer perception of synthetic preservatives; and (2) foodborne diseases are a growing public health problem worldwide, calling for more effective preservation strategies (Hyldgaard et al., 2012; Djenane et al., 2013). EOs serve as preservatives through their antimicrobial and antioxidant mechanisms as many of them are effective in retarding microbial growth, food oxidation, and rancidity, making them promising natural preservatives to be used in food industry. Thus, it is highly desirable to employ natural preservatives with antioxidant and antimicrobial properties to avoid the side effects associated with the use of synthetic preservatives (Saatchi et al., 2014). Accordingly, nutmeg essential oil and its various oleoresin extracts displayed antimicrobial and antioxidant properties that make them a promising natural food preservative to replace the synthetic ones (Gupta et al., 2013; Kapoor et al., 2013).

Essential oils and various oleoresins (acetone, ethanol, methanol, butanol, and water extracts) extracted from nutmeg demonstrated antimicrobial activities against both Gram-positive and Gram-negative bacteria as well as against pathogenic fungi (Omoruyi and Emefo, 2012; Piaru et al., 2012a; Gupta et al., 2013; Ibrahim et al., 2013; Valente et al., 2015). Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Streptococcus mitis*, and *Streptococcus salivarius*) (Omoruyi and Emefo, 2012; Gupta et al., 2013; Ibrahim et al., 2013; Shafiei et al., 2012), Gram-negative bacteria (*Aggregatibacter actinomycetemcomitans*, *Escherichia coli*, *Klebsiella pneumonia, Porphyromonas gingivalis, Proteus* spp, *Pseudomonas aeruginosa, Pseudomonas putida, Salmonella typhi, Shigella dysenteriae*, and *Yersinia enterocolitica*) (Omoruyi and Emefo, 2012; Piaru et al., 2012a; Shafiei et al., 2012; Gupta et al., 2013; Al-Mariri and Safi, 2014), and fungal species (*Aspergillus fumigatus*, *Aspergillus niger, Aspergillus flavus*, and *Aspergillus ochraceus*) (Gupta et al., 2013; Valente et al., 2015) are susceptible to nutmeg essential oil and the different oleoresins. Myristicin (Figure 2) has been shown to be the major antifungal constituent in nutmeg essential oil against *Aspergillus species* (Valente et al., 2015).

One of the modern ways to improve the hygienic safety of manufactured food products is to exploit the antimicrobial properties of natural plant extracts, allowing for a decrease of the use of chemical antimicrobial agents, which constitute a potential human health hazard. In this regard, the antimicrobial properties of EOs have been known for a long time and continue to be the subject of several studies that evaluate their microbial potential as alternatives to chemical agents in food industries (Djenane et al., 2013). In this regard, nutmeg is a promising natural preservative agent to be used in the food industries.

The EOs and various oleoresin extracts from nutmeg also demonstrated significant antioxidant activities. The oils and oleoresins displayed radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$); prevented oxidation of oil model systems, such as linoleic acid and mustard oil; and showed strong reducing power and ferrous ion chelating properties. The exhibited antioxidant activities were as potent as—and in some cases, more potent than—those of known antioxidants.

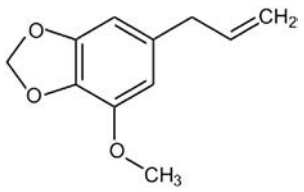

FIGURE 2 Structure of myristicin from nutmeg.

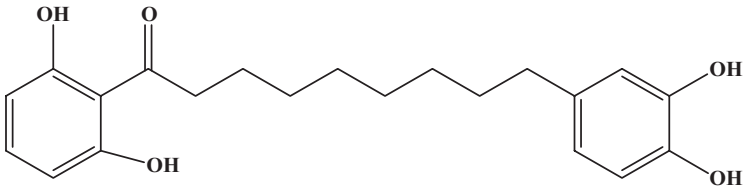

FIGURE 3 Structure of malabaricone C from nutmeg Hou et al. (2012).

Antioxidant compounds have also been isolated from nutmeg oil. One of these compounds is Malabaricone C (Figure 3) which exhibited stronger antioxidant activity than the commonly used synthetic antioxidants (Hou et al., 2012; Gupta et al., 2013; Kapoor et al., 2013). Because antioxidants help prevent oxidation of foods, especially fats and oils, and protect cells from free-radical damage, food manufacturers use antioxidants as food additives to help guard against food degradation and enhance the health profile of functional foods.

Both natural (ascorbic acid and tocopherols) and synthetic (propylgallate, tertiary butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene) antioxidants act as food preservatives. However, there are some arguments about the safety and adverse effects of the synthetic antioxidants when used as food additives. In fact, in recent years, researchers have focused on spicy and medicinal plants for extracting natural antioxidants, which play an important role in the food industry to combat food deterioration. Thus, the combined antioxidant and antimicrobial properties of essential oils and oleoresin extracts from nutmeg are very important assets to protect both microbial and oxidation-induced food deterioration; these properties make nutmeg a promising natural food preservative to replace the synthetic ones (Kapoor et al., 2013). In addition, nutmeg produces a relatively high percentage of essential oil. The total essential oil content of plants is generally very low and rarely exceeds 1%; however, in some cases, such as clove (*Syzygium aromaticum*) and nutmeg (*Myristica fragrans*), it reaches more than 10% (Djilani and Dicko, 2012). Nutmeg and mace contain 7–14% essential oil (Nutmeg, 2014) which could make nutmeg a better candidate for a natural food preservative.

A range of essential oil components have been accepted by the European Commission for their intended use as flavorings in food products. The registered flavorings include linalool, thymol, eugenol, carvone, cinnamaldehyde, vanillin, carvacrol, citral, and limonene—all of which are considered to present no risk to the health of the consumer. The U.S. Food and Drug Administration (FDA) also classifies these substances as generally recognized as safe (GRAS). The crude essential oils classified as GRAS by FDA include, amongst others, clove, oregano, thyme, nutmeg, basil, mustard, and cinnamon.

Despite the demonstrated potential of essential oils and their constituents in vitro, their use as preservatives in food has been limited because high concentrations are needed to achieve sufficient antimicrobial activity. In many food products, the hydrophobic essential oil constituents are impaired by interactions with food matrix components, such as fat, starch, and proteins. Furthermore, the antimicrobial potency of essential oil constituents also depends on pH, temperature, and the level of microbial contamination. Extrapolation of results from in vitro tests to food products is thus difficult at best, and a lower performance of the antimicrobial compound must be expected; before being added to food products, it is useful to investigate how essential oils or their constituents interact with food components in vitro.

With this view, consideration of the issue of the safe use and application of food ingredients is very important. A main obstacle to using essential oil constituents as food preservatives is that they are most often not potent enough as single components, and they cause negative organoleptic effects when added in sufficient amounts to provide an antimicrobial effect. The intense aroma of essential oils, even at low concentrations, can cause negative organoleptic effects exceeding the threshold acceptable to consumers. Even though nutmeg crude essential oil is classified as GRAS by the FDA (Hyldgaard et al., 2012), there are some claims that prolonged use or a large dose of nutmeg can have health risks. For example, long-term consumption of nutmeg may have an adverse effect on visual and auditory sensibilities (Adjene and Nwose, 2010). Therefore, although nutmeg essential oils, oleoresins, and their constituents seem to have attractive applications in food products to prolong the shelf-life of foods by limiting microbial growth or food oxidation, consideration of safe usage and application should not be ignored.

TABLE 2 Extraction of Essential Oil From Different Parts of Nutmeg (*Myristica fragrans*)

Origin/Parts	Technique	Apparatus	Yield (%)	References
Indonesia/seeds	Hydrodistillation	.....	6.85	Muchtaridi et al. (2010)
Nigeria/seeds	Hydrodistillation	Clevenger	1.46	Ogunwande et al. (2003)
Brazil/seeds	Hydrodistillation	.....	7.1	Valente et al. (2011)
Italy/seeds	Super critical fluid extraction (SEF)/ Hydrodistillation	Extraction vessel and separator vessels/ clevenger		Piras et al. (2012)
India/seeds/fruit	Hydrodistillation	Clevenger	3.9–16.5	Maya et al. (2004)
Pakistan/seeds	Hydrodistillation	.....	12.0	Atta-ur-Rahman et al. (2000)
China/seeds	Hydrodistillation	Clevenger	2.4	Du et al. (2014)
Croatia/seeds	Hydrodistillation	Clevenger	4.92	Jukic′ et al. (2006)
Malaysia/pericarp/ fruit/seed	Hydrodistillation	Clevenger	2–3	Choo et al. (1999)
France/commercial	.....	.....		Schenk and Lamparsky (1981)
Sri Lanka/seeds	Steam distillation	Clevenger	10–15	Sarath-Kumara et al. (1985)

ESSENTIAL OIL PRODUCTION

The extraction of essential oils from plants are carried out by different methods, of which hydrodistillation is the most common method; however, other methods including supercritical fluid extraction, maceration, solvent extraction, enfleurage, and mechanical pressing are often employed for certain products (Surburg and Panten, 2006). The yields of essential oil extracted from nutmeg by different methods have been reported to vary from 1.46% to 16.5%, depending on the processing method and geographical origin of the plant (Table 2). The characteristic odor of spice is due to the nutmeg essential oil, which is a steam distillate that appears as a pale-yellow, nearly colorless liquid (Marzuki et al., 2014).

CHEMICAL COMPOSITION OF NUTMEG ESSENTIAL OIL

The essential oil from nutmeg is obtained through extraction mainly from seeds, but it can also be produced from mace, leaves, and pericarp (Peter, 2001; Helen et al., 2012; Choo et al., 1999). The compositions of the essential oil from different parts of the nutmeg plant have been reported to be quantitatively different, but no significant qualitative variation has been seen (Schenk and Lamparsky, 1981; Choo et al., 1999). The essential oil contains from 5% to 15% volatile oils comprised >80% monoterpenes, >5% monoterpene alcohols, and >5% aromatic and other miscellaneous compounds. The major components found in monoterpene are α-pinene, sabinene, 1,8-p-methadiene, β-pinene, 1,4-p-menthadiene, and camphene; the aromatic fraction contains myristicin, elemicin, safrole, while the minor constituents include methyleugenol, eugenol, isoeugenol, and toluene (Schenk and Lamparsky, 1981; Piras et al., 2012; Piaru et al., 2012b).

The composition of nutmeg essential oil has been reported to have wide variation based on the finding using gas chromatography (GC), retention index (RI), and mass spectrometry (MS) with different amounts of myristicin [1], α-pinene [2], sabinene [3], limonene [4], elemicin [5], eugenol [6], safrol [7], and β-pinene [8], as shown in Table 3.

The analysis of nutmeg essential oils reported from different geographical origins differs widely in composition. The composition of essential oil using GC–MS reported from Indonesian nutmeg has been found to contain the following major components: sabin (21.38), 4-terpineol (13.92), myristicin (13.57), α-pinene (10.23), limonene (5.57), safrol (4.28), and α-terpineol (3.11) (Muchtaridi et al., 2010). GC–MS analysis of Nigerian nutmeg essential oil by Ogunwande et al., 2003 found sabinen (49.09), α-pinene (13.19), α-phellandrene (6.72), perpinen-4-ol (16.43), p-cymene (3.30), and myrcene (3.09). Valente et al. (2011) reported the following six major components of essential oil by GC–MS from Brazilian nutmeg: sabinen (25.0), α-pinene (14.1), β-inene (12.4), myristicin (10.9), terpinen-4-ol (7.6), and limonene (6.3). GC–MS analysis of Italian-origin nutmeg essential oil was found to contain myristicin

TABLE 3 Chemical Composition of Nutmeg (*Myristica fragrans*) Essential Oil

Origin (Parts)	Major Components	Yield (%)	References
Indonesia (seeds)	Sabin	21.38	Muchtaridi et al. (2010)
	4-Terpineol	13.92	
	Myristicin	13.57	
	α-Pinene	10.23	
	Limonene	5.57	
	Safrol	4.28	
	α-Terpineol	3.11	
Nigeria (seeds)	Sabinen	49.09	Ogunwande et al. (2003)
	α-Pinene	13.19	
	α-Phellandrene	6.72	
	Terpinen-4-o1	6.43	
	p-Cymene	3.30	
	Myrcene	3.09	
	β-Pinene	2.42	
Brazil (seeds)	Sabinen	25.0	Valente et al. (2011)
	α-Pinene	14.1	
	β-Pinene	12.4	
	Myristicin	10.9	
	Terpinen-4-ol	7.6	
	Llimonene	6.3	
Italy (seeds)	Myristicin	32.8	Piras et al. (2012)
	Sabinene	16.1	
	α-Pinene	9.8	
	β-Pinene	9.4	
	β-phellandrene	4.9	
	Safrole	4.1	
	Terpinen-4-ol	3.6	
India (seed)	Myristicin	1.1–45.6	Maya et al. (2004)
	Sabinene	6.3–45.5	
	Elemicine	1.0–29.7	
	Safrol	0.1–22.1	
China (seeds)	β-phellandrene		Du et al. (2014)
	Safrole	0.1–22.1	
	Terpinen-4-ol		
	Elemicine	1.0–29.7	
	Eugenol	19.9	
	Methylisoeugenol	16.8	

TABLE 3 Chemical Composition of Nutmeg (*Myristica fragrans*) Essential Oil—cont'd

Origin (Parts)	Major Components	Yield (%)	References
	Methyleugenol	16.7	
	Sabinene	11.8	
	Terpinen-4-ol	8.5	
Pakistan (seeds)	Terpinen-4-ol	31.3	Atta-ur-Rahman et al. (2000)
	ɣ-Terpinene	7.8	
	Myristicin	7.1	
	α-Pinene	4.9	
	Elemicine	4.8	
	β-Pinene	4.6	
Croatia (seeds)	Sabinene	25.4	Jukic′ et al. (2006)
	α-Pinene	15.8	
	β-Pinene	13.4	
	Terpinen-4-ol	6.2	
France (commercial)	Sabinene	55.3	Schenk and Lamparsky (1981)
	α-Pinene	12.7	
	β- Pinene	11.9	
	D-limonene	6.2	
Malaysia/pericarp	Terpinen-4-ol	19.1	Choo et al. (1999)
	α-Pinene	15.2	
	Myrcene	9.1	
	Limonene	9.1	
	ɣ-Terpinene	9.1	
	β-Pinene	9.0	
Sri Lanka (seeds)	Sabinene	43.42	Sarath-Kumara et al. (1985)
	α-Pinene	17.48	
	β-Pinene	12.13	
	Limonene	3.22	
	myristicin	3.06	
	Elemicin	1.54	

(32.8), sabinene (16.1), α-pinene (9.8), β-pinene (9.4), β-phellandrene (4.9), safrole (4.1), and terpinen-4-ol (3.6) as the major components. Indian nutmeg essential oil from seeds, as reported by Maya et al. (2004), has been found to contain myristicin (1.1–45.6) as the main compound together with sabinene (6.3–45.5), elemicine (1.0–29.7), and safrol (0.1–22.1); analysis of essential oil extracted from the nutmeg fruit by GC–MS was found to have a different composition from that of seeds, with sabinene (29.4) being the major component along with β-pinene (10.6), α-pinene (10.1%), and terpenen-4-ol (9.6), as described by Kapoor et al. (2013). GC–MS analysis of Chinese-origin nutmeg essential oil reported by Du et al. (2014) revealed the presence of eugenol (19.9), methylisoeugenol (16.8), methyleugenol (16.7), sabinene (11.8), and terpinen-4-ol (8.5%) as the major components. Based on MS database only, the following major components were reported by Atta-ur-Rahman et al. (2000) in nutmeg essential oil obtained in

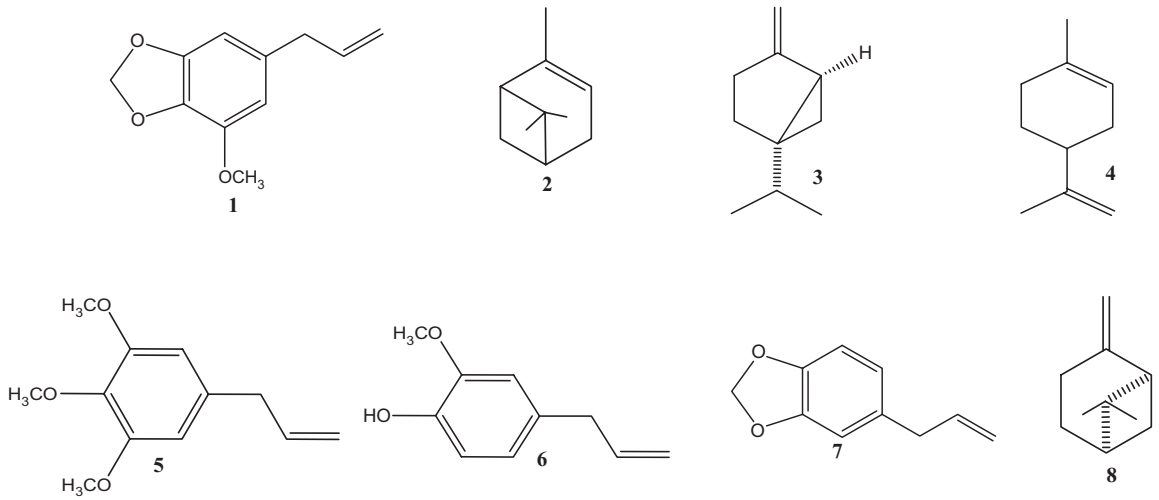

FIGURE 4 Chemical structures of important constituents of nutmeg essential oil: myristicin [1] α-pinene [2], sabinene [3], limonene [4], elemicin [5], eugenol [6], safrol [7], and β-pinene [8].

Pakistan: terpin-4-ol (31.3), ɣ-terpinene (7.8), myristicin (7.1), α-pinene (4.9), and β-pinene (4.6). Based on an MS and RI database, Jukic' et al. (2006) identified the following major components of nutmeg essential oil from Croatia: sabinene (25.4), α-pinene (15.8), β-pinene (13.4), and terpin-4-ol (6.2). Investigation of the composition of an essential from commercially obtained nutmeg in France by Schenk and Lamparsky (1981) using GC reported the following composition: sabinene (55.3), α-pinene, (12.7), β-pinene (11.9), and D-limonene (6.2). The major components in essential oil of the pericarp of Malaysian nutmeg was found by Choo et al. (1999) using RI and MS database: terpin-4-ol (19.1) was the major compound, along with α-pinene (15.2), ɣ-terpinene (9.1), myrcene (9.1), limonene (9.1), β-pinene (9.0), and sabinene (1.2). The analysis of essential oil extracted from nutmeg fruit from Malaysia reported terpineol-4 (21.3), α-pinene (8.5), myristicin (6.8), and γ-terpinene (3.5) (Piaru et al., 2012b). Nutmeg essential oil of Sri Lankan origin, analyzed by Sarath-Kumara et al. (1985) using GC, found sabin (43.42) as the major compound, along with α-pinene (17.48), β-pinene (12.13), limonene (3.22), myristicin (3.06), and elemicin (1.54).

Nutmeg essential oil components vary by origin: Indonesia (32 compounds), Nigeria (37 compounds), Brazil (28 compounds), Italy (30 compounds), India (38 compounds), China (27 compounds), Pakistan (32 compounds), Croatia (17 compounds), France (17 compounds), Malaysia (50 compounds), and Sri Lanka (39 compounds). The chemical structures of some important constituents of nutmeg essential oil are given in Figure 4.

SUMMARY POINTS

- Nutmeg (*Myristica fragrans* Houtt.) is a spice seed that belongs to the family Myristicaceae.
- Nutmeg has a characteristic pungent and pleasant fragrance, with a slightly warm and sweet taste.
- Nutmeg is used to flavor many kinds of baked goods, breads, confections, puddings, dairy products, meats, sausages, sauces, vegetables, and beverages.
- Because of its aroma, nutmeg essential oil has been used as a natural flavoring extract and as a perfume in the cosmetic industry.
- Nutmeg is a highly valued spice, known since antiquity for its aromatic, aphrodisiac, and curative properties.
- Owing to its demonstrated antimicrobial and antioxidant properties, nutmeg essential oil is considered to be a promising biopreservative.
- Nutmeg has high nutritional value and is rich in carbohydrates, proteins, dietary fiber, vitamins A, C, E, and minerals such as calcium, copper, iron, magnesium, manganese, zinc, and phosphorus.
- Nutmeg is known to have multiple medicinal uses and applications, including treatment of gastrointestinal conditions such as stomach ulcers, indigestion, liver disorders, emmenagogue, nervine, digestive, diuretic, diaphoretic, and food flavoring properties.
- Nutmeg essential oils from different geographical origins differ widely in their composition and commonly contain phytochemicals such as limonene, sabinene, α-pinene, β-pinene, myristicin, sabinene, and safrol.

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Chapter 70

Onion (*Allium cepa*) Essential Oils

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List of Abbreviations

ABTS$^{\bullet-}$ 2,2′-Azino-di-[3-ethylbenzthiazoline sulfonate (6)] diammonium salt
CFU Colony forming units
Cys Cysteine
DPPH$^{\bullet}$ 2,2-Diphenyl-1-picrylhydrazyl
IC_{50} Half maximal inhibitory concentration
MBC Minimum bactericidal concentration
MIC Minimum inhibitory concentration
POD Peroxidase
PPO Polyphenol oxidase

INTRODUCTION

Onion (*Allium cepa* L.) is a famous spice commodity grown all over the world. The distinctive flavor of alliums have established the plant as an essential part of international cuisine, and it is also used widely in food processing. Onion has been used in traditional medicinal practice to treat and prevent several diseases and disorders (Rabinowitch and Currah, 2002). The bioactive properties and characteristic flavor of onion have been attributed to its sulfur compounds, which are present in the volatile fraction (onion oil). One method of production of onion oil is steam distillation of crushed onion bulbs, which consist of a complex mixture mostly formed by mono-, di-, tri-, and tetra-sulfides with different alkyl groups (Hosoda et al., 2003).

Alk(en)yl sulfides are perceived as odorants like sulfur, fried onion, garlic, and/or cooked cabbage (Blank, 2002). Thus, in the food industry, onion oil is traditionally and extensively used to impart flavors to processed food (soups, meat, table sauces, salad dressings, and savories) without the difficulties of handling a large bulk of fresh bulbs (Lawless, 2012). In addition to its flavoring properties, it has been demonstrated that onion oil has antioxidant, antibrowning, and antimicrobial properties that could enhance the quality and safety of the treated food, while being a natural additive that consumers prefer (and hence, the food industry has to provide). The mode of action of its constituents is diverse: sulfur compounds can act as electron donors and react with free radicals, stopping oxidative reactions in food products (Ye et al., 2013); the antibrowning and antimicrobial mechanisms are attributed to physicochemical interaction among sulfur compounds and sulfur residues in key enzymes of the browning process and microbial metabolism, respectively (Vazquez-Armenta et al., 2014). This chapter discusses the applications of onion oil in the food industry as a flavor enhancer, antioxidant, antibrowning agent, and antimicrobial agent.

BOTANICAL ASPECTS

Onion (*A. cepa* L.) is a popular spice commodity grown all over the world and consumed in various forms. It has been in cultivation for more than 4000 years (Peter, 2001). The five greatest producers of onion are China, the United States, Turkey, Egypt, and Russia (USDA, 2011). This extensive production is attributed to onion's distinctive flavor, which as established this plant as an essential part of the cuisine of the world; it is also used widely in food processing (Peter, 2001). There is a lot of controversy regarding the taxonomic position of *Allium* and related genera. In a recent classification, the genus occupies the following taxonomic context (Brewster, 2008): class, *Monocotyledones*; order, *Asparagales*; family, *Alliaceae*; and genus, *Allium*.

The genus *Allium* includes wild and cultivated species. *Allium cepa* does not exist in the wild; the wild species *Allium vavilovii* has been identified as the closest relative. Cultivated types of *A. cepa* fall into two broad horticultural groups—the

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common onion group and the *Aggregatum* group, which are distinguished by bulb phenotypes, dormancy, and photoperiod requirements that are different from those of common onion (McCallum, 2007). The most phenotypically diverse is the common onion group, which includes hundreds of open pollinated varieties, land races, and commercial F_1 hybrids (Peter, 2001). This group has cylindrical, opposite leaves and bulbs formed from several leaf bases covered by thin, dry skins. The leaf sheaths form a pseudostem that surrounds the hollow flower scape. Axillary daughter bulbs form tufts or short rhizomes; a progressive reduction of rhizomes within the section culminates in the baseplate disc of onion (Brewster, 2008).

USAGE AND APPLICATIONS

Onion has been used in traditional medical practice to treat and prevent several diseases and disorders (Rabinowitch and Currah, 2002). Health benefits in the treatment of many different major and minor disorders have been claimed in traditional medicine, but precise medical data are lacking to support most of these effects. In folk medicine, onions have been used against different infectious diseases for many centuries (Rabinowitch and Currah, 2002); it has been reported to have anticholesteremic, hypolipidemic, antidiabetic, anti-inflammatory, antibiotic, and anticancer properties (Kumari et al., 1995; Rabinowitch and Currah, 2002). One of the best investigated effects of onion is its antiasthmatic activity, which is attributed to its anti-inflammatory activity (Rabinowitch and Currah, 2002). In addition, a patented pharmaceutical/veterinary preparation has been created from a mixture of onion and coconut (*Cocos nucifera*) for controlling flatworms (plathelminthes) and/or threadworms (nematodes) in animals or humans (Mehlhorn et al., 2011). A method for treating or preventing human viral infection by consuming dehydrated onion material was claimed also in a patent (Goren et al., 2002).

The bioactive properties and characteristic flavor of onion have been attributed to sulfur-containing compounds, which are the main constituents of its essential oil (Figure 1). One method of production of onion oil is steam distillation of crushed onion bulbs (Hosoda et al., 2003). The water-insoluble extractive obtained is a dark-yellow or brownish-yellow liquid with an intense flavor. It consists of a complex mixture of volatile sulfur compounds, mostly mono-, di-, tri-, and tetra-sulphides with different alkyl groups (Hosoda et al., 2003). The yield of extraction of onion oil varies from 0.002% to 0.03% (Brewster, 2008).

USAGE AND APPLICATIONS IN FOOD SCIENCE

The odor and flavor properties of onion oil and its components have been used in the food industry to add traditional flavors in novel foods. Alk(en)yl sulfides, the main constituents of onion oil, are perceived as odorants like sulfur, fried onion, garlic, and/or cooked cabbage (Blank, 2002). Manufacturers of essential oils commonly standardize the extraction and determine the spice equivalency to yield the same aroma and flavor in different batches (Farrell, 1998). Generally, 1 g of onion oil is equivalent to the flavor of 4.4 kg of fresh onions or 500 g onion powder (Brewster, 2008). After this process, onion oil can be incorporated into carriers (salt, carbohydrates, or other edible material) to get dry soluble, fat-based soluble, encapsulated, and liquid soluble spices (Farrell, 1998). The main flavoring forms are onion powder, onion salt, encapsulated essential oil, and oleoresins; the composition and amount of sulfur compounds can vary among them (Brewster, 2008).

Onion oil has been used as a food additive to improve the sensorial, antioxidant, and microbiological quality of a treated product (Table 1). The application of onion oil in fresh cut potatoes was sensorially accepted after 10 days of storage (Vazquez-Armenta et al., 2014). At day 10, the potato slices treated with 2.5 and 5 mg/mL received a higher score ($p > 0.05$; like moderately) than the sample control and the slices treated with 0.5 mg/mL. Comparing these results with the remaining volatiles in potato tissues, it is noticed that dipropyl disulfide was the highest compound detected at day 10 when 5 and 2.5 mg/mL were used. The major comments from panelists were that the treated produce presented a slight onion-seasoned flavor. Therefore, the volatile aromatic constituents of essential oils can be absorbed by the food matrix, which may in turn change their organoleptic characteristics.

In addition to flavoring properties, it has been demonstrated that onion oil has antioxidant, antibrowning, and antimicrobial properties that could enhance the quality of the treated food. Ye et al. (2013) reported the capacity of onion oil to inhibit the 2,2′-Azino-di-[3-ethylbenzthiazoline sulfonate (6)] diammonium salt $(ABTS)^{\bullet -}$ and 2,2-Diphenyl-1-picrylhydrazyl $(DPPH)^{\bullet}$ radicals and chelating metal properties, with IC_{50} values of 0.67, 0.63, and 0.51 mg/mL, respectively. Similarly, these authors reported that the reducing power of the onion oil was dose dependent. On the other hand, Mehlhorn et al. (2011) reported IC_{50} values for $DPPH^{\bullet}$ of 0.09 mg/mL. These studies demonstrated that onion oil can act as an electron donor and react with free radicals to convert them to more stable products.

Ponce et al. (2008) evaluated the antioxidant and antimicrobial effects of onion powder in fresh pork loin (*M. longissimus dorsi*) and belly during storage. The application of onion powder decreased the lipid peroxidation, reducing the production of free fatty acids and thiobarbituric acid reactive substances in pork belly, as well as the peroxide value in pork loin compared to the control. In addition, the development of volatile oxidation products (alcohols, saturated and unsaturated aldehydes) was

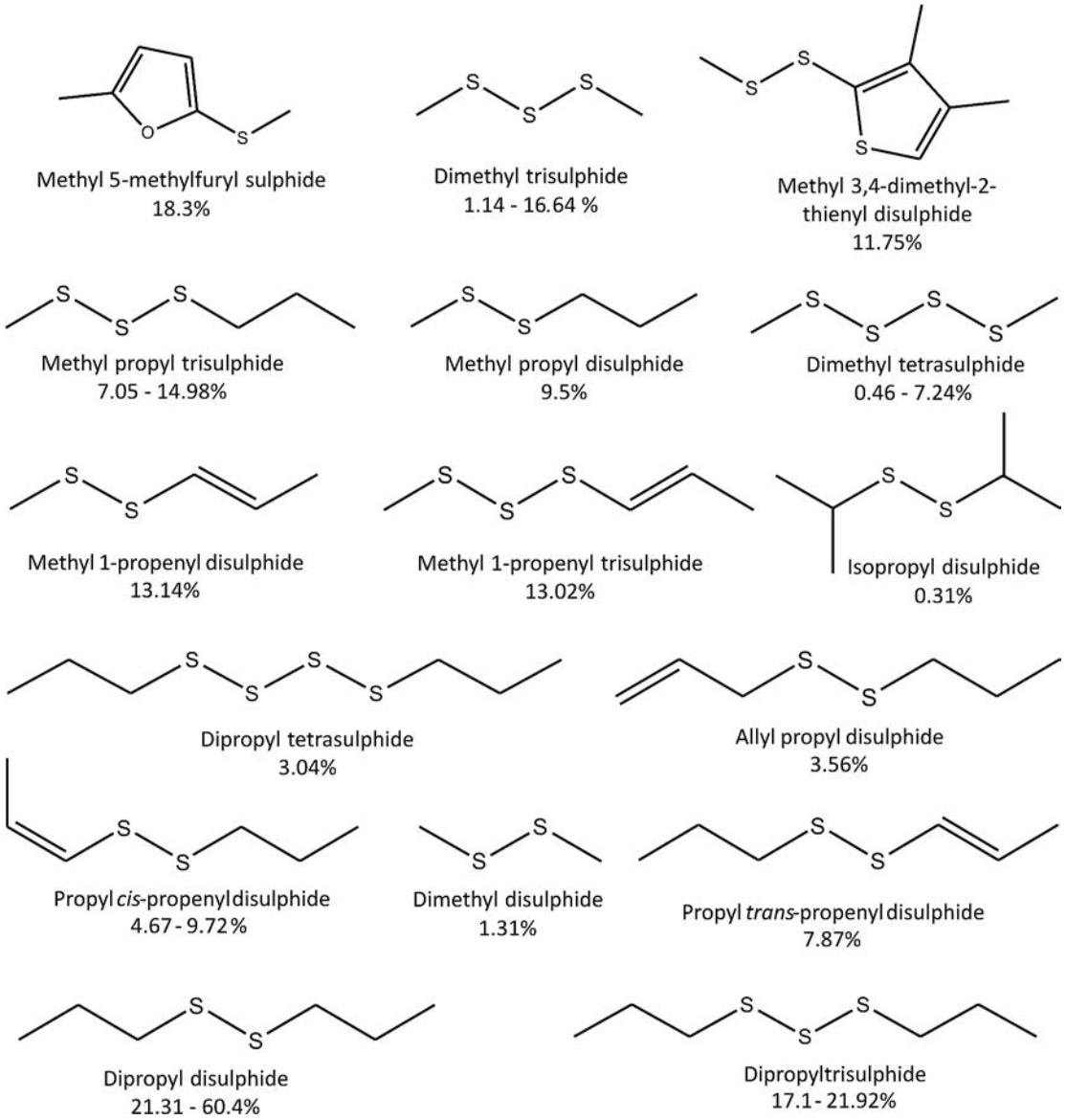

FIGURE 1 Main volatile compounds identified in onion oil.

also inhibited in both enhanced meats. Regarding the antimicrobial effects, onion powder reduced the total bacteria counts and *Enterobacteriaceae* in pork belly, whereas in loin cuts no differences were found ($p > 0.05$) for *Enterobacteriaceae* counts.

Vazquez-Armenta et al. (2014) evaluated the effects of onion oil (0.05, 0.25, and 0.5 mg/mL) on microbial growth, browning decay, and sensorial appeal of cut potatoes stored for 15 days at 4 °C. Dipropyl disulfide and dipropyl trisulfide were the main constituents identified in onion oil, and its application at a dose of 0.5 mg/mL was the most effective to prevent browning (38.5% inhibition respect to control) at the end of storage; it also inhibited polyphenol oxidase (PPO) activity (39% respect to control) after the treatment. Higher concentrations of added onion oil concentration resulted in lower microbial growth of the treated product, achieving a reduction of 1.27 log CFU/g for the 5 mg/mL treatment. Furthermore, the odor and flavor appeal of cut potatoes were well received by the panelists after 10 days of storage. Similarly, Hosoda et al. (2003) evaluated the inhibitory effect of onion oil and its components in browning of shredded lettuce, observing 20% and 70.4% of browning inhibition with the application of 0.05% and 0.2%, respectively. The evaluation of its main constituents showed that trisulfides are better inhibitors than disulfides; however, the sensorial impact of the treatment was not evaluated. In the same manner, chitosan edible film containing 1% onion oleoresin was applied on butternut squash slices during 5 days of storage, resulting in the inhibition of PPO and peroxidase (POD) activities of 61–98% and 40–91%, respectively (Ponce et al., 2008).

TABLE 1 Sensorial, Antimicrobial, and Antioxidant Properties of Onion Oil

Food Matrix	Properties			References
	Sensorial	Antimicrobial	Antioxidant	
Fresh cut potato	Enhance of odor and flavor appealing Inhibition of PPO activity and browning development during cold storage	Reduction of 1.27 log CFU/g of mesophilic bacteria	ND	Vazquez-Armenta et al. (2014)
Fresh pork loin and belly cuts	ND	Reduction in mesophilic bacteria and *Enterobacteriaceae*	Inhibition of lipid peroxidation and volatile compounds production	Ponce et al. (2008)
Shredded lettuce	Inhibition of browning development	ND	ND	Hosoda et al. (2003)
Butternut squash slices	Inhibition of PPO and POD activities	ND	ND	Ponce et al. (2008)

ND, not determined; POD, peroxidase; PPO, polyphenol oxidase.

Enzymatic inhibition of potato PPO by the presence of sulfur volatiles of onion oil could be explained by the possible molecular interactions among them. The helical bundle accommodates the catalytic dinuclear copper center of potato PPO and is surrounded by helices, α1 and α4, and several short β-strands. Two disulfide bridges (Cys 11–Cys 28 and Cys 27–Cys 89) help to anchor the loop-rich N-terminal region of the protein (residues 1–50) to helix α2 (Klabunde et al., 1998). These sulfur amino acid residues present in PPO could be the target of the sulfur volatiles of onion oil; a previous study has clearly demonstrated that sulfur compounds can act as a slow-binding competitive inhibitor of mushroom tyrosinase (Pérez-Gilabert and García-Carmona, 2001). Similarly, Steven et al. (1981) proposed that dimethyl disulfide reacts with trypsin and papain by a disulfide exchange reaction, thus forming inactive complexes. This model serves to illustrate mechanisms by which some sulfides are capable of modulating enzymatic reactions.

Food safety has become increasingly important due to foodborne illness outbreaks around the world. Food is an excellent vehicle in which many pathogens can proliferate and reach appropriate colonization sites in a new host (Newell et al., 2010). In addition, it has become clear that antimicrobial compounds are losing their effectiveness due to the evolution of pathogen resistance; for this reason, there is therefore a continuing need to search for natural compounds with antimicrobial activity. In this context, onion oil represents an alternative to common disinfectants due to its antibacterial properties, which are summarized in Table 2.

Benkeblia (2004) reported the in vitro antibacterial effect of essential oils (50–500 mL/L) of three types of onion (green, yellow, and red) against *Staphylococcus aureus* and *Salmonella enteritidis*; the red onion oil was most effective in a dose-dependent manner. Ye et al. (2013) reported the minimum inhibitory and minimum bactericidal concentrations (MIC and MBC, respectively) of onion oil extracted by supercritical-CO_2 extraction against *Escherichia coli*, *Bacillus subtilis*, and *S. aureus* with values of 0.27, 0.18, and 0.18 mg/mL for MIC, and 0.54 mg/mL for MBC, respectively. In the same manner, Conner and Beuchat (1984) reported MIC and MBC of onion oil against *E. coli*, *S. aureus*, *Listeria monocytogenes*, *S. enteritidis*, and *Campylobacter jejuni* of >10 mg/mL. In addition, onion oil at 200 ppm inhibited growth of *Clostridium botulinum*; however, at 10 ppm, the germination of spores was suppressed (Ismaiel and Pierson, 1990).

Onion oil also has been demonstrated to possess antifungal properties against yeast and molds (Table 3). Ye et al. (2013) reported the sensibility of *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Aspergillus niger*, *Monascus purpureus*, and *Aspergillus terreus* to onion oil. The MIC values of onion oil against the tested microorganisms ranged from 0.18 to 1.80 mg/mL and minimal biocidal concentrations from 1.80 to 3.6 mg/mL, respectively. Film formation on soy sauce by *Zygosaccharomyces rouxii* SS1 was completely prevented for 30 days by the addition of 40 ppm of onion oil (Kim et al., 2004b). Onion oil at concentrations as low as 25 ppm in recovery media reduced the populations of sublethally heat-stressed (44–54 °C) yeast (*Candida lipolytica*, *Debaryomyces hansenii*, *Hansenula anomala*, *Kloeckera apiculata*, *Lodderomyces elongisporus*, *Rhodotorula rubra*, *S. cerevisiae*, and *Torulopsis glabrata*) compared to populations recovered in media containing no oil (Conner and Beuchat, 1984). These authors suggested that the yeasts were either metabolically or structurally damaged as a result of being exposed to elevated temperatures and that onion oil avoided the repair of the injury.

TABLE 2 Antibacterial Activities of Onion Oil

Effective Against	MIC	Inhibition Zone (mm)	References
Escherichia coli	0.27 mg/mL	13.4*	Ye et al. (2013)
Bacillus subtilis	0.18 mg/mL	19.3*	
Staphylococcus aureus	0.18 mg/mL	17.4*	
E. coli	ND	6**	Zohri et al. (1995)
Klebsiella pneumoniae	ND	12**	
Bacillus anthracis	ND	10**	
B. cereus	ND	26**	
Micrococcus luteus	ND	16**	
S. aureus	ND	14**	
S. aureus	25 ppm	ND	Kim et al. (2004a)
E. coli	>1%	5.3***	Smith et al. (1998)
S. aureus	>1%	6.7***	
L. monocytogenes	>1%	5.6***	
Salmonella enteritidis	>1%	6.7***	
Campylobacter jejuni	>1%	5.5***	
S. aureus	ND	5.6****	Benkeblia (2004)
S. enteritidis	ND	7.1****	

Onion oil concentration: *15 mg/disc; **0.5 mg/disc; ***25 µl/disc; ****50 ml/L; ND, not determined.

TABLE 3 Antifungal Activities of Onion Oil

	Evaluated Activity			
Effective Against	MIC	Inhibition Zone (mm)	Inhibition of Mycotoxin Production (%)	References
Rhodotorula glutinis	0.36 mg/mL	11.4*	NA	Ye et al.(2013)
Saccharomyces cerevisiae	0.36 mg/mL	12.9*	NA	
Candida tropicalis	0.36 mg/mL	15.1*	NA	
C. tropicalis	ND	10**	NA	Kocić-Tanackov et al. (2009)
S. cerevisiae	ND	21**	NA	
Candida utilis	25 ppm	ND	NA	Kim et al. (2004a)
Aspergillus niger	0.54 mg/mL	4.1*	ND	Ye et al. (2013)
Aspergillus terreus	1.80 mg/mL	12.7*	ND	
Aspergillus flavus	ND	ND	94.9*** (aflatoxin)	Zohri et al. (1995)
Aspergillus parasiticus var. *globosus*	ND	ND	76.2*** (aflatoxin)	
Aspergillus versicolor	ND	ND	82.6**** (sterigmatocystin)	
Penicillium rubrum	ND	ND	73.4**** (rubratoxin A)	
A. versicolor	ND	ND	83.29***** (sterigmatocystin)	Kocić-Tanackov et al. (2012)

ND, not determined; NA, not applicable; Onion oil concentrations: *15 mg/disc; **1%; ***500 ppm; ****100 ppm; *****0.05 µg/mL.

It has been reported that the antifungal activity of onion oil depends of the variety and tested concentrations. Kocić-Tanackov et al. (2009) established that the oil extracted from the Kupusinski jabuåar variety was more effective at inhibiting the growth of *S. cerevisiae* and *C. tropicalis*, and *Aspergillus tamarii* and *Penicillium griseofulvum* than the Junski srebrnjak variety in a dose-dependent manner. Beside growth inhibition, it has been reported that onion oil could inhibit the mycotoxin biosynthesis. Onion oil applied at 0.01, 0.02, 0.05, and 0.11 μg/mL reduced or inhibited the growth of *Aspergillus versicolor* and biosynthesis (a mutagenic and carcinogenic toxin). Onion oil at 0.05 μg/mL completely inhibited mycelial growth of *A. versicolor* and sterigmatocystin biosynthesis in a 21-day period (Kocić-Tanackov et al., 2012). Similarly, this oil (100–500 ppm) reduced the aflatoxin production of *Aspergillus flavus* and *Aspergillus parasiticus* in 50.6–94.9% and 38.3–76.2%, respectively (Zohri et al., 1995). The sterigmatocystin production in *A. versicolor* and rubratoxin in *Penicillium rubrum* was completely inhibited by 200 ppm of onion oil (Zohri et al., 1995).

The antimicrobial properties of onion oil have been attributed to alk(en)yl sulfides derived from S-alk(en)yl-L-cysteine sulfoxides. The number of carbons in alk(en)yl group and sulfur atoms in molecules influenced antimicrobial activity of sulfides and antimicrobial activity of onion oil appear to be determined by the concentrations of individual constituents of sulfides (Kim et al., 2004a). Sulfur compounds may be involved in the inhibition of the bacterial activity of key enzymes (xanthine oxidase, succinate dehydrogenase, and triose phosphate dehydrogenase) (Kyung and Lee, 2001). This information reveals the diverse properties that onion oil can have in the food industry.

SUMMARY POINTS

- The odor and flavor properties of onion oil are used in the food industry to add traditional flavors in novel foods.
- Onion oil can stabilize free radicals and act as an anti-rancidity agent in food systems.
- The sulfur compounds in onion oil can inhibit enzymatic browning in fresh produce.
- Onion oil possesses antibacterial activity against food pathogens and spoilage bacteria.
- Yeast and mold growth and mycotoxin biosynthesis can be inhibited by onion oil.

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Chapter 71

Oregano (*Origanum* spp.) Oils

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List of Abbreviations

OEO Oregano essential oil
CFU Colonies forming units
EO Essential oil

INTRODUCTION

There is a large number of species worldwide that have been designated the name of oregano, most of them belong to the genera *Origanum* of family Lamiaceae (v*ulgare*, *viride*, *virens*, *majorana*, and *onites*) and *Lippia* of the family Verbenaceae (*graveolens*) (Martínez-Rocha et al., 2008). Oregano essential oil (OEO) has been found to be among the most effective antimicrobial and antioxidant agents (Fernández-Pan et al., 2012). Studies have shown that OEO is effective as an antioxidant and flavoring agent in functional food or nutraceutical products (Loizzo et al., 2009), retarding lipid oxidation (Handl et al., 2008). This makes oregano a source of essential oil (EO) with potentially extensive use in the food industry, especially those adopting the natural alternatives to assure food safety and quality.

Oregano is used mainly as flavoring or seasoning agents in some of the most accepted cuisines around the world, traditionally used in Italian, Greek, and Mexican dishes. Flowering tops are used in beers and ales, and fresh and dried leaves can be added to soups, casseroles, sauces, stew, stuffing, eggs, olives, teas, tomato-based dishes, and strong-flavored foods like chili and pizza (America, 2005). Reflecting on all the culinary uses and functional properties of oregano and the antimicrobial and antioxidant activity of OEO, it must be used its potential as a food industry additive.

BOTANICAL ASPECTS

Of the various types of oregano present worldwide, many belong to the family Lamiaceae, which comprises about 260 genera and 7000 species (Cosge et al., 2009). Most of them belong to the genera *Origanum* (family Lamiaceae) and *Lippia* (family Verbenaceae) (Martínez-Rocha et al., 2008). There are different types of oregano from the genus *Origanum*: *Origanum vulgare* L, *Origanum viride*, and *Origanum virens* representing the Mediterranean region. *Origanum vulgare* is native from the islands, in Crete, in southern Greece, and in coastal areas of the mainland Greece at altitudes up to 1500 m. It is a perennial shrub exhibiting high oil concentration (1.1–8.2%, v/w, depending on its habitat), about 10 times higher in comparison with its relative species (*O. vulgare* subsp *vulgare*, *O. vulgare* subsp *viridulum*, *O. vulgare* subsp *gracile*, *O. vulgare* subsp *viride*) (Karamanos and Sotiropoulou, 2013). In Mexico, a plant complex consisting of aromatic woody-based herbs or shrubs from various genera of the families Lamiaceae (*Calamintha*, *Hedeoma*, *Hyptis*, *Mesosphaerum*, *Monarda*, *Origanum*, *Plectranthus*, and *Poliomintha*), Verbenaceae (*Lantana* and *Lippia*), *Asteraceae* (*Brickellia*), and Fabaceae (*Dalea*) are regarded as oregano (Martínez-Rocha et al., 2008). The most popular Mexican oreganos are *Lippia graveolens* Kunth (Syn: *Lippia berlandieri* Schauer) and *Poliomintha longiflora* A. Gray (Rivero-Cruz et al., 2011), these species have been discussed in detail in Chapter 63. Because that there are many types of oregano, it is important to analyze their chemical composition and bioactive properties, even when similar components are reported among species (Table 1).

USAGE AND APPLICATION

Due to the variability in chemical and aroma characteristics, *Origanum* plants belonging to different species and ecotypes are widely used in agriculture and the pharmaceutical and cosmetic industries as culinary herbs, flavouring substances of

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TABLE 1 The Essential Oil Components in *Origanum* spp

Oregano Species	Compounds	References
Origanum vulgare	Carvacrol Thymol Cymene α-Pinene	Govaris et al. (2010) Gutierrez et al. (2008) Dimitrijević et al. (2007) Rosato et al. (2009) Hussain et al. (2011) Pozzatti et al. (2010)
Origanum majorana	Terpinen-4-ol γ–Terpinene cis-Sabinene-hydrate Thymol p-Cymene 4-Thujanol	Busatta et al. (2008) Giordani et al. (2008) Gutierrez et al. (2008) Hussain et al. (2011)
Origanum onites	Thymol Cavracrol Myrcene α-Terpinene γ-terpinene ϱ-cymene	Pizzale et al. (2002)

food products, alcoholic beverages, and perfumery for their spicy fragrance (Şahin et al., 2004). In folk medicine, oregano is used to treat various ailments such as a respiratory disorders, dyspepsia, painful menstruation, rheumatoid arthritis, and scrofulosis, and as an expectorant, carminative, aromatic for whooping and convulsive coughs, and to treat urinary tract disorders (Teixeira et al., 2013). The bioactive components of oregano are beneficial as flavoring or seasoning agents in some of the most accepted cuisines around the world. Oregano is traditionally used in Italian, Greek, and Mexican dishes. Flowering tops are used in beers and ales, and fresh and dried leaves can be added to soups, casseroles, sauces, stew, stuffing, eggs, olives, teas, tomato-based dishes, and strong-flavored foods like chili and pizza (America, 2005). Reflecting on all the culinary uses and functional properties of oregano is important to analyze and discuss the antimicrobial and antioxidant activity of OEO and its potential as a food additive (Table 2).

USAGE AND APPLICATION IN FOOD SCIENCE

Applications and Quality Issues

The antioxidant properties of oregano and their major components have been proved to be effective in retarding the process of lipid peroxidation in fatty foods (Lagouri et al., 2010). The rates of increment of the peroxide value and conjugated dienes in cottonseed oil, during frying of potato chips, were depressed when oregano was added, with the protective action of oregano extract 2 g/L being considerably greater than that of ground oregano 2 g/L and addition the stability of chips during storage was enhanced by the addition of oregano to the frying oil (Houhoula et al., 2003). Another study evaluated four commercial varieties of oregano from Argentina: Compacto, Cordobes, Criollo, and Mendocino, on changes in positive attributes of fruity, pungent, bitterness, and oregano flavor, and negative attributes as rancid flavor in extra virgin olive oil with addition of these essential oils. The result of this study showed that the presence of oregano essential oil (0.05%) Cordobes type, preserved sensory quality of extra virgin olive oil, prolonging the shelf-life of this product (Asensio et al., 2012).

Milk protein-based edible films containing 1% (w/v) oregano was applied on beef muscle slices, resulting in stabilized lipid oxidation (Oussalah et al., 2004). Fried salted peanuts with 20 g/kg OEO and olive oil showed higher oxidative stability during storage, evaluated by the peroxide and conjugated diene values and was observed that intensity rating of roasted peanut flavor decreased in all fried-salted peanut samples through storage (Olmedo et al., 2009). In addition, the combination of oregano extract 4412 ppm and olive oil 1126 ppm in fresh potatoes resulted in a reduction of acrylamide content, up to 49%, after heating (Kotsiou et al., 2010). The successful combination of OEO, thyme oil pepper oil, and clove oil (all EO from 0.05–1%) resulted in a patent claimed to stabilize polyunsaturated fatty acids (Cloughley and Horrobin, 1996). The addition of oregano spice increased the oxidative stability of tuna salad, although addition of spices had an antioxidative effect, the taste introduced to the product by the addition might be unappreciated in this type of products (Sørensen et al., 2010).

TABLE 2 Antimicrobial, Antioxidant, and Sensorial Effects of *Origanum* spp. Essential Oils Added to Food Products

		Effects			
Oregano Species	**Food Treated**	**Antimicrobial**	**Antioxidant**	**Flavor**	**References**
Origanum vulgare	Cotton seed/ potatoes	ND	Protection against oxidation	Enhancing stability	Houhoula et al. (2003)
Origanum vulgare ssp *hirtum*	Extra virgin olive oil	ND	Reduce oxidative rancidity	Preserved sensory quality	Asensio et al. (2012)
Origanum syriacum var. *bevanii*	Tomato seedling	Antifungal	ND	ND	Soylu et al. (2007)
Origanum vulgare	Peanuts	ND	Higher oxidative stability	Oxidized and cardboard flavor intensities increased	Olmedo et al. (2009)
Origanum marjorana	Sausage	Bacteriostatic	ND	ND	Busatta et al. (2008)
Origanum vulgare	Vegetables Salad	Antimicrobial	ND	ND	De Azeredo et al. (2011)
Origanum onites	Lettuce	Antibacterial	ND	ND	Gündüz et al. (2012)
Origanum vulgare	Tuna salad	ND	Increased the oxidative stability	ND	Sørensen et al. (2010)
Coridothymus capitatus	Protein edible film	Antibacterial	ND	ND	Fernández-Pan et al. (2012)
Origanum vulgare	Sea bream	ND	Strong antioxidant activity	Acceptable sensory scores	Goulas and Kontominas (2007)
Origanum onites L.	Chicken	ND	ND	Desirable and compatible	Oral et al. (2009)
Origanum vulgare	Sausages	Bacteriostatic	ND	Increase sensorial appeal	Busatta et al. (2007)
Origanum onites L.	Fresh cut tomatoes and cucumbers	Sanitizers	ND	Without sensorial defect	Sagdic et al. (2012)
Origanum vulgare subsp *hirtum*	Sheep meat	ND	ND	Acceptable	Govaris et al. (2010)
Oregano sp.	Bolognese sausages	ND	ND	Increased the shelf-life and not found unpleasant by the panellists	Viuda-Martos et al. (2010)
Origanum vulgare	Rainbow trout	ND	ND	Pleasant	Pyrgotou et al. (2010)
Origanum vulgare	Tomato	Antibacterial	ND	ND	Du et al. (2009)
Origanum vulgare	Apple	ND	ND	ND	Lopez-Reyes et al. (2010)
Origanum vulgare	Lettuce	Antibacterial	ND	ND	Gutierrez et al. (2009)
Oregano sp.	Chicken	ND	ND	Shelf-life extension	Ntzimani et al. (2010)
Origanum vulgare	Beef	Antibacterial	Stabilized lipid oxidation		Oussalah et al. (2004)

ND, not determined.

The odor–flavour properties of OEO have been used to add sensorial appeal to food products. The odor sensory impressions of oregano have been defined as lemony/lemon balm, in addition to fruity and sweet descriptors (Bansleben et al., 2010). Sea bream (*Sparus aurata*) treated with OEO (0.8% v/w) received an odor score significantly higher or similar to control samples (Goulas and Kontominas, 2007). The application of OEO (0.345, 0.69, 1.725, 3.45 mg/g) on sausages showed that the addition of this oil may be a promising route to increase sensorial appeal, in addition to the observed bacteriostatic effect (Busatta et al., 2007). Hydrosols obtained from three Lamiaceae plants (thyme, summer savory, and oregano) were effective sanitizers for fresh-cut tomatoes and cucumbers to provide their microbial safety without causing any sensorial defect (Sagdic et al., 2012). Sensory evaluation showed that the addition of oregano EO at 0.6 or 0.9% in minced sheep meat was acceptable, and attribute scores were higher for the EO at 0.6 than 0.9% (Govaris et al., 2010). OEO (0.02%) treatment in conjunction with orange dietary fiber (1%) and vacuum packaging conditions increased the shelf-life of bologna sausages, it should be noted that despite the marked aroma of oregano, this was not found unpleasant by the panellists, who valued the samples containing this EO almost equally with the controls (Viuda-Martos et al., 2010). The sensorial effect of salt, OEO (0.2%) and packaging on fresh rainbow trout fillets during storage at 4C yielded distinctive and pleasant odor and flavor to trout fillets (Pyrgotou et al., 2010). The effect of oregano (*Origanum onites*) EO on the extension of shelf-life of overwrap packed fresh chicken drumsticks reported that OEO gave a characteristic desirable odor and taste to chicken meat, very compatible to cooked chicken flavor, when its concentration was at 0.1% (Oral et al., 2009). It was evaluated the use of vacuum packaging combined with OEO (0.2% and 0.4%) in fresh Mediterranean octopus, concerning the sensorial effect of OEO gave a characteristic, desirable and pleasant odor to the treated samples until days 17 and 23 of storage (Atrea et al., 2009). Choosing the right combination of aromas between the antimicrobial and antioxidant OEO and the food, the quality, safety, antioxidant status, and flavor could be improved. We have to consider that new appeals and tastes are always welcome for modern consumers especially when the sources are of natural origin, like OEO. Evidently, more studies considering the sensorial benefits and the combination of flavors with other food products should be conducted.

Application in Safety Issues

Several techniques are being explored to facilitate the addition of OEO as antimicrobial food additive. The use of antimicrobial edible film could be a means to improving food safety and extending the shelf-life of food systems by controlling the release of antimicrobials on food surfaces. Antimicrobial properties of whey protein isolate based or cellulose-based filter paper films containing oregano and sage EOs (5, 10, 20, 40, and 80 g/kg) were tested against *Listeria innocua, Staphylococcus aureus,* and *Salmonella enteritidis*, and the highest inhibition zones (aproximately 3750 mm^2) were against *L. innocua* (Royo et al., 2010). The addition of essential oil of oregano in tomato-based edible film was effective to inhibit the bacterial growth of *S. enterica* and *Listeria monocytogenes.* Moreover, this edible film showed good physical properties for food applications (Du et al., 2009). The application of oregano films on meat surfaces containing 10^3 UFC/cm^2 of *Eschericia coli* O157:H7 or *Pseudomonas* spp. showed that oregano film was effective against both bacteria, showing a 0.95 log reduction of *Pseudomonas* spp. levels and 1.12 log reduction of *E. coli* O157:H7 (Oussalah et al., 2004).

Incorporation of oregano oil (1%) to a whey protein-edible film was effective at inhibiting the bacterial growth of *L. innocua*, *S. aureus*, *S. enteritidis*, and *Pseudomonas fragi.* Low concentrations of OEO have shown a wide spectrum of action against both pathogen and spoilage bacteria (Fernández-Pan et al., 2012). Promising opportunities are presented by these films with direct application on food systems. In another study, the use of oregano essential oil (500 ppm) was effective to effectively reduce populations of *Salmonella* spp. inoculated on iceberg lettuce until 2.28 UFC/g, but showed browning and softening of the lettuce leaf surface after 10 min of treatment with oregano oil (Gündüz et al., 2012).

OEO and its principal compounds have been applied also as antimicrobials in fruit and vegetable produce. OEO and carvacrol (0.67 and 0.067%) were used to treat apple, mango, orange, and tomato juices inhibiting the growth of *E. coli* O157:H7 (Friedman et al., 2007). Treatments of apples with EOs from oregano (1 and 10%) showed significant efficacy at reducing *Botrytis cinerea* and *Penicillium expansum* growth (Lopez-Reyes et al., 2010). EOs from *O. vulgare* L. and *Rosmarinus officinalis* L. (80–0.003 μL/mL) inhibited bacterial microflora associated with minimally processed vegetables (De Azeredo et al., 2011). Carvacrol and thymol (10, 25, 50, 100, 250, and 500 μL/L of carvacrol, thymol or the mix (1:1) of carvacrol + thymol) applied to lemon, resulted in low fungal decay induced by *Penicillium digitatum* and *Penicillium italicum* (Pérez-Alfonso et al., 2012). OEO (400 ppm) combined with thyme (6000 ppm) was evaluated for control of *Enterobacter cloacae* on lettuce (Gutierrez et al., 2009). *Listeria* strains were more sensitive than spoilage bacteria, and oregano and thyme were the most active EOs. The average efficacy of EOs against *Listeria* spp. was in the following order: oregano ≥ thyme > lemon balm, while the efficacy order of EOs against the spoilage bacteria was: oregano ≥ thyme > marjoram.

OEO (0.2% v/w) or its principal components have also been used in conjunction with other technologies, such as vacuum-packaging with ethylenediaminetetraacetic acid (1.5% w/w) and lysozyme solution (1.5% w/w) on semicooked coated chicken meat, extending shelf-lives by 7–8 days compared with controls (Ntzimani et al., 2010). In another investigation carvacrol

(0.3–0.5%), caprylic acid (0.25–1.0%), and ε-polylysine (0.125–1.0%) were applied on breaded chicken products and showed a reduction in *Salmonella* populations (Moschonas et al., 2012). Pectin edible films that contained carvacrol (0.5, 1.5, and 3%) and cinnamaldehyde (0.5, 1.5, and 3%) caused the inactivation of *L. monocytogenes* on ham and bologna (Ravishankar et al., 2012). Dipped carp fillets with carvacrol (0.5%) and thymol (0.5%) delayed bacterial growth and extended the shelf-life of the fillets (Mahmoud et al., 2004). The studies of the antimicrobial activity of *Origanum majorana* (1.15, 2.3, 5.75 mg/g) in fresh sausage showed that its addition to fresh sausage exerted a bacteriostatic effect at concentrations lower (1.15 mg/g) than the minimum inhibitory concentration (2.3 mg/g) (Busatta et al., 2008). EO of *Origanum syriacum* var. *bevanii* (0.4, 0.8 1.6, 2.4, and 3.2 µg/mL) had a marked antifungal effect against soil contamination with *Sclerotinia sclerotiorum*, both oils reduced sclerotial viability, increasing the number of surviving tomato seedling by 69.8 and 53.3%, respectively (Soylu et al., 2007). In other study, four pathogenic bacteria (*E. coli* O157:H7, *S. enterica*, *B. cereus*, and *S. aureus*) were inoculated in a dough made from corn flour with carvacrol (0.5, 2, and 5%) and all the strains were completely inactivated within 24 h (Ortega Morente et al., 2010). This information reveals the antimicrobial potential of OEO to be used as a food additive.

Considering the technological use of this oil, a wider range of food matrices could be treated to take advantage of its antimicrobial efficacy, like fresh meat, seafood, and several types of whole and fresh-cut fruits and vegetables. To achieve a more effective treatment of food products using OEO several technologies must be contemplated: nanoemulsions, nanocapsules, vapors, edible films based in different polymers; in conjunction with other preserving technologies like low temperatures, modified atmosphere packaging, and irradiation. The generation of this information will profound on the antimicrobial knowledge and effective uses of the OEO.

SUMMARY POINTS

- Essential oil from *Origanum* spp. has shown efficacy retarding lipid oxidation in food matrices.
- Oregano essential oil possesses strong antimicrobial activity against food pathogen bacteria highlighting its potential as a tool to achieve food safety.
- Oregano essential oil has shown efficacy in reducing microbial growth of deteriorative microorganisms (bacteria, yeast, molds), representing the potential to increase shelf-life of food.
- Oregano essential oil can be considered as a rich source of bioactive compounds and its addition to food matrices transmit these benefits; this approach can be use as a tool to generate functional foods.
- The sensorial approach of the use of oregano essential oil must be studied further to relate the dose–response of the different application that can present.

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Chapter 72

Patchouli (*Pogostemon Cablin* Benth) Oils

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List of Abbreviations

EO Essential oil
PC *Pogostemon cablin*

INTRODUCTION

The commercial importance of medicinal and aromatic plants is now in great demand due, in part, to climatic features. India is a country with diverse agro-climatic zones, and has a wealth of different types of flora and fauna. The introduction, acclimatization, and cultivation of a huge number of aromatic plants occurred with minimum effort. Patchouli is an important medicinal and aromatic herb which is considered as one of the vital intercrop with immense export potential. Generally this plant is a shade loving and an annual crop. The name patchouli is derived from the Tamil word *patchai* ("patch" means green and "ellai" means "leaf"). Commonly in India, the plant is known as pachholi, panadi, pacchathene, etc. The patchouli plant is native to tropical south-east Asian countries, but is widely cultivated throughout the world. It was introduced into Europe in around 1844 when shawls were wrapped with patchouli. Several species are cultivated exclusively in India and other parts of the world for their oil but *Pogostemon cablin* (PC) is one that yields the best grade of oil. The oil is extracted from shade dried leaf of patchouli through steam distillation (Donelian et al., 2009) or by hydrodistillation through Clavenger apparatus (Das et al., 2011). The oil content varies from 2.5 to 3.5% in shade dried leaves in commercial distillations. The oil is thick and deep reddish brown in color. It is having camphoraceous odor. Interestingly, the value and demand of the oil in the perfumery market is increased because of unavailability of synthetic substitute for patchouli oil (Ramachandra et al., 2002) and hence this oil is used as a base material in perfume industry. Patchoulol or patchouli alcohol ($C_{15}H_{26}$) and norpatchoulenol are the main chemical components which are responsible for strong, long-lasting fragrant aroma of the oil and other minor constituents like caryophyllene, α, β-patchoulene, pogostol, seychellene, and cycloseychellenel are also present (Figure 1) (Akhila and Nigam, 1984).

BOTANICAL ASPECTS

Plant Profile

Kingdom: Plantae (plants)
Subkingdom: Tracheobionta (vascular plants)
Superdivision: Spermatophyta (seed plants)
Division: Magnoliophyta (flowering plants)
Class: Magnoliopsida (dicotyledons)
Subclass: Asteridae
Order: Lamiales
Family: Lamiaceae (mint family)
Genus: *Pogostemon* Desf. *pogostemon*
Species: *Pogostemon cablin Benth Patchouli*

Patchouli (PC) is a branched, erect, perennial aromatic herb belongs to the family Lamiaceae. The plant requires deep, well-drained, fertile, slightly acidic, deep loamy soil, rich in humus and nutrients. An altitude of 800–1000 m above the sea

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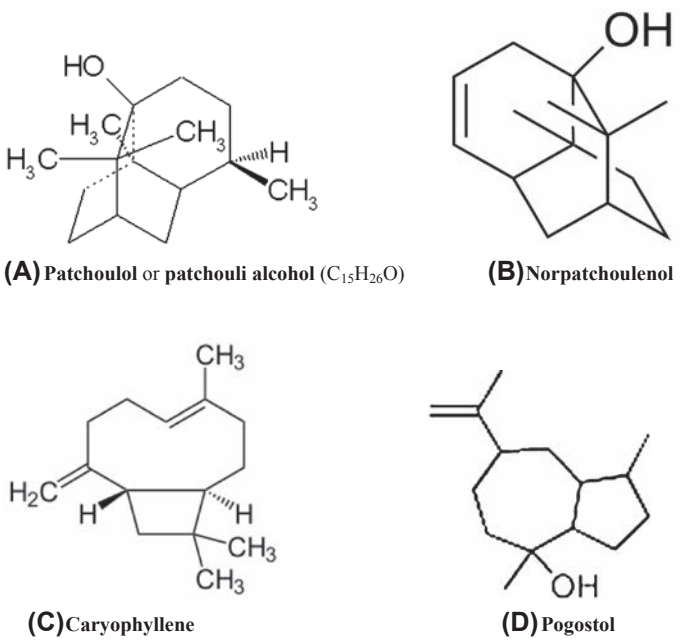

(A) Patchoulol or **patchouli alcohol** ($C_{15}H_{26}O$) **(B) Norpatchoulenol**

(C) Caryophyllene **(D) Pogostol**

FIGURE 1 Some of the important chemical structures of the PC EO. (A) Patchouli alcohol (http://institutenuklir.wordpress.com/2011/10/14/peningkatan-kualitas-minyak-nilammetode-1/). (B) Norpatchoulenol (http://en.m.wikipedia.org/wiki/File:Norpatchoulenol.svg). (C) β-Caryophyllene (http://en.wikipedia.org/wiki/File:Beta-Caryophyllen.svg). (D) Decahydro-1,4-dimethyl-7-(1-methylvinyl)azulen-4-ol (http://www.chemicalbook.com/ChemicalProductProperty_EN_CB52202618.htm).

FIGURE 2 (A) Patchouli leaf (http://www.indiamart.com/ultrainternational/). (B) Patchouli plant with white flowers (http://www.indianetzone.com/16/patchouli_oil.htm).

(A) Patchouli leaf **(B) Patchouli plant with white flowers**

level, pH of the soil range from 5.5 to 7.5, an annual rainfall about 300–400 cm, and relative humidity of 75% are optimum for good growth. A temperature range from 20 to 35 °C is ideally suited for patchouli cultivation.

The plant grows to about 1.0–1.5 m height. The leaves are about 7–10 cm long, ovate, covered with trichomes all over the epidermis and variable green color in full sun light but bright green in the shade. Leaves are leathery, dentate margins, pale to purplish green in color. The flowers are small, delicate, and borne both in axillary and terminal spikes. They are generally pinkish in color. The seeds are highly fragile in nature. The flowers are in bloom during the autumn season (Figure 2).

The plant is grown extensively in the tropical climates of Indonesia, Malaysia, Singapore, China, Brazil, and India. In India, the plant is cultivated in the coastal regions of Tamil Nadu, Karnataka, Maharashtra, Gujarat, Goa, Kerala, Andhra Pradesh, Assam, and West Bengal (Bown, 2001) (Figure 3). Karnataka is a leading producer of patchouli. After the initial stray attempts to grow the crop, its systematic cultivation was started in 1962 by Central Institute of Medicinal and Aromatic Plants (CIMAP) (Kumar et al., 1986). Botanists first described patchouli in 1845 (Das, 2010).

Propagation of the crop is through stem cutting due to difficulties of flowering in India and hence, in the nursery, the bed stem cutting method is adopted for mass cultivation. After nursery bed raising, the healthy plantlets are transplanted in the field when they are about 2 months old. The ideal time for planting is late March to May followed by September to

FIGURE 3 Cultivation of patchouli in different states of India. Prepared by author.

October. This plant can be maintained for more than one year, but the yield declines after about three to four cuttings. A massive constraint is the presence of pests and diseases caused by various organisms during the Patchouli cultivation like yellow mosaic virus (Sastry and Vasanthakumar, 1981) and *Potyvirus* (Peanut stripe virus) (Singh et al., 2009), leaf roller (*Pachyzacia stultalis*), and a myriad bugs (*Pachipeltis* spp), mites, and other nematodes. They can be controlled by spraying of pesticides like methyl parathion, Dimethoate 30 EC, Ethion, Furadan granule, etc., at recommended doses. The leaves

TABLE 1 Some of the Important Manufacturer and Exporter Companies for Patchouli Oil in India

Name of the Company	Situated	Products
Crystal Aromatics	New Delhi	Manufacturer and exporter of patchouli essential oil (PC EO)
Karnataka Aromas	Bangalore	Pure PC EO
Triveni Aromatics and Perfumery Pvt Ltd	Vapi, Gujarat	Pure PC EO
Falcon	Bangalore	Production of PC EO
Nikita Extracts	Ahmedabad	Manufacturer of PC EO
Sagar Aromatics	Mumbai	Production of cosmetics with PC EO
Nice Exporters	Coimbatore	Production of cosmetics with PC EO
Aromatic Herbals Pvt Ltd	Kolkata	Supplier of PC EO
Aditi International	Mumbai	Production of cosmetics with PC EO
Sumedh Organic Agri Biz	Bangalore	Exporters and suppliers of patchouli oil

Source is various internet websites, for example, http://dir.indiamart.com/impcat/patchouli-oil.html.

are harvested preferably in morning time after 3–4 months after planting and then dried in shade. The dried leaves are packed in gunny bags and stored for 3 months. Some of the manufacturing industries in India are listed in Table 1.

There is no established variety of patchouli under cultivation but two types of patchouli are well known: true Patchouli (*P. cablin*) and Java Patchouli (*Pogostemon heyneanus*). Three patchouli germplasms namely Johore (IIHR- PP-1), Java (IIHR-PP-4), and Singapore (IIHR-PP-5) were multiplied and planted in field trials for morpho–economical evaluation. Johore (IIHR-PP-1) has been found to be promising with respect to growth of plants, herbage, and essential oil (EO) yield (Das, 2010).

USAGE AND APPLICATIONS

Patchouli herb is believed to have many health benefits to people. The healing properties of this plant are discussed in this section. Patchouli EO has remarkably versatile traditional, medicinal, and cosmetic uses. Indonesia is the major producer and exporter of its oil, whereas Malaysia, China, and Brazil are also large producers. The Indian demand for patchouli EO is around 220 tons valued at 6,159,021 US$ while global demand is to the tune of 1600 tons of oil per annum with a value of 44,792,880 US$ (Vijayakumar, 2004).

Traditionally the fresh leaves are very effective in healing burns, calming nerves, and controlling appetite. In the early 1800s, Patchouli was used as a moth repellant in Asia and in Europe. One of the earliest known uses for patchouli was as protection from bed bugs and moths. Patchouli is traditionally used as a shampoo when the leaves are crushed with lemongrass. Fresh leaves were crushed and the juice was applied to burns, snake bites, and cuts to cure the wounds.

An EO possesses various medicinal and health benefit activities like cicatrisant, febrifuge, antiinsecticidal, fibrinolytic, and antithrombotic activity (Sumi, 2003; Eunkyung et al., 2002), antifungal, and bacteriostatic properties (Kukreja et al., 1990; Pattnaik et al., 1996). In aromatherapy, it is used to calm nerves, and relieve depression and stress (Bowel et al., 2002). Different pharmacological activities like antiinflammatory, antiseptic, astringent, diuretic, sedative, antimutagenic, etc., were also reported (Ichikawa et al., 1989; Miyazawa et al., 2000). Inhaling patchouli EO produced a decreased response in the sympathetic nervous system as results it lowered systolic blood pressure and acts as good tonic for veins (Haze et al., 2002). An infusion of leaves is used for relief of menstrual cramps (Wei and Shibamoto, 2007).

Commercially, PC EO is extensively used in the perfume and cosmetics (Hasegawa et al., 1992; Maheswari et al., 1993) industries. In formulation it can be used for bad breath and halitosis in a gargle form, and the raw leaves are ingested for an aphrodisiac action. In a lotion or cream, PC has long been used in skin care activity. It helps to alleviate acne-prone conditions, eczema, inflammation, cracked, chapped, and irritated skin. Furthermore, the oil can be blended (with basil, bergamot, geranium, juniper, lavender, etc.) and used as massage oil or diluted in a bath for constipation, dermatitis, and overweight. Due to antifungal properties of patchouli EO, it is used for treating athlete's foot. In hair care formulations, patchouli EO helps alleviate signs of dandruff and balances oiliness due to antifungal properties. Bath soap formulations with oil relaxed the mind. Dried leaves are used for scenting wardrobes.

PC is used widely in modern perfumery and modern scented industrial products such as paper towels, laundry detergents, and air fresheners. Ground, shade-dried patchouli obtained from residual EO was used in different types of incense sticks manufacture by blending the patchouli spent charge powder (Ramya et al., 2013).

USAGE AND APPLICATIONS IN FOOD SCIENCE

EOs are beneficial to the industries, particularly the food and beverage industries, due to their uniform quality and colorless appearance. The flavor and fragrance industries have grown due to increased demand for ethnic foods, innovation in beverage products, growth in processed food consumption, and an increasing demand for natural fragrances. Hence, the major primary markets for EOs includes in soft drink and food industries in India. PC added a new dimension to its culinary use in the food and beverage industries. With an increasing trend for preference of natural products, demand for natural fragrances and flavorings such as patchouli continues to grow, despite market competition by synthetic substitutes which have the advantages of lower production costs, stable pricing, and regular supply in food beverages. EOs are used extensively as they are generally recognized as safe flavoring agents in foods, beverages, and confectioneries according to strict good manufacturing practice and flavorist standards. Therapeutic grade EOs are generally safe for human consumption in small amounts. After approval from the Food and Drug Administration, the therapeutic grade EO of PC is approved as a food additive and flavoring agent and can be use in different formulations. The fresh leaves of PC are used as a seasoning and an EO (obtained from steam distillation) is used in food supplements and other soft drinks in very low concentration of around 2 ppm (Bauer et al., 1997). Dried patchouli dried leaves are extensively used as a flavor ingredient in major food products including alcoholic and nonalcoholic beverages like in preparations of soda, pop, coke, soda pop, fizzy drink, tonic, seltzer, mineral, or carbonated beverages.

PATCHOULI CABLIN EO IN FOOD FLAVORING AND FOOD PRESERVATIONS

The interest in EOs and their application in food preservation has been amplified years by an increasingly negative consumer perception of synthetic preservatives. Furthermore, food-borne diseases are a growing public health problem worldwide which can be minimized by effective preservation strategies and that is due to the action of antibacterial properties of EOs. In the nineteenth century the application of EO in aroma and flavor ingredients increased and about 300 were used commercially in the flavor and fragrances market (Burt, 2004) in foods. Maintaining or creating nutritional value, texture, and flavor is an important aspect of food preservation. Usually the darkish shades of patchouli oil are preferred for food products. Combinations of PC EO and other EOs with different preservation techniques are better alternatives for food protection. In Asia and South America, PC EO is often blended with anise and clove oil as a breath freshener and used in making delicious foods. PC EO is having antimicrobial activity that helps for preservation of foods. Generally, the chemical pickling method is used for preserving food in an edible antimicrobial liquid. This method is carried out by placed the foods in an edible liquid that inhibits or kills bacteria and other microorganisms. PC EO is helpful in preserving the foods like cucumbers, peppers, corned beef, herring, and eggs and also some mixed vegetables like piccalilli. The oil of PC also acts as a good masking agent for unpleasant tastes and smells in the food products like meat and other meat products. The oil of PC helps in the inhibition of oxidative deterioration in the meat products due to its antioxidant activity (Hussain et al., 2011) and is also used in preserving meat and meat products by canning process. Due to the presence of long-lasting strong sweet, woody, and spicy fragrance of the PC EO, it preserves the appearance, flavor, and texture of the meat products to a large extent. The EO of PC was added to low tar cigarettes and tobacco for enhancing flavor. Fresh leaves are used as seasoning to improve the flavor of foods like salad dressings, soups, sauces, etc.

PATCHOULI CABLIN EO IN BAKERY PRODUCTS

The fragrances of baked products are made richer and smoother with the modification of freshly distilled PC EO into a syrupy form. Flavor is really the ingredient that helps the baker to add uniqueness to its product. A number of EOs are available to add flavor to baked products in which PC EO plays a vital role with its strong sweet fragrance. The EO of PC is used as a fundamental ingredient in baking and is also used to add moisture and texture to baked products like cakes, cookies, puddings, etc. Naturally occurring antimicrobial compounds for food preservation getting increasing attention due to consumer awareness about natural food products and a growing concern of microbial resistance toward conventional preservatives. Hence, due to the presence of natural antioxidants and antimicrobial activities of PC EO, it increases shelf life of some bakery products like cakes because PC EO inhibits oxidation of cakes that become rancid after prolonged storage owing to the oxidation of polyunsaturated fatty acids. Biscuits are a major baked food product, contributing over 70%

of the total production in the bake industry. The flavor of PC EO is used as one of the important ingredients to make creams that are used in biscuit preparations. A cream is prepared and applied onto sheets to form a sandwich. The manufacture of prepacked sandwiches is now a large industry, consuming large quantities of bread that can be stored with the help of the vapor of PC EO. Further packaging of the baked products is also carried out with the vapor form of PC EO. An antifungal activity of the PC EO helps to minimize the fungal growth of the back products over longer storage (Kocevski et al., 2013). Some of the baked products are used for longer storage with the help of PC EO (vapor form). Baked products include cookies, namkeen, dry cake, vegetable patties, biscuits, etc.

PATCHOULI CABLIN EO IN ALCOHOLIC AND NONALCOHOLIC BEVERAGES

The demand for India's alcoholic beverage products like beer, wine, white wine, grapes wine, brandy, whiskies, rum, gin, etc. are increasing in global market. India is the third largest country in the market for alcoholic beverages in the world. During the composition of a new alcoholic spirit the EOs must be added to alcohol dropwise, dissolved, and only then can juices, syrup, and water be introduced. This method is followed for enhancing flavor in alcoholic preparations like natural EO from PC is added to vodkas and liqueurs like brandy as a primary or supplemental flavor because PC EO has a very strong taste and flavor. PC EO is also used in nonalcoholic beverages like fruit and vegetable juices and tonic preparations. The development of soft drink industries in India is of great importance because it is a major consumer of EOs, especially of PC EO. The EO of PC is used for preparing soft drinks and help in preservation without spoiling the appearance of the beverage or causing peculiar taste in the soda, pop, fizzy drink, etc. Patchouli is used in tonic form and corrects metabolic functions like decomposition of food and absorption of nutrients by toning up the liver, stomach, and intestines, helping stronger and healthier growth. The tonic is also used for proper excretion, regulates the endocrine secretion of hormones and enzymes, and also tones up the nervous system by boosting an immunity to protect from infections.

PATCHOULI CABLIN EO IN SELTZER, MINERAL, OR CARBONATED BEVERAGE

Seltzer means mineral water, soda water, or carbonated water. These sparkling waters with natural flavors contain EOs for calorie-free refreshment. Both carbonated water and mineral water are usually calorie-free. Therapeutic grade PC EO is added to mineral water in dropwise to enhance the flavor and increase calcium level in bones. PC EO is preferred to add in to water because of its sweet pleasant taste and it is also acts as antioxidant. One of the benefits of adding therapeutic grade PC EO to water is to eliminate toxins, petroleum residues, metals, and other harmful substances without any side-effects. PC EO also helps to energize, uplift the mind and spirits, and boost health. PC EO is also added in soda water to increase metabolism and soothe digestive issues.

PATCHOULI CABLIN EO IN OTHER FOOD PRODUCTS

Chewing gums and all kinds of mouth refreshing confectioneries are another range of confectioneries generally containing sweetening composition, food grade acid composition, cooling agents, and flavoring agents, especially EO of herbals plants. Chewing gum is a functional food, having a significant impact on oral hygiene over the short-term. PC EO is used in chewing gum and bubble gum for enhancing flavor. The product does not just mask foul mouth odor, it also helps in kills bacteria due to its antibacterial activity that are linked with bad breath. The products also provide a sensation of mouth moistening and freshness upon consumption. The PC EO helps keep mood fresh and relieves tension when chewing bubble gum or chewing gums.

SUMMARY POINTS

- The *Patchouli cablin* plant is native to the Philippines and Malaysia and widely cultivated in other countries, including India.
- Patchouli essential oil is a raw material of great importance in the perfumer's palette.
- Postharvest handling of the patchouli herbage play an important role in obtaining the patchouli essential oil both in terms of oil yield and quality.
- Planting on raised beds provides better survival and yield.
- Partial shade prolongs the life of cropping.
- Steam distillation is the preferred process for the extraction of essential oils from dried *Patchouli cablin* plant material from the consumer point of view.

- Patchouli is beneficially made use of in aromatherapy, perfumery, cosmetics, and incense stick production industries.
- Dried leaves of *Patchouli cablin* are extensively used as a flavor ingredient in major food products including alcoholic and nonalcoholic beverages.
- Therapeutic grade essential oil from PC is used in flavor food supplements, beverages, candy, baked products, puddings, and other soft drinks at very low concentrations.

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Chapter 73

Pune-sa (*Nepeta*) Oils

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List of Abbreviations

MIC Minimum inhibitory concentration
BHT Butylated hydroxytoluene
DPPH 2,2-Diphenyl-1-picrylhydrazyl

INTRODUCTION

Plants have served humans for a great variety of their needs such as shelter, clothing, food, flavors, fragrances, and medicines. Lamiaceae is included among the most important plant families because of its ethnomedicine and medicinal values based on concentration of essential oils (Venkateshappa and Sreenath, 2013). Moreover, many plants of the family Lamiaceae are highly aromatic and these plants produce volatile oils. It has been reported that the volatile oil of the Lamiaceae family of plants are employed in the pesticide, flavoring, pharmaceutical, fragrance, perfumery, and cosmetic industries (Venkateshappa and Sreenath, 2013).

Although there have been modern improvements in food production techniques the question about food safety still remains a vitally important public health issue. Essential oils derived from plants have been used as flavoring agents in foods and beverages (Helander et al., 1998). Moreover, due to their antimicrobial potential, they are used as natural food preservatives. Essential oils showed antimicrobial activity due to the presence of various terpenoid and phenolic compounds. Interestingly, these terpenoids showed antibacterial or antifungal activity in their pure form as well (Helander et al., 1998). In Iran the genus *Nepeta* is described by the common Persian name of "Pune-sa" (Formisano et al., 2011). This chapter gives an overview of the antimicrobial (antibacterial and antifungal) and antioxidant activity of the volatile oils derived from *Nepeta* species.

BOTANICAL ASPECTS

The genus *Nepeta* belongs to the family Lamiaceae, a family of flowering plants. The Lamiaceae family was traditionally considered closely related to Verbenaceae but later studies demonstrated that many genera categorized in Verbenaceae were related to Lamiaceae (Venkateshappa and Sreenath, 2013). The Lamiaceae plants are aromatic and these include culinary herbs such as basil, rosemary, marjoram, mint, sage, and thyme (Venkateshappa and Sreenath, 2013).

The genus *Nepeta* is represented in Iran by 67 species. Of these 38 are endemic and 31 are native in Iran. Moreover. *Nepeta cataria* and *Nepeta crassifolia* Boiss. & Buhse are perennial herbs that are found wild in Azerbaijan, Golestan, Khorasan, Mazandaran, Qazvin, and Tehran provinces of Iran. Thirty three *Nepeta* species are present in Turkey including 38 taxa, of which 17 are endemic (Farjam, 2012).

The main chemical components are monoterpenes, nepetalactone derivatives, iridoid glucosides, sesquiterpenes, abietane-type diterpenes, isopimarane-type diterpenes, ursanes-type, oleanane-type, lupine-type triterpenes, flavonones, flavanols, phenols, and caffeic acid phenethyl esters.

USAGE AND APPLICATIONS

Plants of the *Nepeta* genus are extensively employed in folk medicine as an antispasmodic, diuretic, antiasthmatic, antiseptic, antitussive, ethnobotanical effect, diaphoretic, vulnerary, antispasmodic, tonic, and febrifuges (Farjam, 2012). Some plants of the *Nepeta* genus have been reported to have astringent properties and are used as a topical remedy for scorpion

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FIGURE 1 *Nepeta cataria*.

and snake bites. *Nepeta crispa* Willd. is known locally as Mofarrah and this plant has been used in Iranian traditional medicine for many years. It has been reported that an infusion and beverage obtained from *N. crispa* has been used in Iranian traditional medicine as a relaxant, sedative, carminative, and also for nervous and respiratory disorders (Sonboli et al., 2004).

Nepeta cataria (Figure 1) is commonly called "catnip" or "catmint" due to its irresistible action on cats. *Nepeta cataria* is employed in herbal teas because of its lemony mint flavor. Traditionally, *N. cataria* is employed for the treatment of colic, cough, diarrhea, asthma, and bronchosis. *Nepeta cataria* (catnip) is used as a tea as a sedative and soporific. It has been reported that fresh and dried leaves of *N. cataria* are used for flavoring sauces, juices, soups, and cooked foods. Moreover, *N. cataria* is also used as a tincture, infusion, and poultice. *Nepeta cataria* flowering tops and leaves are used as antispasmodic, antitussive, astringent, carminative, diaphoretic, semmengogue, refrigerant, sedative, stimulant, stomachic, and tonic (Adiguzel et al., 2009). *Nepeta cataria* is employed as a fortifier, a disinfectant and is also used for treatment of colds (De Pooter et al., 1987). Nepetalactone was reported from the volatile oil catnip and this compound has proven to be useful in digestive complaints, migraine headaches, and nervous disorders.

It has been reported in the literature that extract of *Nepeta koperdaghensis* is employed as a diuretic, a bacteriostatic, and a cure toward eczema-type skin disorders (De Pooter et al., 1987). On the other hand, *Nepeta hindostana* reduced the level of serum lipids and lipoproteins and due to this property *N. hindostana* might thus supplement remedies toward atherosclerosis (De Pooter et al., 1987). *Nepeta glomerulosa* Boiss. subsp. *carmanica* is a herbaceous wild plant and native to Iran and is used in Iranian folk medicine to treat pneumonia (Sajjadi and Ghassemi, 1999). *Nepeta ispahanica*, *Nepeta binaloudensis*, *Nepeta bracteata*, *Nepeta pogonosperma*, and *Nepeta pungens* are used as a medicinal herb in Iranian traditional medicine. Moreover, *Nepeta caesarea* is employed as a herbal tea for the treatment of gastric disorders. It has been reported that fresh leaves of *Nepeta troodi* and *Nepeta parnassica* are used for toothache and also for treatment of contusions and rheumatic pains (Formisano et al., 2011).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Antimicrobial Activity

Alim et al. (2009) evaluated the antimicrobial potential of the essential oils of *Nepeta nuda* against bacterial and fungal strains such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella thyphi*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Bacillus subtilis*, *Corynebacterium diphteriae*, and *Candida albicans*. The results showed that the essential oils of this plant demonstrated strong activity against *K. pneumoniae*, *S. aureus*, and *C. albicans* with inhibition zones of 38 ± 1.5 mm, 22 ± 0.8 mm, and 22 ± 0.5 mm, respectively (Table 1). On the other hand, essential oils of *N. nuda* demonstrated moderate antimicrobial activity toward *E. coli*, *C. diphteriae*, *P. vulgaris*, and *B. subtilis*. However, the essential oil did not show any activity toward *P. aeruginosa* (Alim et al., 2009). It is interesting to note that the major components of essential

TABLE 1 Antimicrobial Activity of the Essential Oil of *Nepeta* Species and Diameter of Inhibition Zone (mm)

Microorganism	*Nepeta nuda*[a]	*Nepeta tuberosa*[b]	*Nepeta atlantica*[b]	*Nepeta cataria*[b]	*Nepeta granatensis*[b]
Staphylococcus aureus	22 ± 0.8	23.33 ± 8.81	7.50 ± 1.44	10.00 ± 3.53	68.00 ± 12.00
Staphylococcus aureus ATCC 25923	–	4.37 ± 0.62	18.43 ± 7.92	5.00 ± 0.00	22.50 ± 6.29
Salmonella enteritidis	–	20.00 ± 0.00	20.00 ± 0.00	53.33 ± 13.33	80.00 ± 0.00
Escherichia coli	12 ± 0.6	30.0 ± 5.77	7.96 ± 4.07	16.60 ± 3.33	70.00 ± 10.00
Escherichia coli ATCC 25922	–	6.25 ± 1.25	13.13 ± 4.25	16.25 ± 3.75	35.00 ± 15.00
Pseudomonas aeruginosa	6 ± 0.0	40.0 ± 0.00	22.50 ± 6.29	37.50 ± 15.47	40.00 ± 0.00
Salmonella thyphi	16 ± 0.8	–	–	–	–
Klebsiella pneumonia	38 ± 1.5	–	–	–	–
Proteus vulgaris	11 ± 0.4	–	–	–	–
Bacillus subtilis	12 ± 0.9	–	–	–	–
Corynebacterium diphteriae	13 ± 0.1	–	–	–	–
Candida albicans	22 ± 0.5	–	–	–	–

[a]*Agar disc diffusion method, diameter of inhibition zone (mm) including disk diameter of 6 mm.*
[b]*Data represent the MICs (μL/mL) ± standard error of the means for four experiments.*

oils of *N. nuda* are *trans*-caryophyllene (23.9%), isopulegone (12.6%), cis-sabinol (10.1%), and β-pinene (10.0%). In another study, the major compounds of the oil of *N. nuda* were demonstrated to be β-caryophyllene oxide (21.8%), spathulenol (13.8%), allo-aromadendrene (9.0%), and β-caryophyllene (5.4%) (Kokdil et al., 1998).

Zenasni et al. (2008) reported the antimicrobial activities of four *Nepeta* species: *Nepeta atlantica*, *Nepeta tuberosa*, *N. cataria*, and *Nepeta granatensis* (Table 1). Results of their antimicrobial activity showed that the volatile oils from *N. tuberosa*, *N. atlantica*, and *N. cataria* demonstrated antimicrobial activity toward all strains of tested bacteria and minimum inhibitory concentrations (MICs) were in the region of 4.37–53.33 μL/mL. The essential oils obtained from *N. granatensis* showed moderate antibacterial activity with MICs ranging from 22.5 to 80 μL/mL. The MICs of essential oils from *N. tuberosa*, *N. atlantica*, and *N. cataria* toward *S. aureus* (ATCC 25923) were 4.37, 7.50 and 5.00 μL/mL, respectively, while the MIC of essential oils obtained from *N. granatensis* was of the order of 22.50 μL/mL. Moreover, the bacterial strains *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were more sensitive to the essential oils of *N. tuberosa*, *N. atlantica*, and *N. cataria* and this was evident from their MICs values which ranged from 4.37 to 16.25 μL/mL. On the other hand, the MICs ranged from 7.96 to 30 μL/mL for isolated *E. coli* and *S. aureus*. It is interesting to note that *Salmonella enteritidis* and *P. aeruginosa* (ATCC 27853) were less sensitive to essential oils of four plants (MICs below 53.33 μL/mL). Of further note was that the oil of *N. granatensis* comprises a small amount of nepetalactone and this same oil showed comparatively low antibacterial potential toward tested microorganisms (MICs ranged from 22.5 to 80 μL/mL).

The major component of the essential oils of *N. atlantica* (74%), *N. tuberosa* (82%), *N. cataria* (82%), and *N. granatensis* (42%), was neptalactone (the stereoisomer 4a-α, 7-α, 7a-β-nepetalactone and the dihydronepetalactone). Moreover, *N. atlantica* is relatively rich in β-caryophyllene (8.2%) and farnesol (2.5%), while *N. granatensis* is characterized by other components (eucalyptol 24%, α-pinene 6.3%, and α-phellandrene 5.8%). Additionally, *N. cataria* is rich in limonene (4.1%) and terpinene (4.2%). It was postulated that the antibacterial potential of the oils of *Nepeta* species may be due to their major component compound, that is, nepetalactone. However, this does not exclude the possibility that other components may be responsible for the antibacterial activity of these essential oils. This is to an extent evident in part by the differences observed in the sensitivity of tested bacteria to essential oils from *Nepeta* species which have different concentrations of nepetalactone. It has been found (Zenasni et al., 2008) that *N. granatensis* oils contain 42% nepetalactone and 24% eucalyptol. On the other hand, some authors have reported that eucalyptol demonstrated antibacterial activity against *S. aureus*.

Stojanovic et al. (2005) reported that the activity of the ethanol oil solution of *Nepeta rtanjensis* at a 1:10 dilution was higher than the activity of the reference antibiotic (Table 2). It is interesting to note that *E. coli* (5.5 mm) and *S. aureus* (5.0 mm) were particularly nonresistant at that dilution. On the other hand, a 1:30 dilution demonstrated nearly equal activity

TABLE 2 Antimicrobial Activity of the Oils of *Nepeta rtanjensis* (Growth Inhibition Zones, mm)

Microorganisms	*Klebsiella pneumoniae*			*Pseudomonas aeruginosa*			*Salmonella enteritidis*			*Escherichia coli*			*Staphylococcus aureus*			*Aspergillus niger*		
Dilution	**1:10**	**1:30**	**1:60**	**1:10**	**1:30**	**1:60**	**1:10**	**1:30**	**1:60**	**1:10**	**1:30**	**1:60**	**1:10**	**1:30**	**1:60**	**1:10**	**1:30**	**1:60**
Bacteriostatic	4.8	3.0	3.0	6.5	3.5	3.0	9.0	8.0	8.0	9.0	6.0	6.0	10.5	9.0	6.3	6.0	3.5	3.5
Bactericidal	4.8	0.5	0.0	3.5	1.0	0.0	4.5	0.5	0.0	5.5	2.0	0.0	5.0	3.0	1.0	0.5	0.0	0.0

TABLE 3 Antimicrobial Activity of *Nepeta crispa* Essential Oil (Inhibition Zone, mm)

	Oil Dilutions				
Microorganism	**1**	**1:2**	**1:4**	**1:8**	**1:6**
Bacillus subtilis	26.5	22	18	14	10.5
Enterococcus faecalis	10.5	9.5	7	Na	Na
Staphylococcus aureus	19.5	16	12.5	9	7
Staphylococcus epidermidis	15	12	9.5	8	7
Escherichia coli	16	12.5	9	8	7
Klebsiella pneumoniae	10.7	7	–[a]	–	–
Pseudomonas aeruginosa	9.5	7	–	–	–
Candida albicans	28	23	16	12	9
Saccharomyces cerevisiae	24	20	16	12	–
Aspergillus niger	26	22	14	10	–
Microsporium gypsium	26	21	16	10	–

[a](–) Inactive.

as the standard antibiotic toward all the microorganisms. More importantly, the oil demonstrated bacteriostatic activity even as a 1:60 dilution. However, essential oil samples inhibited the growth of the colonies but no fungicidal activity in the case of the strains of *Aspergillus niger*. It was postulated that the antimicrobial potential of the essential oils could be directly associated to nepetalactone, 1,8-cineole, and γ-thujaplicin.

Sonboli et al. (2004) tested the antimicrobial activities of essential oils of *N. crispa* against seven Gram-positive and Gram-negative bacteria and four fungi (Table 3). The data indicated that the oil showed moderate-to-strong antibacterial activity toward all the tested bacteria and strong activity toward the fungi. It is interesting to note that the absolute essential oil and 1:2 oil dilutions exhibited antimicrobial activity toward all the tested bacteria, especially *C. albicans*, *B. subtilis*, *Saccharomyces cerevisiae*, *A. niger*, and *Microsporium gypsium* as well as the four fungi tested. On the other hand, the 1:4 oil dilution showed activity toward all tested microorganisms, except two Gram-negative strains, *K. pneumoniae* and *P. aeruginosa*. However, 1:8 and 1:16 oil dilutions did not show antimicrobial activity toward two Gram-negative (*P. aeruginosa* and *K. pneumoniae*) and a Gram-positive (*Enterococcus faecalis*) bacteria. Most importantly the essential oil of *N. crispa* at different dilutions also demonstrated a similar antimicrobial activity to that of standard antibiotics. It was postulated that the antimicrobial activity of the essential oils could be associated with 1,8-cineole and nepetalactone which are present in 47.9 and 20.3%, respectively, in *N. crispa*.

Salehi et al. (2007) reported the antibacterial activity of *N. ispahanica* toward different bacterial species, *S. aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *B. subtilis* ATCC 9372, *E. faecalis* ATCC 15753, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27852, and *K. pneumoniae* ATCC 3583 (Table 4). The results indicated that the essential oils of *N. ispahanica* showed strong antibacterial activity toward *S. aureus*, *S. epidermidis*, and *B. subtilis* with MIC values of 1.9, 7.6, and 7.6 mg/mL, respectively. Moreover oils from *N. ispahanica* showed moderate activity toward *E. faecalis*, *K. pneumonia*, and *E. coli* and no activity was observed toward *P. aeruginosa*. It is interesting to note that the oil showed the same or higher antibacterial activity as ampicillin in some cases. On the other hand, 1,8-cineole, which is the major component of the oil from *N. ispahanica*, showed moderate-to-high antibacterial activity toward most of the tested bacteria. Rigano et al. (2011) evaluated the in vitro antibacterial activity of *Nepeta glomerata* oil toward different bacteria species (Table 4) and the results indicate clearly that the oil exhibited activity toward Gram-positive bacteria but with lower MIC values (25–100 mg mL^{-1}). The bacteria most affected by the action of the essential oils were *Streptococcus faecalis*, *S. epidermidis*, and *B. subtilis*. Celik et al. (2008) reported that essential oils from *Nepeta cadmea* showed antimicrobial activity against the tested microorganisms (Table 4).

Grbić et al. (2008) reported on the antifungal activity of essential oils of *N. rtanjensis* and the data illustrated that the oils displayed strong antifungal activity toward all the examined micromycetes (Table 4). It has been observed that both *Alternaria* species exhibited the highest sensitivity to this oil with MIC values in the range of 0.6–0.8 μg mL^{-1}. On the other

TABLE 4 Antimicrobial Activity of the Essential Oil of *Nepeta* Species

Microorganism	*Nepeta ispahanica*[a]	*Nepeta glomerata*[a]	*Nepeta cadmea*[b]	*Nepeta rtanjensis*[d]
Bacillus subtilis	7.6 ± 0.9	50	6 ± 0	–
Staphylococcus aureus	1.9 ± 0.3	100 (>100)	6 ± 0	–
Staphylococcus epidermidis	7.6 ± 1	50 (100)	–	–
Enterococcus faecalis	>15.2 ± 0.5	25 (50)	–	–
Escherichia coli	15.2 ± 0.5	>100 (>100)	2 ± 0	–
Klebsiella pneumoniae	>15.2 ± 0.3	>100	n.a.[c]	–
Pseudomonas eruginosa	–	>100	–	–
Morganella morgani	–	–	7.5 ± 0	–
Bacillus cereus	–	–	7.5 ± 0	–
Alternaria sp. 1	–	–	–	0.8
Alternaria sp. 2	–	–	–	0.6
Cladosporium cladosporioides	–	–	–	1.0
Trichoderma iride	–	–	–	1.4
Bipolaris spicifera	–	–	–	1.0

[a]*MIC (mg/mL).*
[b]*Agar disc diffusion method, diameter of inhibition zone (mm).*
[c]*not active.*
[d]*MIC/μg mL⁻¹.*

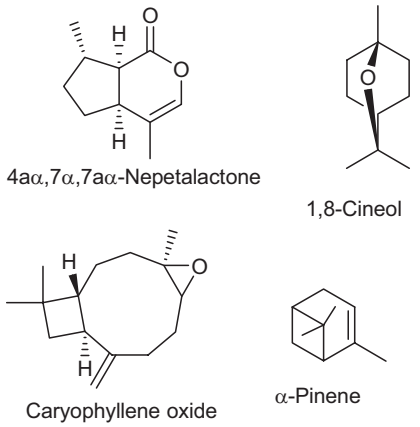

FIGURE 2 Structure of 4aα, 7α, 7aα-nepetalactone, 1,8-cineol, caryophyllene oxide, and α-pinene.

hand, the MIC value of *N. rtanjensis* oil for *Cladosporium cladosporioides* and *Bipolaris spicifera* was 1.0 μg mL^{-1} and the highest MIC (1.4 μg mL^{-1}) of the same oil was reported toward *Trichoderma viride*. It is interesting to note that standard Quadris exhibited lower antifungal activity than *N. rtanjensis* oil. It has been reported that essential oils of *N. pungens* collected from Iran showed significant antibacterial activity toward *S. aureus* (Farjam, 2012).

Nepeta species could be divided into two groups: nepetalactone-containing and nepetalactone-free species. The most frequent component reported in *Nepeta govaniana*, *N. cadmea*, *Nepeta cephalotes*, *Nepeta racemosa*, *Nepeta binaludensis*, and *Nepeta sulforiflora* is 4aα, 7α, 7aα-nepetalactone (Figure 2). It has further been reported that 4aα, 7α, 7aα-nepetalactone was present in the oils of *N. nuda* spp. *albiflora* and *N. rtanjensis* as well as *Nepeta asterotrichus* and *Nepeta sintenisii* oils. The main compound of the nepetalactone-free species is 1,8-cineol and this is present in *Nepeta heliotropifolia*. On the other hand, caryophyllene oxide was the main compound of the essential oil of *Nepeta cilicia*, *Nepeta betonicifolia*, and *N. nuda*. ssp. *nuda*, while α-pinene was present in the oil of *N. glomerulosa* and caryophyllene oxide in *Nepeta fissa* oil (Grbić et al., 2008).

TABLE 5 Antioxidant Activities of Essential Oils of *Nepeta* Species

Nepeta EO	DPPH IC_{50}	Lipid Peroxidation IC_{50}	Inhibition Ratio of Linoleic Acid Oxidation
Nepeta Foliosa	20.10 ± 1.85 (mg/mL)	2.13 ± 1.27 (mg/mL)	n.t
Nepeta flavida	42.8 ± 2.19 (μg/ml)	n.t.[a]	n.t
Nepeta cataria	n.a[b]	n.t.	27.0 ± 2.1

DPPH, 2,2-Diphenyl-1-picrylhydrazyl.
[a]*not tested.*
[b]*not active.*

Baranauskiene et al. (2003) studied the sensory properties of *N. cataria* (catnip) and the most prevailing descriptors of catnip herb were "floral, sweet," "citrus, lemon," "herbaceous, spicy," "fruity, esteric," and "minty." The aroma profile mainly depends on the composition of essential oils and geranyl acetate, 1,8-cineole, citronellyl acetate, *cis*- and *trans*-linaloloxides, and *p*-cymene were present at high concentrations in essential oil of catnip herb. It has been suggested that these major constituents are crucial to the overall herb aroma profile.

Antioxidant Activity

Ricci et al. (Giamperi et al., 2009) reported on the antioxidant activity of the essential oils of *Nepeta foliosa* and the results indicated rather weak activity (Table 5). However, essential oils of *N. foliosa* showed strong lipid peroxidation inhibition and activity of the essential oil was similar to butylated hydroxytoluene (IC_{50} = 2.13 μm g/mL and 3.86 μg/mL, respectively).

Tepe et al. (2007) reported on the antioxidant activity of essential oils of *Nepeta flavida* and their data demonstrated strong antioxidant activity with an IC_{50} value of 42.8 ± 2.19 μg/mL. On the other hand, inhibition activity of the essential oil of *N. flavida* toward linoleic acid oxidation was 86.3 ± 1.69%. Tepe et al. reported that the major components of the essential oil of *N. flavida* were 1,8-cineole and linalool. The relative antioxidant activities of 1,8-cineole and linalool demonstrated that 1,8-cineole was strongly active in both tested systems while linalool proved to be inactive. It has been reported that some plant essential oils showed antioxidant activity due to the presence of 1,8-cineole (Tepe et al., 2007). The essential oil of *N. nuda* showed weak antioxidant activities (Alim et al., 2009). Adiguzel et al. (2009) reported on the antioxidant activity and their results showed that essential oils of *N. cataria* did not have any antioxidant activity but exerted a moderate activity with an inhibition ratio of linoleic acid oxidation at 27% (Table 5).

SUMMARY

- Essential oils of the genus *Nepeta* such as *N. nuda*, *N. atlantica*, *N. tuberosa*, *N. cataria*, *N. granatensis*, *N. rtanjensis*, *N. crispa*, and *N. ispahanica* showed significant antimicrobial activity against the growth of food poisoning organisms.
- Oils of *N. foliosa* and *Nepeta flavida* showed the most promising antioxidant activity.
- The antimicrobial and antioxidant activity of *Nepeta* (Pune-sa) essential oils confirms the potential use of these essential oils in the food industries.
- The antimicrobial and antioxidant activity of *Nepeta* (Pune-sa) essential oils also confirms the potential use of these essential oils in the preservation of foodstuffs against bacteria and fungi.
- These oils may also be valuable for increasing the shelf life of foodstuffs.
- The reviewed studies clearly demonstrate that *Nepeta* (Pune-sa) essential oils present a great potential for the food industry.

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Chapter 74

Rockroses (*Cistus* sp.) Oils

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INTRODUCTION

Cistus plants, also known as rockroses, are a Mediterranean native genus of shrubs included in the Cistaceae family, which is formed by a total of eight genera. The *Cistus* genus (25 different species according NCBI Taxomony Browser; http://www.ncbi.nlm.nih.gov/taxonomy) is the most numerous, common, and well known, but *Helianthemum* and *Halimium* genera are also important for their number of species, relevance, and phytochemical composition. *Cistus* species are disseminated over different areas of the Mediterranean area Portugal, Spain, Canary Islands, north-west Africa, Italy, Greece, and Turkey (Barrajon-Catalan et al., 2011), following a particular distribution pattern for each species, depending on climatological and soil conditions. Some of the *Cistus* species are endemic and other species are widespread in the Mediterranean area. Some *Cistus* plants such as *Cistus ladanifer* adapt easily to wildfires, as their seeds resist them and raise rapidly forest biomass increasing the risk of wildfires in some areas of Spain (Barrajon-Catalan et al., 2011).

Most *Cistus* species are very fragrant and sweet-smelling, and are much appreciated in the perfume industry and for ornamental purposes. Volatile compounds are found not only in essential oils, but also in aqueous and hydroalcoholic extracts (Greche et al., 2009). All *Cistus* species secrete essential oils in different amounts (Kintziou et al., 2001). Moreover, in some species (*C. ladanifer*), the leaves are coated with a highly aromatic resin called labdanum, which is sometimes mistakenly considered as an essential oil in the literature. Labdanum also shows a high content of volatile compounds which have been deeply studied (Weyerstahl et al., 1998; Greche et al., 2009). As is often the case for plants, *Cistus* essential oils are normally obtained by steam distillation, but in the case of *C. ladanifer*, supercritical CO_2 extraction has also been used (Rincón et al., 2000), yielding an essential oil with higher quality and purity.

BOTANICAL ASPECTS

Cistus plants are perennial shrubs with evergreen, opposite, simple, usually slightly rough-surfaced, 2–8 cm long leaves. Their flowers are hermaphroditic, actinomorphic, and hypogynous, with three or five sepals (usually with the outer sepals smaller than the inner ones) opposite to petals. They present five petals with colors ranging from white to purple and dark pink depending on the subgenus, with a conspicuous dark red spot at the base of each petal in a few species. Figure 1 shows pictures corresponding to diverse colored flowers of representative members of the *Cistus* genus. The androecium has numerous fertile stamens and the gynoecium is formed by a three or five carpellate ovary (although *C. ladanifer* displays 6 to 12 carpels) and a solitary style with a single capitate or discoid stigma. From an ecological point of view, *Cistus* plants present insect-dependent pollination, flower-dependent reproduction, and spring-dependent phenology, and characteristics that demonstrate their adaptation to Mediterranean ecosystems (Guzmán and Vargas, 2005). *Cistus* plants grow in different ways, from a woodland understory, to be the dominant species in evergreen scrub. Co-occurring species of *Cistus* are also very frequent, particularly in mountain ranges (Guzmán and Vargas, 2005).

Taxonomical and phylogenic relationships among these species have been based primarily on vegetative and reproductive characters. At the present, these taxonomic data have been significantly improved using genetic approaches (Guzmán and Vargas, 2005). The polyphenolic composition has been also utilized to establish different chemotypes, therefore permitting classification of the members of *Cistus* genus, which confirms taxonomic and evolutionary studies (Barrajon-Catalan et al., 2011). As shown in Figure 2, the *Cistus* genus is divided into three subgenera: *Cistus*, *Leucocistus*, and *Halimioides*. The first includes purple flowered plants and the last two comprise species showing white flowers. Whereas the *Cistus*

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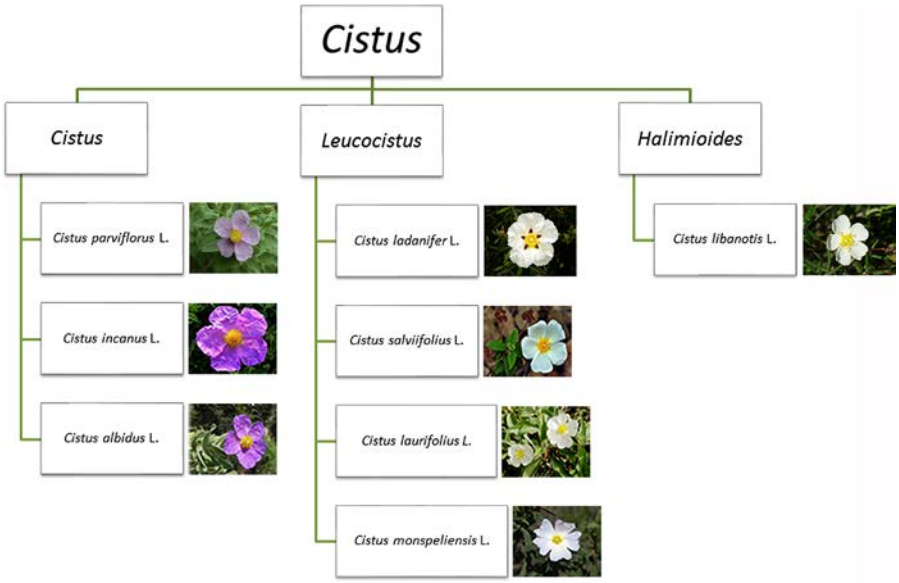
FIGURE 1 Flowers from representative *Cistus* plants. Three species of *Cistus* flowers are shown as an example of white (*Cistus salviifolius, Leucocistus* subgenus, A); purple (*Cistus incanus*, *Cistus* subgenus, B); and dark red spotted white flowers (*Cistus ladanifer*, *Leucocistus* subgenus, C). The intensity and exact color vary between species, especially for *Cistus* subgenera, which present different tones of purple on each species. The presence of a red spot in *Cistus ladanifer* is not always observed, as occurs in *Cistus ladanifer* var. *albiflorus*. (Unpublished figure).

FIGURE 2 Taxonomic relationships between *Cistus* species with characterized essential oils. Taxonomic relationships between the different *Cistus* species (grouped in subgenus) with reported data on their essential oils. A photographic detail of the flowers is shown close to the name of each species. (Unpublished figure).



subgenus is almost devoid of ellagitannins and contain mostly flavonoids, *Leucocistus* and *Halimioides* subgenera are enriched in ellagitannins and contain smaller amounts of flavonoids. Previously reported phylogenetic and taxonomic studies (Guzmán and Vargas, 2005) pointed out the relationship between the members of these subgenera. These are summarized in Figure 2, showing the most representative species with characterized essential oils of each subgenus. Essential oils and resins in plants have several vital functions, they contribute to pollination, act as insect repellent agents, protect against fungal or bacterial attack, and prevent against water evaporation and ultraviolet (UV) damage. It has been proposed that morphological leaf traits and labdanum secretion in white-flowered *Cistus* linage have been influenced by UV radiation, therefore determining their successful adaptation to different Mediterranean habitats (Guzmán et al., 2009).

USAGE AND APPLICATIONS

Traditional folk medicine has used *Cistus* species, their extracts, and/or essential oils for a large variety of purposes such as antiinflammatory, antiulcerogenic, wound healing, antimicrobial, cytotoxic, or vasodilator remedies. They have also been used as an antidiarrheic, antacid, and antispasmodic by simple decoction of their leaves (Zidane et al., 2013). Studies have revealed information on the possible candidate compounds for all these effects, but new activities are being discovered and attributed to various *Cistus*-derived products. These include antimicrobial, antioxidant, antitumor, antinociceptive, analgesic, and neuroprotective effects (De Andres et al., 1999; Kupeli and Yesilada, 2007; Barrajon-Catalan et al., 2010; Loizzo et al., 2013).

The compounds in essential oils and exudates may have also allelopathic effects by inhibiting the growth of another species and reducing their germination, due to phytotoxicity over plants and soil (Verdeguer et al., 2012).

That is the case for some flavonoids found in *C. ladanifer* leafs and in soils where this plant was grown (Chaves et al., 2001). Although labdanum cannot be considered an essential oil, it contains a high percentage of volatile compounds, most of them shared with the corresponding essential oil. Both labdanum and essential oil, especially obtained from *C. ladanifer*, are very much appreciated in the perfume industry being an essential component in about 30% of modern perfumes. Its fragrance is reminiscent of gray amber and it combines perfectly with the fragrance families of amber, chypre, and fern due to its excellent fixative properties. Labdanum from *C. ladanifer* is also used as an additive to aromatize some types of tobacco.

Undoubtedly, the major potential of *Cistus* essential oil is related to its antimicrobial capacity. Several studies have reported the potential antimicrobial activity of essential oils and extracts derived from *Cistus* plants, especially those from *C. ladanifer* (Greche et al., 2009; Zohra and Atik, 2011; Tomás-Menor et al., 2013), but also those derived from other species such as *Cistus incanus* (Demetzos et al., 1995, 1997), *Cistus albidus* (Tomás-Menor et al., 2013), *Cistus monspeliensis* (Bouamama et al., 1999), *Cistus salviifolius* (Demetzos et al., 2002; Tomás-Menor et al., 2013), *Cistus laurifolius* (Kintziou et al., 2001), and *Cistus parviflorus* (Demetzos et al., 1990; Güvenç et al., 2005). Nevertheless, still much research must be done in order to use these materials for food preservation purposes or as ingredients for cosmetics and toiletries.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Cistus essential oils and resins have not been used in food science and industry regularly, only marginal use as a flavoring agent for food or active ingredient for food supplements is reported. Labdanum oleoresin from *Cistus* spp. was included by the Council of Europe in the list of substances granted approval (COE No. 134). The United States Food and Drug Administration (FDA, 2014) also approved it for use as a food additive or flavoring agent (FDA Code of Federal Regulations, title 21, vol 3; 21CFR172.510) in low quantities (2–14 ppm). *Cistus incanus* extract is also used as an ingredient in food supplements with immune boosting properties. No further reports on an established use of *Cistus*-derived products in food purposes are available. Nevertheless, two new potential uses for *Cistus* oils and other *Cistus*-derived products are emerging from the literature that can be exploited in the future:

1. Antioxidant or radical scavenging activity: Not only the essential oils, but also all *Cistus*-derived products (labdanum, aqueous, alcoholic and hydroalcoholic extracts) possess significant antioxidant activity (Amensour et al., 2010; Barrajon-Catalan et al., 2010; Guimarães et al., 2010; Zidane et al., 2013), which may have a potential use to prepare ingredients focused to preserve foods from oxidation or to inhibit lipid peroxidation (Jerónimo et al., 2012). In fact, some novel cosmetic products already include *Cistus* ingredients as antioxidants in their formulations. Table 2 shows values of reported antioxidant activity of *Cistus* derived products, that is, leaf extracts and essential oils.
2. Antimicrobial activity: *Cistus* essential oils and extracts have a proven antimicrobial activity which has been reported against several Gram-positive and Gram-negative bacteria and also against some fungal infections (Demetzos et al., 1997; Bouamama et al., 1999; Kintziou et al., 2001; Greche et al., 2009; Barrajon-Catalan et al., 2010; Rauwald et al., 2010; Zohra and Atik, 2011; Tomás-Menor et al., 2013). This reported activity would allow the use of *Cistus* essential oils as preservative agents in the food industry, but first, adequate dosage and toxicity assessments must be established before food safety authorities approve their use.

Nowadays, the use of green chemistry is encouraged as an alternative to conventional chemicals in order to reduce health and environmental risks. The use of *Cistus* spp. ingredients as preservative or antimicrobial agents may suppose an option to reduce the use of chemicals in food or cosmetic sectors (Anastas and Eghbali, 2010).

In addition to their proven antioxidant and antimicrobial activities, the composition of the most representative *Cistus* essential oils and extracts is described, and their potential use in food science and industry is discussed.

Cistus ladanifer L

Cistus ladanifer (Figure 1(C)) essential oil is the most deeply characterized one among all *Cistus* essential oils and its chemical composition has been thoroughly studied (Pascual et al., 1982; Mariotti et al., 1997; Robles et al., 2003; Gomes et al., 2005; Oller-López et al., 2005; Costa et al., 2009). Although there are differences between the reported data probably due to the plant origin, variety, or seasonal variations, the most abundant compounds include α-pinene, viridiflorol, borneol, trimethyl cyclohexanone, and camphene (Figure 3, Table 1). As mentioned, *C. ladanifer* is the only essential oil which has been obtained by supercritical CO_2 extraction with small differences in composition when compared with traditional stem distillation (Rincón et al., 2000).

TABLE 1 Main Terpene Compounds of the Essential Oils Derived from Different *Cistus* Species. References for Each Species Have Been Included

Species	Main Compounds	References
Cistus ladanifer	α-Pinene, viridiflorol, trimethyl cyclohexanone, borneol and camphene	Pascual et al. (1982), Mariotti et al. (1997), Robles et al. (2003), Gomes et al. (2005), Oller-López et al. (2005), Costa et al. (2009), Zidane et al. (2013)
Cistus incanus	Manoyl oxide, viridiflorol, carvacrol, and cadinene	Demetzos et al. (1995), Paolini et al. (2009), Loizzo et al. (2013)
Cistus albidus	α-Zingiberene, α-curcumene, α-cadinol, and α-bisabolol	Robles and Garzino (1998), Maccioni et al. (2006), Paolini et al. (2008)
Cistus salviifolius	Camphor, viridiflorol, longiborneol, phylloclad-ene, abietatriene, and *cis*-feruginol	Demetzos et al. (2002)
Cistus monspeliensis	Manoyl oxide, bisabolol, 1,8-cineole, bornyl acetate, and α-pinene	Robles and Garzino (2000), Angelopoulou et al. (2001a), Angelopoulou et al. (2002), Oller-López et al. (2005), Viuda-Martos et al. (2011)
Cistus laurifolius	Borneol and nonacosane	Öğütveren and Tetik (2004a)
Cistus libanotis	Camphene, α-pinene, β-pinene, sabinene, γ-terpinene, bornyl acetate, terpineol, and borneol	Loizzo et al. (2013), Zidane et al. (2013)
Cistus parviflorus	Carvacrol, caryophyllene oxide, abietatriene, 4-epi-dehydroabietol, dehydro abietol, *cis*-ferruginol, 8-α-13-oxy-14-ene-epilabdane, and manoyl oxide	Angelopoulou et al. (2001b), Öğütveren and Tetik (2004b)

Labdanum has also been well characterized (Weyerstahl et al., 1998; Greche et al., 2009) but its oral ingestion is very limited due to reported neurotoxic, hepatotoxic, and nephrotoxic effects. However, it has been traditionally used at low doses as an ingredient in sedative tea, coffee, and other infusions to prevent insomnia and anxiety.

Cistus ladanifer essential oil and other derivatives have shown significant antioxidant and antimicrobial properties. Probably, *C. ladanifer* materials are the most deeply characterized among all of the *Cistus* species in relation with these activities (Table 2). The antimicrobial activity of the essential oil has been proven against various bacterial and fungal strains, whereas labdanum resinoid showed poor activity (Greche et al., 2009). A remarkable activity of *C. ladanifer* essential oil against pathogenic bacteria such as *Listeria monocytogenes* was reported (Zohra and Atik, 2011). The highest antimicrobial activity against a variety of Gram-positive and Gram-negative clinical pathogens was observed for the most polar fraction of the essential oil, primarily containing mono- and sesquiterpene alcohols, which even inhibited multidrug-resistant *Enterobacter aerogenes* (Guinoiseau et al., 2011). A modest capacity of this essential oil has also been reported to inhibit the growth of *Helicobacter pylori*, which may allow its use as food additive to improve present therapies (Bergonzelli et al., 2003). *Cistus ladanifer* leaves have also been used as an additive for feeding to improve lamb meat quality. A deceased level of meat deterioration induced by lipid oxidation (Jerónimo et al., 2012) and increased content of unsaturated fatty acids in muscle were observed (Jerónimo et al., 2010), without affecting meat color or sensory properties. Because in these studies complete leaves and stems were utilized, it must be presumed that the terpene fraction from oil together with polyphenols are the responsible for such effect. However, other studies showed that *C. ladanifer* leaves exudates may impair mouth skeletal muscle relaxation through inhibition of the sarcoplasmic reticulum Ca^{2+}–ATPase (Sosa et al., 2004), therefore these effects and the potential responsible compounds must be further carefully studied.

Cistus incanus L

Cistus incanus (also known as *Cistus creticus*, *Cistus villosus*, or pink or hairy rockrose) (Figure 1(B)) essential oil is probably the second most characterized one among all *Cistus* essential oils. All the *C. incanus*-derived products are deeply characterized in their composition by high pressure liquid chromatography and gas chromatography–mass spectrometry (GC–MS), as occurred with *C. ladanifer*. Several studies have reported the characterization of materials obtained from this plant: extracts derived from leaves using different solvents (Barrajon-Catalan et al., 2011; Skorić et al., 2012), labdanum

TABLE 2 Antioxidant Activity of Different *Cistus*-Derived Products Obtained from the Bibliography

Product	Assay→Value (Units)	References
Aqueous extract of *Cistus ladanifer* leaves	TEAC→35.85 ± 1.25 mmol TE/100 g DW	Barrajon-Catalan et al. (2010)
	FRAP→117.72 ± 4.38 mmol Fe^{2+}/100 g DW	
	ORAC→3329.0 ± 182.1 μmol TE/g	
	TBARS→73.13 ± 8.12% of inhibition (0.375 mg/mL)	
Aqueous extract of *Cistus salviifolius* leaves	TEAC→365.85 ± 5.28 mmol TE/100 g DW	Tomás-Menor et al. (2013)
	FRAP→435.77 ± 5.87 mmol Fe^{2+}/100 g DW	
	ORAC→5459.00 ± 66.53 μmol TE/g	
	TBARS→0.007 ± 0.003 mg/mL (50% inhibition)	
Essential oil of *Cistus ladanifer*	DPPH scavenging→36.28 ± 0.36 mg/mL	Guimaraes et al. (2010)
Essential oil of *Cistus incanus*	DPPH scavenging→828.1 ± 3.6 μg/mL (IC_{50})	Loizzo et al. (2013)
	TEAC→395.1 ± 3.5 μg/mL (IC_{50})	
	FRAP→0.4 ± 0.09 μM Fe (II)/g	
Essential oil of *Cistus salviifolius*	DPPH scavenging→814.7 ± 3.4 μg/mL (IC_{50})	Loizzo et al. (2013)
	TEAC→394.7 ± 3.8 μg/mL (IC_{50})	
	FRAP→5.2 ± 0.8 μM Fe (II)/g	
Essential oil of *Cistus libanotis*	DPPH scavenging→499.9 ± 3.1 μg/mL (IC_{50})	Loizzo et al. (2013)
	TEAC→272.5 ± 2.7 μg/mL (IC_{50})	
	FRAP→19.4 ± 1.6 μM Fe (II)/g	
Essential oil of *Cistus monspeliensis*	DPPH scavenging→991.9 ± 4.4 μg/mL (IC_{50})	Loizzo et al. (2013)
	TEAC→4.5% at 396 μg/mL (IC_{50})	
	NA	

Each row shows the name of the *Cistus* species, the kind of sample (solvent used or essential oil), the result for different antioxidant assays, and the bibliographic source. DW: dry weight; TE: Trolox equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; NA: not assayed; TEAC: Trolox equivalent antioxidant capacity; TBARS: thiobarbituric acid-reactive substances assay; ORAC: oxygen radical absorbance capacity; FRAP: Ferric reducing ability power. A detailed description for each antioxidant assay can be obtained from Barrajon-Catalan et al. (2010).

oleoresin (Demetzos et al., 1994), and essential oil (Demetzos et al., 1995; Paolini et al., 2009; Loizzo et al., 2013). Although the reports on essential oil composition are slightly different, diterpenes such as manoyl oxide, bicyclic sesquiterpenes such as cadinene, oxygenated sesquiterpenes such as viridiflorol, and burnesol and oxygenated monoterpenes such as carvacrol are the most abundant compounds in almost all the analysis (Figure 3, Table 1).

The essential oil of *C. incanus* is not used as a food additive at industrial level, but their infusions and teas are very popular and have been traditionally used to treat respiratory infections and colds. Studies have reported their antiviral capacity against avian influenza A virus in cellular and animal models (Droebner et al., 2007; Ehrhardt et al., 2007) and in a randomized, placebo–controlled human trial (Kalus et al., 2009). There are some commercial teas and nutraceutical products containing *C. incanus* extracts or their leaves. As this product was not used as food ingredient in the European Union before May 1997, a safety assessment, and probably its consideration under Novel Food Regulation, would be required before being authorized for food use (European-Commission, 2014).

The antimicrobial activity of *C. incanus* essential oil is well documented against a panel of Gram-positive and Gram-negative bacteria (Demetzos et al., 1995, 1997). A more detailed study has reported that volatile compounds from essential oil showed stronger growth inhibitory effect than leaf extracts against *Borrelia burgdorferi* (Rauwald et al., 2010). Moreover, diterpenes isolated from labdanum of *C. incanus* showed potential antileishmania activity, which has allowed designing of semisynthetic potent diterpenes with selective leishmanicidal activity (Fokialakis et al., 2006). The antimicrobial activity of extracts derived from *C. incanus* leaves is also well documented. Extracts obtained with different solvents

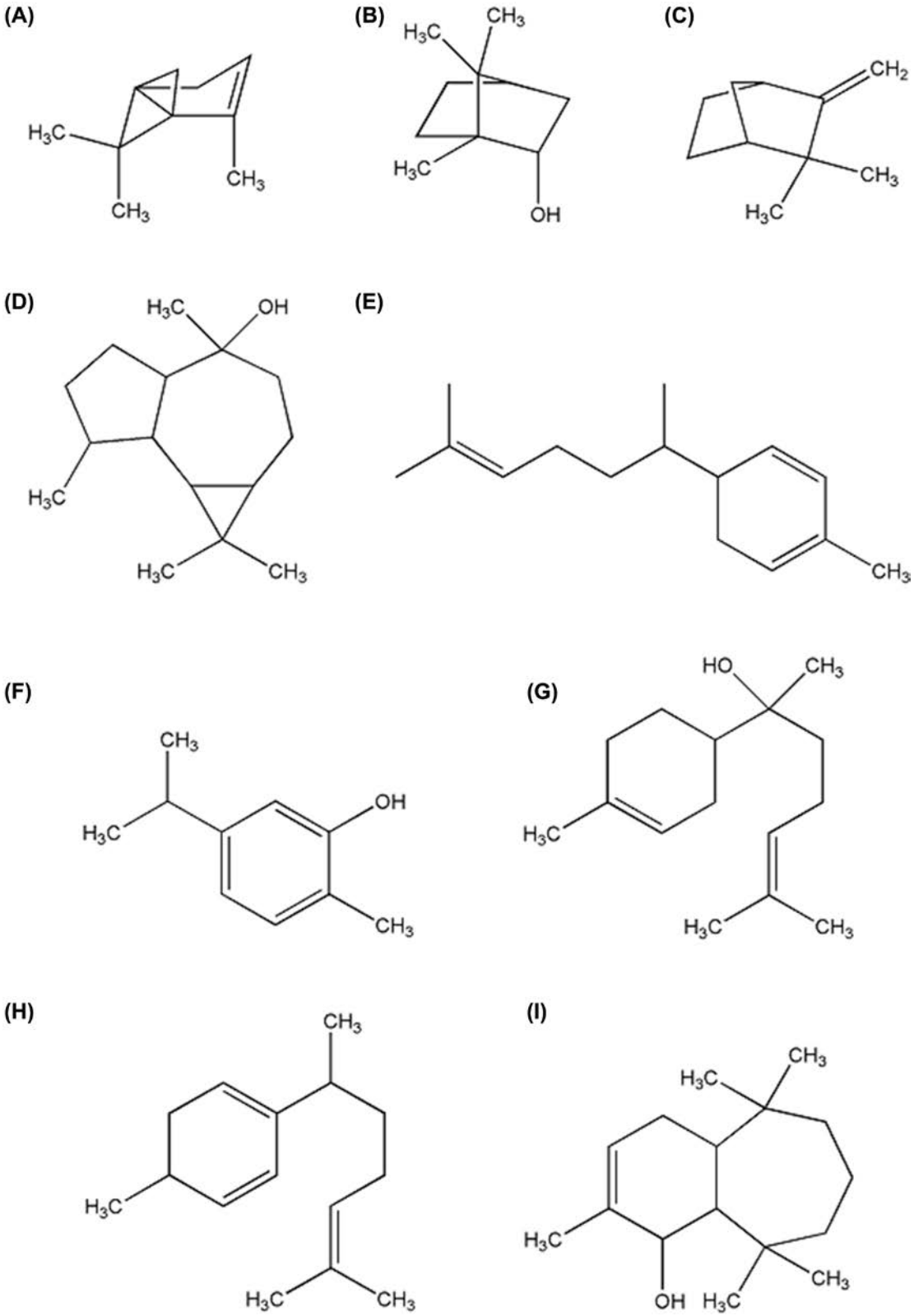


FIGURE 3 Chemical structures of the main terpene compounds in *Cistus* essential oils. Chemical structures of the main terpenes in *Cistus* essential oils: α-pinene (A); borneol (B); camphene (C); viridiflorol (D); zingiberene (E); carvacrol (F); bisabolol (G); curcumene (H); and longiborneol (I). (Unpublished figure).

exhibited growth inhibitory activity against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*) (Güvenç et al., 2005), and also antifungal activity (Bouamama et al., 1999).

A study demonstrated that essential oils of various *Cistus* species, including *C. incanus*, possess acetylcholinesterase and/or butyrylcholinesterase inhibitory activity, which can be useful for prevention and treatment of Alzheimer's disease. This promising discovery opens new alternatives to the development of pharmaceutical and nutraceutical formulations focused to increase the therapeutic arsenal for Alzheimer's patients (Loizzo et al., 2013).

Cistus albidus L

Cistus albidus (white rockrose) essential oil has also been well characterized by different studies (Robles and Garzino, 1998; Maccioni et al., 2006; Paolini et al., 2008). This oil mostly contains sesquiterpenes, being α-zingiberene the most abundant oil followed by other such as α-curcumene, α-cadinol, and α-bisabolol (Figure 3, Table 1), which reach a significant abundance. The percentages of these compounds vary depending on their seasonal and geographical origin. There are no current food applications for *C. albidus* products beyond its potential antimicrobial and antioxidant activities. However, some studies have reported their potential use in ethnomedicine (Tomás-Menor et al., 2013) and ethnoveterinary (Benítez et al., 2012).

Cistus salviifolius L

Little work has been done on the composition of *C. salviifolius* (Figure 1(A)) essential oil. However, a complete GC–MS analysis of the composition of essential oils derived from several populations of this plant has been reported (Demetzos et al., 2002). These essential oils were characterized by the presence of high percentages of oxygenated sesquiterpenes, such as viridiflorol and longiborneol (Figure 3, Table 1). The oxygenated monoterpene camphor was also detected as the major compound in most samples analyzed. Finally, among the diterpenes, phyllocladene, abietatriene, and *cis*-feruginol were the most abundant ones (Demetzos et al., 2002).

No special application has been described for *C. salviifolius* in the food industry. Like other *Cistus* species, the antimicrobial activity of *C. salviifolius* is well documented both for extracts deriving from leaves (Tomás-Menor et al., 2013) and the essential oil (Demetzos et al., 2002). Leaf extracts are more potent against Gram-positive microorganisms than against Gram-negative ones. Accordingly, Gram-positive bacteria (*Staphylococcus epidermidis* and *S. aureus*) were more susceptible to different chemotypes of essential oils derived from *C. salviifolius* than Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*). Among three different chemotypes identified in Crete (Greece), the Gram-positive antimicrobial capacity seems to be related to the chemotype bearing high camphor content. *Cistus salviifolius* essential oil has also exhibited neuroprotective activity by inhibiting acetylcholinesterase and, to a lesser degree, butyrylcholinesterase (Loizzo et al., 2013). Therefore, it could be a potential ingredient to prevent neurodegenerative disorders.

Cistus monspeliensis L

Cistus monspeliensis, also known as black-rockrose, is probably much less known than other species, but its essential oil has been deeply analyzed in numerous studies (Robles and Garzino, 2000; Angelopoulou et al., 2001a, 2002; Oller-López et al., 2005; Viuda-Martos et al., 2011). Once again, there are differences between the different reports probably due to soil, climate, and origin factors. Diterpenes such as manoyl oxide are the main constituents in all the samples. Other compounds such as the sesquiterperne α-bisabolol, and monoterpenes such as 1,8-cineole (eucalyptol), bornyl acetate, and α-pinene are also abundant in this essential oil (Figure 3, Table 1).

Essential oils from *Cistus monspeliensis* present moderate cytotoxic activity against leukemia cell lines (Angelopoulou et al., 2001a). They also show potential neuroprotective activity, but to a lesser degree than other *Cistus* species (Loizzo et al., 2013). In contrast, no antimicrobial activity was found for *C. monspelliensis* essential oil compared with *C. parviflorus* (Demetzos et al., 1990). The antiparasitary activity against leishmania of a triterpene isolated from *C. monspeliensis* has also been reported (Fokialakis et al., 2006).

Cistus laurifolius L

The essential oil of *C. laurifolius*, also known as mountain rockrose, is also poorly characterized compared with other *Cistus* species. The main components include terpenes such as borneol and other compounds such as the paraffin with

pheromone activity nonacosane (Figure 3, Table 1) (Öğütveren and Tetik, 2004a). Although the antiinflammatory and antinociceptive activities have been described for *C. laurifolius*-derived products (Kupeli and Yesilada, 2007), only a potential antimicrobial activity has been reported for its essential oil (Kintziou et al., 2001).

Cistus libanotis L

Also known as Lebanon rockrose, *C. libanotis* essential oil has been well characterized in several studies (Loizzo et al., 2013; Zidane et al., 2013). Monoterpenes represent the main family of compounds with camphene, α-pinene, β-pinene, sabinene, and γ-terpinene as the major compounds. Oxygenated monoterpenes such as bornyl acetate, terpineol, and borneol are the most representative (Figure 3, Table 1). There are no references on the food potential application of *C. libanotis* essential oil. The essential oil of *C. libanotis* has shown stronger butyrylcholinesterase inhibitory activity than other *Cistus* essential oils, which could be explained through the synergic interaction among the terpene compounds in the oil. This may support its functional role in the prevention of neurodegenerative diseases (Loizzo et al., 2013). *Cistus libanotis* essential oil also exhibited stronger antioxidant capacity when compared to *C. creticus*, *C. salviifolius* or *C. monspeliensis* essential oils, as compared by using different radical scavenging tests (Table 2) (Loizzo et al., 2013).

Cistus parviflorus

This *Cistus* species is a particular one, their flowers are purple, but their botanical characteristics are closer to the white flowered *Cistus*, suggesting that it could have originated as a hybrid species in the past (Guzmán and Vargas, 2005, 2009). Its essential oil is well characterized by GC–MS (Angelopoulou et al., 2001b; Öğütveren and Tetik, 2004b). Monoterpenes and bicyclic sesquiterpenes such as carvacrol and caryophyllene oxide, respectively, as well as diterpenes such as abietatriene, 4-epi-dehydroabietol, dehydro abietol, and *cis*-ferruginol were found in high percentage composition. Labdane-type diterpenes such as 8-α-13-oxy-14-ene-epilabdane and manoyl oxide mixture of isomers were also found (Angelopoulou et al., 2001b; Öğütveren and Tetik, 2004b) (Figure 3, Table 1). The antimicrobial activity for *C. parviflorus* essential oil has been documented (Demetzos et al., 1990), but no reference to other activities has been published so far.

SUMMARY POINTS

- All *Cistus* species secrete essential oils in different amounts, which are mostly composed of monoterpenes, sesquiterpenes, and diterpenes.
- Labdanum, the exudate secreted by leaves and stems, is not an essential oil; however, it shares volatile compounds and activities.
- Depending on the species, pinene, borneol, camphor, and carvacrol (monoterpenes), viridiflorol and zingiberene (oxygenated sesquiterpenes), and manoyl oxide and abietatriene (diterpenes) are the most abundant and common compounds of *Cistus* essential oils.
- Antimicrobial and antioxidant activities are the most characterized and well documented for most *Cistus* essential oils. Nevertheless, still further research into safety must be done in order to use these materials for food preservation purposes.

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Chapter 75

Rose (*Rosa*×*damascena* Mill.) Essential Oils

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INTRODUCTION

Destruction of vegetable and fresh fruits and food deterioration during storage is a real environmental problem as well as in the food industry. Using synthetic preservatives in foodstuffs to prevent the food contamination and deterioration over a long period may cause problems like carcinogenicity and other toxicities. Therefore, food industries are highly interested in replacing the synthetic preservatives usually used in foodstuff with natural preservatives like plant essential oils. It has been shown that plant essential oils have the ability to control the rate of food and fruit deterioration as well as controlling various contaminations in foodstuff.

Rosa×*damascena* Mill. (in fact the accepted name of the plant is *Rosa* × *damascena* Herrm.) is one the most important species of the Rosaceae family and it is a medicinal plant. This plant and the essential oil extracted from this plant have various applications in perfume and in food industries. They are used as antibacterial, antioxidant, and as flavoring agents. They are also having various pharmacological properties.

BOTANY

Rosa×*damascena* Mill. (derived from *Rosa gallica* L. and *Rosa moschata* Herm.), a member of the *Rosa* genus from Rosaceae family, has the common name of summer damask rose.

This species is a low, deciduous plant, with wide runners and is branched above ground. The length of the shoots of *R.*×*damascena* shrub is generally between 0.5 and 1.5 m and it is covered with tall, recurvate, or erect thorns and stem glands of various sizes. The leaves are usually divided into five, rarely seven, ovate to elliptic leaflets, and are long, glandular, grey–green above and are bluer below, leaflets grow together at the leaf stem and terminate in free tips (Figure 1).

Inflorescence is usually rather many-flowered, corymbiform, or corymbiform–paniculate. The flowers are usually solitary, more rarely in two and three, on 2–3 cm long thickly glandular pedicles, the calyx is round to pear-shaped and usually thickly covered with stalked gland and gland bristles. The velvety petals are pink to purple, 2–3 cm long and wide (Figure 2). The style and stigma form the ovary that is surrounded by carpels enclosed in the calyx, forming woolly capitula, the ripe and red-brown false fruit is oblong 1–1.5 cm long, and broadest in the upper part (Figure 3) (Brown, 2002; Fedorov et al., 1941; Khatamsaz, 1992).

Rosa×*damascena* is cultivated in Turkey, Iran, Bulgaria, and Morocco but it has also been cultivated in other areas of the world. A map of the main areas in which *R.*×*damascena* is cultivated is shown in Figure 4.

There are many reports concerning the composition of the essential oil of *R.*×*damascena*. Quercetin, kaempferol, cyanidin, lycopene, rubixanthin, zeaxanthin, xanthophyll, and taraxanthin have been isolated from the hips of the rose plant. The yield of the volatile oil is very low and it is about one part of oil from 3000 parts of flowers. However, the main constituents of the essential oil obtained from the flowers of this plant are citronellol, nerol, geraniol, β-phenylethanol, eugenol, and methyl eugenol (Khare, 2007). Due to the low oil yield from *R.*×*damascena* and also high demand for this volatile oil, it is one of the most expensive volatile oils (Alsemaan et al., 2011; Baydar and Baydar, 2005; Boskabady et al., 2011; Shahbazi and Esmaeili, 2012).

The constituents of the oil extracted from *R.*×*damascena* vary depending on the growing area. Iran, India, Bulgaria, China, and Turkey are the main countries that cultivate this shrub and produce the essential oil of this plant. The main components of the oil obtained from this plant cultivated in different parts of these countries summarized in Table 1.

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FIGURE 1 *Rosa×damascena* shrub. Note: This figure was published on the internet.

FIGURE 2 *Rosa×damascena* flower. Note: This figure was published on the internet.

Although the main compounds of the oil extracted in these countries are citronellol, geraniol, and nonadecane, there are also other compounds like docosane (up to 19%), disiloxane (up to 19%), and heneicosane (up to 18%) reported for Iranian oil. In addition, β-phenyl ethanol (50–86%) was reported for Turkish absolute and geranyl acetate plus citronellol (up to 25%) and heneicosane (up to 11%) was reported for "York and Lancaster" attar (Tucker and DeBaggio, 2009).

USAGES AND APPLICATIONS

Roses are important groups of ornamental plants. These plants have been used extensively in various foods, food industry, nutritional products, and the cosmetic industry. In ancient medicine, these plants were also used as medicinal herbs. The volatile oil obtained from the *R.×damascena* flower is used in perfumery, therefore, it is very valuable oil in the cosmetic industry (Sadraei et al., 2013).

The petals of *R.×damascena* are used to make different products like rosewater, rose oil, and rose absolute. These products are used in perfumery and food industry for flavoring. They used to produce pleasant flavors to foods like sorbets, sweets, confectionery, and deserts (Brown, 2002).

FIGURE 3 *Rosa*×*damascena* fruits. Note: This figure was published on the internet - Gernot Katzer's Spice Pages.

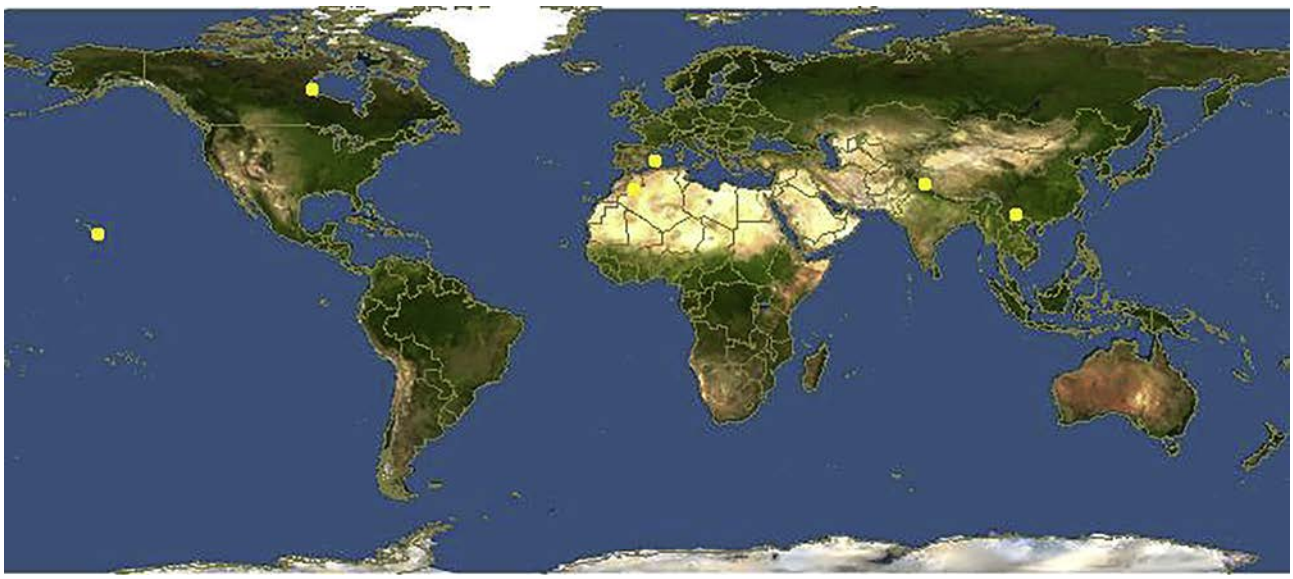

FIGURE 4 Map of most areas in which *Rosa*×*damascena* is cultivated. The cultivated areas are shown by yellow dots. (Gray in print versions). Note: This figure was published on the internet.

A popular *R.*×*damascena* essential oil product is called rose water (Figure 5). Rose water may contain various amounts of rose volatile oil depending to the quality of the product. Rose water is used for various traditions as well as in food industry (Boskabady et al., 2011).

The petals of *R.*×*damascena* are dried and used in different ways in various kind of food and food products like salad, syrups, jams, and jellies (Brown, 2002; Nikbakht and Kafi, 2008; Zargari, 1996). The dried flower buds and petals of *R.*×*damascena* are also used in *Ras El Hanout*, a North African spice mixture, and in potpourris.

TABLE 1 Comparison of the Main Compound of the Oil Obtained From *Rosa × damascena* Cultivated in Different Countries (Tucker and DeBaggio, 2009)

Country and Product	Citronellol (%)	Geraniol (%)	Nonadecane (%)
Bulgarian attar	33–36	16–26	5–14
Iranian oil	15–47	Up to 18	Up to 40
Indian oil	12–36	15–36	Trace to 25
Chinese oil	31–44	16–22	2–17
Gülbirlik rose oil from Turkey	31–44	9–14	8–15

FIGURE 5 Bottles of Iranian rose water. Note: This figure was published in the internet.

Summer damask rose is also a medicinal plant and different parts of this species have been widely used in traditional medicine.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Food deterioration as well as destruction of vegetable and fresh fruits during storage is a major environmental problem. It is also a main concern of the food industry. The high temperature and humid environment rapidly increases the rate of destruction of foodstuff, fresh fruit, and vegetables. Microorganism contamination during storage and some pathogenic fungi species are mainly responsible for this kind of spoilage. Oxidation is another major cause of degradation of materials and foods. Reactive oxygen species (ROS) in lipid per-oxidation are mainly produced by the oxidation of foods by chemical spoilage during the storage, especially in food products containing oils and fats. Damage of ROS produced in foodstuff during the storage to cells can lead to serious problems for the people consuming these food products.

The obligation to use synthetic preservatives in the food industry to prevent food contamination and deterioration can lead to some health and environmental problems like carcinogenicity and other toxicities especially when they are used over a long period. Therefore, the food industries are very keen to replace the synthetic preservatives with natural preservatives with botanical sources like plant essential oils. Replacing the synthetic preservatives with a natural safe botanical source preservative like the essential oils of the plant furnishes the ability to prevent and delay deterioration of foods, vegetables, and fresh fruit in storage and control various contaminations in foods. It is also provides lower risk of developing resistance against pathogenic microorganisms and more safety for the people consuming the food product as well as reducing environmental problems.

Damascus rose essential oil is a safe natural oil which has antioxidant, antifungal, and antibacterial activity. Therefore, this plant volatile oil and its related products can be used as safe natural compounds in the food industry. These products are safely usable in functional foods and/or as dietary supplements. In addition, they can be used as flavoring agents in food industry as well as in perfume industries to provide a better quality to the fragrances.

As an Antimicrobial for Food Preservation

Bacteria, fungi, and yeasts are responsible for destruction and spoilage of foodstuff, fresh fruit, and vegetables by microorganism contamination during the storage. Affecting the foodstuff by various microorganisms and breakdown the food can change the appearance of the foods as well as the taste and odor (Dobre et al., 2011a,b). Volatile oils of the various plants and their components have shown to have antifungal and antibacterial properties. *Rosa*×*damascena* essential oil also has antibacterial activity and therefore can be used as preservative in food preparations.

Ulusoy et al. (2009) studied the antibacterial effect of *R.*×*damascena* products including *R.*×*damascena* essential oil. The antimicrobial activity of the volatile oil of this plant was evaluated using agar diffusion and minimum inhibitory concentration (MIC) tests. In this study, six different pathogenic microorganisms, *Pseudomonas aeruginosa, Chromobacterium violaceum, Escherichia coli*, *Bacillus subtilis, Erwinia carotovora*, and *Staphylococcus aureus*, were used. The *R.*×*damascena* essential oil showed strong antibacterial activity against tested microorganism. The reported MIC (0.25 to >4) suggested high antimicrobial activity for the tested oil. The strong antibacterial activity of *R.*×*damascena* volatile oil showed in this study suggests it can be used as a natural preservative with botanical source in the food industry to preserve foodstuff and it can also be used as an antibacterial agent for disinfecting the various surfaces (Ulusoy et al. 2009).

Aridoğan et al. studied the antibacterial effect of essential oil of *R.*×*damascena* using the disk diffusion method against *P. aeruginosa, S. aureus*, and *E. coli* bacterial strains. Results of this study indicated that the volatile oil obtained from this plant was effective against *S. aureus* (Aridoğan et al., 2002).

The antibacterial activity of volatile oil of extracted from petals of *R.*×*damascena* examined by Basim et al. against three different bacteria strains of *Xanthomonas axonopodis* spp. *vesicatoria*. Results of the study showed remarkable inhibition of growth of these bacterial strains. These results suggested the possible use of essential oil of *R.*×*damascena* as a potential antibacterial agent to control *X. axonopodis* spp. *vesicatoria* strains originating from diseases in plants like pepper and tomato (Basim and Basim, 2003).

Lisin et al. investigated the antimicrobial effect of *R.*×*damascena* essential oil against the yeast *Candida albicans,* Gram-negative *P. aeruginosa* and *E. coli*, and Gram-positive *S. aureus* using various concentration of the oil. The low concentrations of this volatile oil showed inhibitory effects and bactericidal activities against all tested microorganisms (Lisin et al., 1999).

The antimicrobial activity of *R.*×*damascena* essential oil obtained from the plants grown in different geographic area and their compositions were studied by Gochev et al. Results showed high antibacterial activity for the oils and some of their main components like geraniol, citrenellol, and nerol (Gochev et al., 2008).

Özkan et al. investigated the antibacterial activities of fresh and spent flower extracts of *R.*×*damascena*. They used the method of agar diffusion against 15 different species of bacteria. Results of the study showed the effectiveness of both extracts against nearly all the microorganisms tested. A comparison of the results of the two different extracts showed that the fresh flower extract had stronger antibacterial activity relative to the spent flower extract (Özkan et al. 2004).

As an Antioxidant

Chemical degradation of foodstuffs during the storage, especially food products containing oils and fat, is a major problem in food industry. ROS produced in foodstuffs during storage is very reactive and can damage the transient chemical species formed in cells (Mantle et al., 2000). The effect of ROS on food components degrades foods quality and can provide serious problems for those consuming these food products. Therefore, the food industry has to use preservatives in order to prevent the food spoilage. Essential oils are possessing antioxidant activity and can be used as a natural source of antioxidant. The

volatile oil of *R.×damascena* has antioxidant effects and can be a useful and safe antioxidant candidate for use in the food industry.

Nazıroğlu et al. examined the effectiveness of *R.×damascena* essential oil on the level of antioxidant in the cerebral cortex of 32 male rats. Results showed the levels of lipid peroxidation in the male rats cerebral cortex decreased after exposure to *R.×damascena* volatile oil vapor. Therefore, they suggested treatment with this rose volatile oil vapor has highly protective effects in suppressing oxidative stress (Nazıroğlu et al., 2013).

Yassa et al. investigated the chemical composition of the essential oil of *R.×damascena* from Iran, in the population of Guilan. In addition, they examined the antioxidant activity of the essential oil and hydroalcoholic extract of this plant. Results of the study showed strong antioxidant activity for the extract when 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ammonium thiocyanate methods were used. The volatile oil major components were geraniol, 1-nonadecene, *n*-tricosane, and hexatriacontane. The volatile oil also showed good antioxidant effect when tested with the DPPH method. The results indicated that the oil of this plant had higher antioxidant activity compared with vitamin E, butylated hydroxyanisole and butylated hydroxytoluene (Yassa et al., 2009).

Schieber et al. studied the antioxidant activity of flavonol glycosides extracted from essential oil recovered from industrial distillation of *R.×damascena* petals. They concluded that distilled rose petals have antioxidant activity and can be used in food ingredients. Therefore, it is possibly suitable to be used as a safe natural antioxidant with botanical source in food industry (Schieber et al., 2005).

Antioxidant activity of fresh and spent flower extracts of *R.×damascena* obtained after steam distillation was examined by Özkan et al. This investigation showed that the fresh flower extract has stronger antioxidant activity relative to the spent flower extract (Özkan et al., 2004).

Shikov et al. evaluated the antioxidant effects of *R.×damascena* polyphenol-enriched extracts obtained from the by-product of the plant petal on stored canned strawberries. The antioxidant activity was tested using the Trolox equivalent antioxidant capacity and ferric reducing antioxidant power methods.

Results showed that compared with the control sample the antioxidant capacity was higher during and after storage for three months. Therefore, they suggested the rose petal extracts could be used as natural antioxidant in food products and this way reach higher stability for the color during the process of the enzymatic fruit firming (Shikov et al., 2012).

As Flavoring Agent

Rosa×damascena is an important plant as an ornamental plant, and is cultivated in various places like houses, parks, etc. Also, commercially, it is an important plant. Because of various compounds in the petal, hips, essential oil, and in different extracts obtained from this plant, it is extensively cultivated for use in the perfume industry as well as in the food industry. This plant is used for food flavoring as dried flowers, dried bud, and dried petals. In addition to the use of dried flowers of this plant, most of the various products obtained from *R.×damascena*, like hips, essential oil, hydrosol, absolute, aqueous, ethanol extractions of *R.×damascena* flowers, and especially rose water, are traditionally used as flavoring agents in foodstuff. Traditionally in the food industry and in preparation of some food products like liqueurs, jams, jellies, etc., rose petals are used in order to preserve them from contamination as well as flavoring them.

Several different studies have also shown that *R.×damascena* and its products have antioxidant and antimicrobial activity and they are a good natural source to use for keeping foodstuff in good shape, color, and condition for longer and to avoid contamination (Jabbarzadeh and Khosh-Khui, 2005; Nikbakht and Kafi, 2008; Zargari, 1996).

SUMMARY POINTS

- *Rosa×damascena* is an ornamental plant.
- *Rosa×damascena* used in food and cosmetic industry.
- *Rosa×damascena* in an ancient medicinal plant.
- *Rosa×damascena* and its products are extensively used as flavoring agent.
- A popular *Rosa×damascena* essential oil product is rose water.
- From *Rosa×damascena* several different products like rose water, rose oil, and rose absolute are extracted.
- *Rosa×damascena* essential oil has various pharmacological activities.
- *Rosa×damascena* essential oil has antimicrobial activity.
- *Rosa×damascena* essential oil has antioxidant activity.

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Chapter 76

Rose Hip (*Rosa canina* L.) Oils

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INTRODUCTION

Rose hips, also known as rose haw or rose hep, are the fruits of the rose plant (rose bush) belonging to *Rosa* genus in the *Rosaceae* family. Rose hips are typically red to orange in color. The fruits are comprised of 30–35% seeds and 65–70% pericarp. They are well known to have the highest vitamin C content among horticultural crops, fruits, and vegetables. Rose hips have been found to contain appreciable amounts of various bioactive compounds, such as carotenoids, tocopherol, polyphenolics, bioflavonoids, tannins, pectin, sugars, organic acids, amino acids, essential oil (Uggla et al., 2003), folates (Stralsjo et al., 2003), and polyunsaturated fatty acids (Larsen et al., 2003).

Rose hips are remarkable fruits for their traditional pharmaceutical uses, which may be partly attributed to their rich profile of bioactives, especially antioxidant phenolics (Olsson et al., 2005). The seed lipids of rose hips contain high amounts of polyunsaturated fatty acids (Szentmihalyi et al., 2002). Rose hips are popular due to their food, phytomedicine, and cosmo-nutraceutical uses (Uggla et al., 2003). The fruits (rose hips) of *Rosa canina* in particular contain high content of vitamin C and proanthocyanidins and are used for various food and pharmaceutical applications (Osmianski et al., 1986). This chapter mainly focuses on the traditional pharmaceutical and food science applications of rose hips and the essential oil of a widely distributed species of rose hips, *R. canina* L.

BOTANICAL ASPECTS

Rose hips are the berry-like fruits of different species of rose plant/rose bushes belonging to *Rosa* genus in the *Rosaceae* family. The genus *Rosa* L. (Rosaceae) consists of approximately 150 reported species, all of which grow in the northern hemisphere in Europe, Asia, the Middle East, and North America. These deciduous flowering shrubs are widely grown in gardens for their flowers and fruits (Ercisli, 2005). Photographs of the rose hips plant and its ripened fruits are shown as Figure 2 and Figure 3, respectively.

Rosa canina, known as the dog rose, is a variable climbing rose species that grows wildly in Europe, northwest Africa, and Western Asia. This deciduous perennial shrub has a height of about 2–3 m. Its stems are small, sharp, hooked prickles, which help the plant to climb up. The leaves are pinnate, with five to seven leaflets. The flowers of *R. canina* are normally pale pink, but occasionally the color may fluctuate between a deep pink and white. The flower consists of five petals that cover the diameter of 4–6 cm and eventually form a red-orange fruit (hip). The plants show strong resistance to harsh environmental conditions (poor soil, rocky places, and limiting water, etc.). Its flowers are hermaphrodite and can be pollinated by bees, flies, beetles, and Lepidoptera. Interestingly, the plant is self-fertilizing because the flowers can also undergo self-pollination (Plants For A Future, 1996–2010). A photograph of a typical flower of *R. canina* is shown in Figure 4.

USES AND APPLICATIONS

Rose hips (fruits) from rose bushes of *Rosa* genus are important due to their traditional pharmaceutical, nutraceutical, and commercial significance. They are beneficial for health due to their appreciable levels of high-value nutrients, minerals, and bioactives. The fruits, boiled in water, can be used as a diuretic as well as a remedy for common cold (Sen and Gunes, 1996). Rose hips are traditionally employed to treat influenza, inflammation, and chronic pain. Moreover, they are useful for the treatment of skin disorders and ulcer (Guimaraes et al., 2010).

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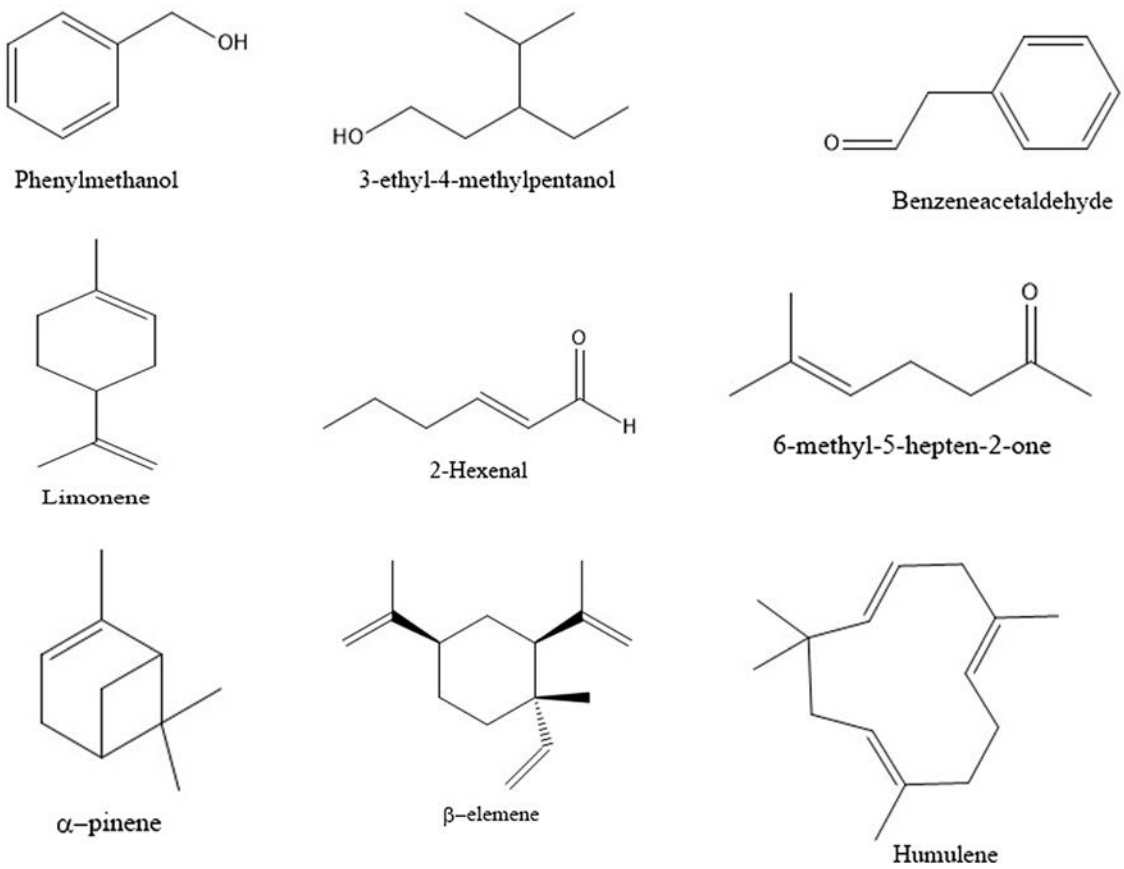

FIGURE 1 Chemical structures of some important constituents of rose hip essential oil.

FIGURE 2 *Rosa canina* plant. *http://commons.wikimedia.org/wiki/File:Rosa_canina.jpg*.

Another healthy function of the rose hips is their essential fatty acids (EFAs), which humans cannot synthesize and must be supplied through diet. The EFAs are long-chain polyunsaturated fatty acids derived from linolenic, linoleic, and arachidonic acids. The EFAs regulate numerous body functions, including blood pressure, blood viscosity, and immune and inflammatory responses (Simopoulos and Salem, 1996). Due to their high therapeutic value, rose hips are widely used as ingredients in different native phyto-medicines (Shnyakina and Malygina, 1975).

FIGURE 3 Ripened fruits of *Rosa canina*. *http://publicphoto.org/fruit/rose-hip-close-up*.

FIGURE 4 The flower of *Rosa Canina* *http://commons.wikimedia.org/wiki/File:Rosa_canina_EnfoqueFlor_2010-5-08_CampodeCalatrava.jpg*.

Rose hips powder, which is marketed as a food supplement in several European countries due to its anti-inflammatory properties, has been shown to reduce knee or hip osteoarthritis in clinical trials. The powder is also used as a remedy to treat rheumatoid arthritis, wounds, and inflammations (Rein et al., 2004). Rose hips, as a rich source of physiologically functional bioactives including carotenoids, phytosterols, tocotrienols, anthocyanins, catechins, and polyphenolics, are reported to protect the body against cancer and cardiovascular diseases (Rein et al., 2004). Fu et al. (2006) reported that compounds found in rose hip species such as *Rosa rugosa* Thunb. flowers have inhibitory activity against human immunodeficiency virus reverse transcriptase. A study conducted by Xie and Zhang (2012) has also shown that extracts from rose hips flowers can inhibit the angiotensin-converting enzyme, thus offering therapeutic potential in hypertension and congestive cardiac failure, particularly when the presence of an additional diuretic component offers added value in lowering blood pressure.

Rose hips (fruits) of *R. canina* are especially well known for their traditional pharmaceutical and therapeutic potential against different diseases (Rein et al., 2004; Orhan et al., 2007). They are used for the preparation of herbal tea with potential uses as a remedy for common cold and infections (Ph. Eur., 2005). *Rosa canina* fruits, being diuretic and laxative, are reported to be effective for digestive problems. As a rich source of natural antioxidants, the fruits are commonly used in nutraceutical and folk medicine preparations to strengthen the body's defensive system against different ailments (Shnyakina and Malygina, 1975). The extracts from *R. canina* fruits have strong anti-inflammatory

and antinociceptive activities (Orhan et al., 2007). Clinically, it is proven that *R. canina* fruit powder is beneficial for hip and knee osteoarthritis patients (Winther et al., 2005).

USES AND APPLICATIONS IN FOOD SCIENCES

Rose hips are typically red or orange with a fruity and tangy flavor similar to that of cranberries; they can be used as fresh or in dried form. As a rich source of minerals (potassium, phosphorous), vitamins (vitamin C), carotenoids, and flavoring components, rose hips have several culinary and food applications (Shnyakina and Malygina, 1975; Oszmianski and Chomin, 1993). The demand for rose hips as a natural and useful material is increasing as new products are emerging in the market, including health supplements, food additives, functional foods, specialty oils, and exfoliates. The high content of added-value bioactive compounds in rose hips allows for the extraction, isolation, and potential applications of several of health-promoting components, such as ascorbic acid, carotenoids, phenolic compounds, anti-inflammatory agents, and polyunsaturated fatty acids (Szentmihalyi et al., 2002; Demir et al., 2014).

Rose hips are a potential fruit commodity to be used for functional food and cosmo-nutraceutical preparations. Rose hips oil, which is mostly cold-pressed from the seeds of wild rose bush species such as *R. canina* and *Rosa moschata*, due to its skin healing potential, is a valuable material for the development of herbal cosmetics and skin care products such as lotions and creams. The oil is one of the richest sources of EFAs (linoleic acid and linolenic acid), with content as high as 70–80% (Tables 1 and 2). The oil has healing power to treat skin problems such as scars, dermatitis, acne, eczema, and burns. Due to its content of EFAs, carotenoids, and vitamin A (retinol) with skin rejuvenating properties, rose hip seed oil acts as a natural skin care remedy, moisturizer, and anti-aging agent. The seed oil is also useful for healing scars and diminishing photoaging and imparts smoothness to skin (Concha et al., 2006).

Rose hips, due to their health promoting components and anti-inflammatory/antioxidant properties, are used as ingredients in probiotic drinks, beverages, soups, and yogurts (Gao et al., 2000; Chrubasik et al., 2008). The rose hip powder, which is rich in vitamin C, is available in many health food stores as a health supplement (Larsen et al., 2003). Moreover, rose hips fruit extracts (RHFE) are a potential source of antimicrobial and antioxidant agents and total phenolics (78–102 mg gallic acid equivalents (GAE)/g dry weight [DW]). The antibacterial activity of RHFE has been evaluated against a panel of bacteria (Yilmaz and Ercisli, 2011). In another study, DPPH free radical scavenging capacity (EC_{50}) of RHFE of the species *R. canina* L. was recorded to be in the range of 25–988 µg/mL, while the total phenolics were noted to be in the range of 1.5–82.2 µg GAE/mg DW (Wenzig et al., 2008).

An investigation revealed that some species of rose hips, including *R. cania*, *Rosa dumalis*, *Rosa gallica*, *Rosa dumalis*, and *Rosa hirtissima* have appreciable free radical scavenging potential and total phenolics (31.08–52.94 mg GAE/g DW). The concentration of phenolic compounds in the tested rose hips species varied widely, ranging from 1.89 µg/g DW [(−)-epicatechin] to 32.18 µg/g DW (4-hydroxy benzoic acid) (Demir et al., 2014). This supports the potential uses of RHFE as a natural antioxidant and antimicrobial additive for food preservation. The presence of a considerably high concentration of natural sugars (glucose and fructose), organic acids (ascorbic, citric, malic acids), and volatiles (alcohols, aldehydes, ketones, terpenoids, and esters) contributes mainly to the taste and organoleptic value as well as flavoring properties of rose hips (Demir et al., 2014). Interestingly, the occurrence of organic acids and flavanols in rose hips helps to inhibit oxidation of vitamin C, thus increasing its stability and bioavailability in humans (Demir et al., 2014).

Rosa canina fruits have long been used in food and nutrition due to their antioxidant potential, which is attributed to the presence of ascorbic acid and phenolics (Hemila, 1992). Typically, the reddish-orange fruit (rose hips) of *R. canina* is used as a vitamin supplement and nutraceutical product (Nowak and Krzaczek, 1994). Both the fresh and dried rose hips from this species are commonly used to make jam, marmalade, fruit juice, and beverages, among others (Uggla and Nybom, 1999), while the dried fruits and roots are employed for making tea (Sen and Gunes, 1996; Ph. Eur, 2005).

TABLE 1 Seed Oil Yield of *Rosa canina* in Comparison with Some Other Species of Rose Hips

Species	Oil Content (%)	Origin	References
Rosa canina	1.2–1.6	Turkey	Demir and Ozcan, (2001)
Rosa canina	7.15	Turkey	Kazaz et al., (2009)
Rosa canina	3.25–6.68	Hungry	Szentmihalyi et al., (2002)
Rosa damascena	2.75	Turkey	Kazaz et al., (2009)
Rosa sp.	15.95	France	Machmudah et al., (2007)

TABLE 2 Fatty Acid Composition of *Rosa canina* Seed Oil in Comparison with Some Other Rose Hip Species

				Fatty Acid Content (%)								
Fatty Acid	*R. canina*	*Rosa* sp.	*R. damascena*	*R. rubiginosa*	*R.subcanina*	*R. dumalis (besseriana)*	*R. inodora*	*R. villosa*	*R. rugosa*	*R. pisiformis*	*R. pulverulenta*	References
C14:0	0.03	–	–	0.052	0.04	0.03	0.05	0.04	0.03	–	–	Nowak (2005)
C15:0	0.03	–	–	0.035	0.03	0.035	0.04	0.04	0.04	–	–	
C16:0	3.54	–	–	2.86	3.09	2.79	3.24	3.07	2.77	–	–	
C16:1, n-9	0.05	–	–	0.035	0.03	0.035	0.05	0.07	0.04	–	–	
C16:1, n-7	0.06	–	–	0.02	0.07	0.069	0.09	0.08	0.18	–	–	
C17:0	0.08	–	–	0.05	0.05	0.06	0.05	0.07	0.05	–	–	
C18:0	2.46	–	–	1.64	1.89	1.77	2.40	1.73	1.04	–	–	
C18:1, Δ6	0.02	–	–	0.03	0.065	0.077	0.05	0.08	0.05	–	–	
C18:1, Δ9	20.30	–	–	14.22	13.88	13.54	18.22	18.41	14.42	–	–	
C18:1, Δ11	0.05	–	–	0.57	0.49	0.43	0.47	0.52	0.72	–	–	
C18:2 Δ9, 12	51.67	–	–	47.20	49.77	53.77	50.29	44.41	50.32	–	–	
C18:3 Δ9,12,15	19.08	–	–	31.40	28.69	25.61	22.79	29.49	27.90	–	–	
C20:0	0.90	–	–	0.74	0.74	0.77	0.77	0.83	0.72	–	–	
C20:1	0.30	–	–	0.36	0.44	0.32	0.30	0.59	0.70	–	–	
C20:2	0.08	–	–	0.13	0.09	0.11	0.07	–	0.16	–	–	
C22:0	0.15	–	–	0.15	0.21	0.20	0.15	–	0.29	–	–	
C22:1	0.07	–	–	0.08	0.03	0.07	0.03	0.17	0.04	–	–	
C12:0	4.80	–	–	–	–	0.00	–	0.00	–	3.58	0.00	Ercisli et al., (2007)
C16:0	16.4	–	–	–	–	24.4	–	26.6	–	19.6	19.3	
cis-C18:2	16.0	–	–	–	–	20.0	–	17.5	–	16.1	16.6	
cis-C18:3	40.5	–	–	–	–	36.6	–	46.9	–	38.0	49.7	
C19:0	4.74	–	–	–	–	0.00	–	0.00	–	0.00	0.00	
cis-C19:1	5.79	–	–	–	–	2.92	–	0.00	–	9.55	11.4	
cis-C21:1	0.00	–	–	–	–	4.24	–	0.00	–	0.00	0.00	
cis-C22:2	6.60	–	–	–	–	0.00	–	0.00	–	0.00	0.00	
C23:0	0.00	–	–	–	–	0.00	–	7.10	–	0.00	0.00	

Continued

TABLE 2 Fatty Acid Composition of *Rosa canina* Seed Oil in Comparison with Some Other Rose Hip Species—cont'd

				Fatty Acid Content (%)								
Fatty Acid	*R. canina*	*Rosa* sp.	*R. damascena*	*R. rubiginosa*	*R.subcanina*	*R. dumalis (besseriana)*	*R. inodora*	*R. villosa*	*R. rugosa*	*R. pisiformis*	*R. pulverulenta*	References
C18:2	–	47.02–49.14	–	–	–	–	–	–	–	–	–	Machmudah et al., (2007)
C18:3	–	33.02–40.21	–	–	–	–	–	–	–	–	–	
C16:0	–	3.83–4.68	–	–	–	–	–	–	–	–	–	
C18:0	–	2.46–2.62	–	–	–	–	–	–	–	–	–	
C16:0	3.60–7.87	–	–	–	–	–	–	–	–	–	–	
C18:0	2.45–3.27	–	–	–	–	–	–	–	–	–	–	
C18:1	16.25–22.11	–	–	–	–	–	–	–	–	–	–	
C18:2	35.94–54.75	–	–	–	–	–	–	–	–	–	–	
C18:3	20.29–26.48	–	–	–	–	–	–	–	–	–	–	
C16:0	5.26	–	5.30	–	–	–	–	–	–	–	–	Kazaz et al., (2009)
C18:0	3.13	–	2.02	–	–	–	–	–	–	–	–	
C18:1	22.14	–	23.91	–	–	–	–	–	–	–	–	
C18:2	48.84	–	54.18	–	–	–	–	–	–	–	–	
C18:3	20.65	–	15.09	–	–	–	–	–	–	–	–	

CHEMICAL COMPOSITION OF ROSE HIPS ESSENTIAL OIL

A few studies have been reported on the yield and chemical composition of rose hips essential oil. According to Nowak (2005), the essential oil of *R. canina*, as analyzed using gas chromatography (GC)/mass spectrometry (MS) method, contained 97 chemical components—mainly vitispiran (isomer) (1.8–17.38%), α-*E*-acaridial (0–13.55%), dodecanoic acid (0.62–11.98%), hexadecanoic acid (2.45–14.26%), docosane (C22) (0–13.29%), β-ionone (0.11–10.97%), 6-methyl-5-hepten-2-one (around 14.49%), myristic acid (0.52–4.05%), and linoleic acid (0–21.95%). The characteristic volatile compounds present in rose hips essential oils are presented in Table 3.

In another study, GC–MS analysis revealed that volatile compounds of rose hip species such as *R. canina* are a complex mixture of alcohols, aldehydes, ketones, monoterpenes, and sesquiterpenes. Among alcohols, 2-hexen-1-ol and 1-hexanol were determined to be the major compounds at level of 4.95–35.13 μg/kg and 10.93–48.02 μg/kg, respectively, while 2-hexenal is the main aldehyde (39.93–219.59 μg/kg). Only two ketones have been identified: 4-octen-3-one (3.09–6.03 μg/kg) and 6-methyl-5-hepten-2-one (13.67–22.36 μg/kg). Among monoterpenes, limonene dominated at a level of 60.99–124.71 μg/kg, followed by α-pinene at 8.42–85.84 μg/kg. Moreover, β-elemene (1.26–42.91 μg/kg) and α-humulene (1.01–10.16 μg/kg) were found to be the dominant among sesquiterpenes (Demir et al., 2014). The structures of some important chemical constituents of rose hips essential oil are shown in Figure 1 (Nowak, 2005).

TABLE 3 Characteristic Compounds in the Essential Oil of *Rosa canina* in Comparison with Other Species of Rose Hips

Species	Main Compounds	Amount	Other Characteristic Compounds	Amount	Reference
R. canina	Vitispiran (isomer)	10.3%	-5-methyl-3-hexanone and 2-heptanone and hexadecanoic acid -α-*E*-acaridial, β-ionone, dodecanoic acid, linolic acid; -6-methyl-5-hepten-2-one	>7% >5% >2%	Nowak (2005)
R. rugosa	*cis*-3-hexenal 6-methyl-5-hepten-2-one	27.5% 14.5%	-vitispiran (isomer) and dodecanoic acid, -nonanal and hexadecanoic acid -5-methylhexanal	>5% ~2.5% 2.3%	
R. vosagiaca	Hexadecanoic acid Vitispiran (isomer)	9.5% 9.3%	-linolic acid, 2- heptanone, β-ionone, dodecanoic acid, -6-methyl-5-hepten-2-one α-*E*-acaridial, myristic acid	>9% >2%	
R. caryophyllaceae	Docosane (C22) Dodecanoic acid	13.3% 12%	-hexadecanoic acid -myristic acid -β-ionone	8.4% >4% >2%	
R. coriifolia	Vitispiran (isomer) α-*E*-acaridial β-ionone	13.4% 12.7% 11%	-5-methyl-3-hexanone and 2-heptanone, dodecanoic acid, hexadecanoic acid -6-methyl-5-hepten-2-one, salicylaldehyde	>4% >2%	
R. subcanina	Linolic acid Vitispiran (isomer) Hexadecanoic acid	22% 16.1% 14.3%	-dodecanoic acid -myristic acid and 2- heptenol	>6% >2%	
R. rubiginosa	Vitispiran (isomer)	17.4%	-hexadecanoic acid, dodecanoic acid, docosane (C22) -α-*E*-acaridial, 6- methyl-5-hepten-2-one, -β-ionone, 5-methyl-3-hexanone and 2 heptanone	>5% >2%	
R. villosa	α-*E*-acaridial	13.6%	-β-ionone, hexadecanoic acid -heptanal, dodecanoic acid, linolic acid	>6% >3%	
R. tomentosa	α-*E*-acaridial Hexadecanoic acid Heptanal	3% 11.7% 9%	-5-methyl-3-hexanone and 2-heptanone, vitispiran (isomer), hydrocarbon -benzaldehyde and a-opinene, β-ionone, unsaturated hydrocarbon	>5% >2%	

SUMMARY POINTS

- Rose hips are the fruits of rose bush (*Rosa* genus) in the *Rosaceae* family.
- Rose hips are well known because they have the highest vitamin C content among horticultural crops, fruits, and vegetables.
- Rose hips are remarkable fruits for their traditional pharmacological applications.
- Rose hips extracts/oils have appreciable antioxidant and antimicrobial potential and thus can be explored as valuable ingredients for functional food and nutraceuticals.
- Rose hips have long been traditionally used as an herbal tea, vitamin supplement, and health food product.
- Rose hips have a rich profile of nutrients and thus are utilized to prepare jam, marmalade, fruit juice, beverages, and drinks.
- Rose hips seed oil is one of the richest sources of EFAs and is a valuable material for the development of herbal cosmetics and skin care products, such as lotions and creams.
- Rose hips volatile essential oil is a complex mixture of alcohols, aldehydes, ketones, terpenoids, and esters.

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Chapter 77

Rosemary (*Rosmarinus officinalis* L.) Oils

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List of Abbreviations

DPPH 2,2-Diphenylpicrylhydrazyl assay
EDTA Ethylenediaminetetraacetate
MIC Minimum inhibitory concentration
PUFA Polyunsaturated fatty acids
SPME Solid phase microextraction
TVC Total viable count
WHO World Health Organization

INTRODUCTION

In recent years, the demand for essential oils (EOs) from medicinal plants has increased, particularly in the case of oil from the rosemary plant (*Rosmarinus officinalis* L.), which has widespread use as a natural food preservative (listed by the European Food Safety Authority (EFSA) in question number EFSA-Q-2003-140 and the U.S. Food and Drug Administration (FDA) at the link http://www.cfsan.fda.gov/~dms/eafus.html). Rosemary oil has antimicrobial, antimycotic, and antioxidant properties and, above all, low cost and ready availability.

The essential oil synthesized by aromatic–medicinal plant species can be obtained through different methods, and the extraction method chosen can potentially affect both yield and composition (Okoh et al., 2010). The most commonly used methods for this purpose are hydrodistillation, solvent extraction, and simultaneous distillation-extraction. New methods have also been developed to recover the components of interest while preventing alterations in the composition of the oils; these include methods such as supercritical fluid extraction and the use of microwaves.

Extraction by means of distillation, based on the volatility of the essential oils, is the most frequently used method to separate out these substances, both industrially and in the laboratory. One of the most popular methods is hydrodistillation using a Clevenger-type system, following the guidelines of the European Directorate for the Quality of Medicines (2002).

BOTANICAL ASPECTS

The genus *Rosmarinus* (family *Labiatae or Lamiaceae*) comprises three different species (*Rosmarinus officinalis*, *Rosmarinus eryocalix*, and *Rosmarinus tomentosus*) found primarily in the western Mediterranean region, with some enclaves on islands in the Aegean Sea and the South of Turkey. It can also be found growing wild in Crimea, Cyprus, Caucasus, Crete, the Macaronesian region, and Central and South America. Its natural habitat spans from areas close to the sea up to 1500 m above sea level (Font Quer, 2001).

Almost all are heliophilous and pioneer plants that quickly colonize open, barren spaces, such as areas of burned brush, fallow land or fields of abandoned crops; they therefore play a fundamental role in the early phases of development of Mediterranean brush.

The name *Rosmarinus* most likely comes from the Greek words *rhops myrinos*, meaning aromatic brush (Figure 1). Morales (2010) described the characterization and taxonomy of the *Rosmarinus* genus, specifying that it is a perennial bush with erect or procumbent hairy stems and spherical glands. The leaves are simple, linear to lanceolate, downward curving, and whole, often with a rough surface, either hairless or tomentose; they have glandular and nonglandular hairs. Glandular hairs include peltated trichomes, predominantly on the abaxial surface, and capitated trichomes, located on abaxial and

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FIGURE 1 Rosemary during the flowering stage.

adaxial surface (Figure 2; Marin et al., 2006). The plants are characterized by a lax inflorescence in axillary or cymose verticillasters with 5–15 flowers.

Rosemary is a plant that can be used in different ways, distinguishing it as an aromatic, medicinal, and culinary plant. Accordingly, rosemary is grown all over the world, particularly in Russia, England, France, Spain, Portugal, the Balkan Peninsula, Tunisia, China, Australia, and within the United States, such as in the state of California (Morales, 2010).

This genus is extremely rich in essential oil, although there are large intraspecies differences in terms of the yield and quality of the oil it produces. Intrinsic factors, particularly genetic inheritance (Zaouali et al., 2012) and/or the phase of development of the plant when harvested (Zaouali et al., 2013; Jordán et al., 2013a), in addition to extrinsic factors, such as soil and climate conditions (Brewer, 2011) explain this large degree of variability.

The chemical composition of rosemary oil has been widely studied, and the presence of different chemotypes has been detected (Figure 3). According to Napoli et al. (2010), from a chemical point of view, rosemary essential oil can be classified as one of three chemotypes: cineoliferum (high 1,8-cineol content), camphoriferum (camphor >20%), or verbenoniferum (verbenone >15%). Even beyond the differences in concentration that may exist among the majority components (1,8-cineol, camphor, borneol, α-pinene) of essential oil from the *Rosmarinus* genus (primarily *R. officinalis*) found in the Mediterranean region, the worldwide variability among individual plants belonging to this species is especially high, as shown in Table 1. This greatly complicates any extrapolation of results concerning the use of these oils in different scientific areas, as they are highly dependent on the origin of the plants and their corresponding chemical composition (Table 2).

USAGE AND APPLICATIONS

Highly prized by ancient peoples, rosemary has been closely associated with love and marriage, as well as birth and death rites. These aromatic shrubs have been widely cultivated since ancient times for medicinal, culinary, and ornamental purposes. In particular, rosemary has a tremendous variety of folk uses and a large body of mythology associated with it. Since antiquity, it has been especially associated with treatments to improve memory and remembrance. The main historical medicinal uses of rosemary have been as a brain tonic and a gentle liver-cleansing medicine. In ancient Greece and Rome, rosemary was believed to strengthen the memory, which accounts for its being known as the herb of remembrance and fidelity. Rosemary was also an essential part of an apothecary's repertoire during the Renaissance (Begum et al., 2013).

In folk medicine, infusions made from its stems and flowers have been used to treat headaches and colds, as a pain reliever for renal colic and dysmenorrhea, and as an antispasmodic (Al-Sereiti et al., 1999). Following essential oil extraction, the distilled water from the flowers is also used as eyewash, to improve digestion, and to alleviate stomachaches (Argueta et al., 1994). The essential oil itself serves as a rubefacient and tonic.

Medicinal plants have played a major role as a source of pharmacologically active substances. Today, rosemary essential oil is a product with good prospects for the pharmaceutical, cosmetic, and food industries, endowed by its chemical

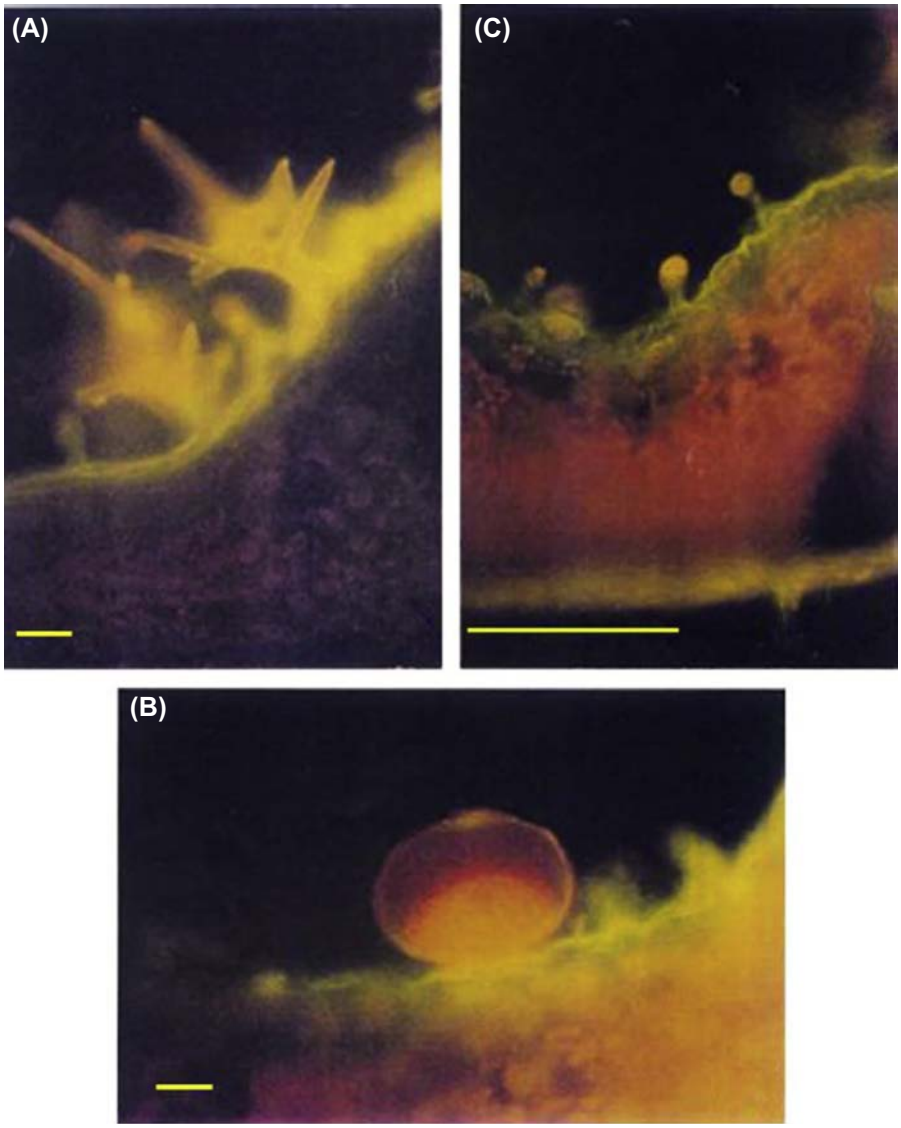

FIGURE 2 Trichomes of rosemary leaves. (A) Nonglandular trichome; bar = 100 μm. (B) Peltate trichomes; bar = 100 μm. (C) Capitate trichome; bar = 1000 μm (Marin et al., 2006).

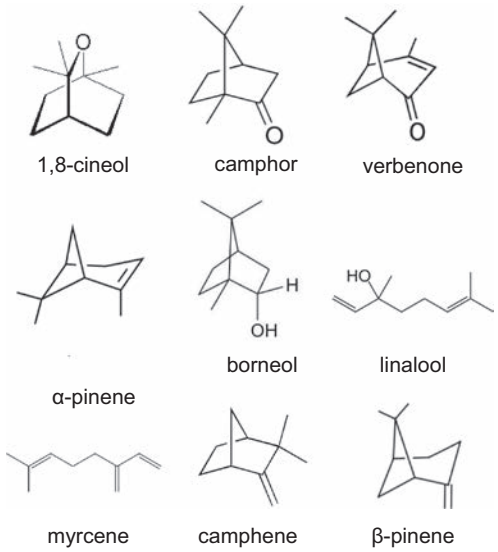

FIGURE 3 Chemical structure of the major components identified in rosemary essential oil.

TABLE 1 Variability in the Chemical Composition of *Rosmarinus officinalis* Essential Oil

Authors	Origin	Chemotype
	Spain	22% 1,8-Cineol, 20% camphor, 19% α-pinene
Angioni et al. (2004)	Sardinia (Italy)	23% α-Pinene, 16% borneol, 9.4% verbenone, 10.4% bornyl acetate
Pitarokili et al. (2008)	Greece	24.1% α-Pinene, 14.9% camphor, 9.3% 1,8-cineol, 8.9% camphene
Özcan and Chalchat (2008)	Turkey	44.02% *p*-Cymene, 20.5% linalool, 16.62% γ-terpinene, 2.64% 1,8-cineol
Diab et al. (2002)	Lebanon	19.1–25.1% 1,8-Cineol, 18.8–38.5% α-pinene
El-Massry et al. (2008)	Egypt	52.8% 1,8-Cineol, 11.9% camphor, 10.2% α-pinene, 7.5% borneol
Zermane et al. (2010)	Algeria	48.9% Camphor
Zaouali et al. (2005)	Tunisia	20–46% 1,8-Cineol, 8.5–30.2% camphor, 6.5–13% α-pinene, 4–25% borneol
Elamrani et al. (2000)	Morocco	Three chemotypes: 37.4% α-pinene, 41–53% camphor, and 58–63% 1,8-cineol
Serrano et al. (2002)	Portugal	16.6–29.5% Myrcene, 8.3–14.5% 1,8-cineol, 14.3–23.1% camphor
Mata et al. (2007)	Portugal	35.4% Verbenone
Jaganmohan et al. (1998)	India	30% 1,8-Cineol, 30% camphor
Porte et al. (2000)	Brazil	26.0% Camphor, 22.1% 1,8-cineol, 12.4% myrcene, 11.5% α-pinene
Martínez et al. (2009)	Mexico	14.1% α-Pinene, 11.5% camphene, 12.0% β-pinene, 7.9% α-phellandrene, 8.6% 1,8-cineol, 3.4% 2-bornanone, 8.7% camphor
Masatoshi and Hiroaki (1997)	Japan	50% 1,8-Cineol and low levels of α-pinene and camphor (10%)

TABLE 2 Biological Activities of *Rosmarinus officinalis* Essential Oil

Authors	Chemotype	Activity
Okoh et al. (2010)	23% Verbenone, 17% camphor, 12% bornyl acetate, 10% 1,8-cineol	Antibacterial: *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae*
Bozin et al. (2007)	22% Limonene, 22% camphor, 13% α-pinene	Antibacterial: *S. enteriditis*, *S. thypi*, *E. coli*, *Sigella sonei*
Pitarokili et al. (2008)	24% α-Pinene, 15% camphor, 9% 1,8-cineol, 9% camphene	Antifungal: *S. sclerotiorum*, *Ph. nicotianae*, *S. epivorum*, *F. oxysporum f.* sp. *Dianthi*, *and F. proliferatum*
Özcan and Chalchat (2008)	44% *p*-Cymene, 21% linalool, 17% γ-terpinene	Antifungal: *A. alternata*, *Botrytis cinerea*, *F. oxysporum*
Sagorchev et al. (2010)	45% 1,8-Cineol, 10% camphor, 13% α-pinene	Specific effects on nerve cells adrenergic receptors
Horváthová et al. (2010)	25% 1,8-Cineol, 19% camphor, 19% α-pinene, 17% *p*-cymene	Enhanced resistance of rat hepatocytes against DNA oxidative damage
Martínez et al. (2009)	14% α-Pinene, 12% camphene, 12% β-pinene, 9% 1,8-cienol, 9% camphor	Antinociceptive effects, antagonist of 5-HT1A receptors
El-Massry et al. (2008)	53% 1,8-Cineol, 12% camphor, 10% α-pinene, 8% borneol	Free radical scavenging activity and lipoxygenase inhibition

composition with beneficial properties that meet society's growing demand for natural products. In fact, among its many uses, rosemary oil is used nowadays as a food flavoring agent, and it is well-known medicinally for its powerful antimutagenic, antibacterial, and chemopreventive properties (Horvàthovà et al., 2010).

The latest research related to the use of rosemary essential oil for medical purposes has mainly been focused on its antibacterial (Al-Mariri and Safi, 2013), antifungal (Carvalhinho et al., 2012), insecticidal (Zoubiri and Baaliouamer, 2011), anticancer (Mothana et al., 2011), antispasmolytic (Ventura-Martínez et al., 2011), anti-inflammatory, and antinociceptive

effects (Takaki et al., 2008), as well as its antioxidant properties (Menghini et al., 2010). All these biological activities make rosemary essential oil very interesting to the medical industry, both as a source of active components and, increasingly, for its potential future use in drug formulations.

Applications of *R. officinalis* in aromatherapy have also been described in several publications. Promising results have been reported regarding the improvement of cognitive function in both healthy adults and patients with Alzheimer's disease (Sayorwan et al., 2013; Pengelly et al., 2012).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Preservatives

Despite modern improvements in slaughter hygiene and food production techniques, food safety is an increasingly important public health issue (WHO, 2002). Crises in the human food sector have undermined consumer confidence in the capacity of the food industry and public authorities to ensure the safety of food. Furthermore, the agricultural and agrifood sectors have suffered important losses, which ultimately affect community taxpayers. For these reasons, the European Commission has made food safety one of its main priorities. Several outbreaks of food poisoning caused by food contaminated with pathogenic microorganisms (such as *Salmonella enteritidis*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*) have largely contributed to this lack of confidence.

In light of this problem, new solutions are required to ensure the safety of our food. Fueled by the growing technological innovation of recent years, the additives used as preservatives and/or stabilizers have played an important part in food safety, although the demand for natural food products has led to an attempt to include alternatives to chemical additives in the food production process.

The Lamiaceae family is one of the most important in terms of the production of essential oils with antimicrobial and antioxidant properties (Tsao and Zhou, 2007). Many articles can be found in the scientific literature regarding the antibacterial activity of rosemary essential oil against foodborne pathogens (Jordán et al., 2013b; Ivanovic et al., 2012). However, the antibacterial activity of a particular rosemary plant differs from that of any other, as a consequence of the different chemotype composition. For this reason, it is necessary to define the chemotype (or the relative abundance of its components) that best acts against the most common foodborne pathogens.

This is in agreement with the statement that the antibacterial activity of oil may be related to the chemical configuration of its components, the proportions in which they are present, and the interactions among them (Bajpai et al., 2012).

Antioxidant Activity

Consumer demand for healthy food products provides an opportunity to develop antioxidants as new functional foods. Along these lines, Ojeda-Sana et al. (2013) have published that rosemary essential oils rich in myrcene exhibit strong antioxidant activity.

Previously, Wang et al. (2008) had compared the antioxidant activities of *R. officinalis* L. essential oil and three of its main components (1,8-cineol, α-pinene, and β-pinene) using the 2,2-diphenylpicrylhydrazyl assay (DPPH·) and β-carotene bleaching assay. In the DPPH· assay, rosemary essential oil showed stronger antioxidant activity than any of the pure components. The strong antioxidant capacities exhibited by these oils suggest that rosemary essential oil could be an important source of natural antioxidants, and therefore a potential alternative to the synthetic preservatives used in the food industry.

Antimicrobial Effect

Public concerns about food safety have led to the search for potent natural antimicrobial components. Among others, rosemary essential oil is well known for its potent bactericidal activity. With this in mind, Gómez-Estaca et al. (2010) and other researchers have tested the antibacterial activity of rosemary essential oil against 18 genera of bacteria, most of which are common food pathogens and bacteria contributing to food spoilage (Table 3). Some authors have also reported a good inhibitory in vitro effect of rosemary essential oil against pathogenic microorganisms such as *E. coli*, *Bacillus cereus*, and *Staphylococcus aureus* (Burt, 2004).

Gram-positive bacteria are more sensitive to essential oils, as the hydrophilic cell wall structures of Gram-negative bacteria have been demonstrated to block the penetration of hydrophobic components through the cell membrane (Burt, 2004). The mechanisms by which some components present in essential oil exert their antibacterial activity are poorly understood. Ojeda-Sana et al. (2013) have reported a bactericidal effect associated with damage to the cell membrane caused by 1,8-cineol. These authors assessed changes in the membrane permeability of *E. coli* and *Enterococcus faecalis* after treatment with α-pinene or 1,8-cineol.

TABLE 3 Qualitative Antimicrobial Activity of Rosemary Essential Oil on Different Bacterial Strains (Gómez-Estaca et al., 2010)

Salmonella choleraesuis	+++
Lactobacillus acidophilus	++
Listeria innocua	++
Citrobacter freundii	+
Escherichia coli	++
Shigella sonnei	+++
Pseudomonas aeruginosa	–
Yersinia enterocolitica	+++
Brochothrix thermosphacta	+
Staphylococcus aureus	+++
Bacillus cereus	+++
Listeria monocytogenes	+
Clostridium perfringens	+++
Aeromonas hydrophila	+++
Shewanella putrefaciens	+
Pseudomonas fluorescens	–
Vibrio parahaemolyticus	++
Photobacterium phosphoreum	+++

+++: totally inhibited; ++: partially inhibited; +: slightly inhibited; -: no inhibited.

Regarding the effectiveness of the essential oil versus the individual components that define its chemotype, it has been shown that a synergistic effect may occur among the volatile components that enhance the oil antibacterial activity as compared to the pure components, mainly α-pinene and 1,8-cineol (Jiang et al., 2011). In addition to these major components, minor compounds may also make a significant contribution to the antimicrobial activity of the oil. Thus, the antimicrobial activity of rosemary essential oil should be attributed to a synergistic effect of its whole volatile fraction.

Brochothrix thermosphacta bacteria are the microflora prevalent in refrigerated meat stored in a modified atmosphere with high-oxygen content. Although these bacteria are sensitive to rosemary essential oil, when used as a component in a modified atmosphere, this oil does not produce the expected results. Indeed, rosemary essential oil not only is ineffective against *B. thermosphacta* at a minimum inhibitory concentration (MIC) of 2, but it also has a detrimental effect on the organoleptic qualities of the meat (Nowak et al., 2012). Therefore, the practical application of essential oils may be limited because of flavor considerations.

All the studies referred to above are related to in vitro microbiological activities, but the application of these essential oils for improving the zoo-technical livestock production deserves special mention. Accordingly, ruminant microbiologists and nutritionists have been exploring alternative methods for modifying the metabolism of ruminants in order to improve feed efficiency and animal productivity.

Antimycotoxigenic Characteristics

Rosemary essential oil could be safely used as a preservative in certain kinds of foods to protect them from toxigenic fungal infections (Rasooli et al., 2008).

Pitarokili et al. (2008) tested the antifungal activity of spontaneous *R. officinalis* essential oil from Greece against five phytopathogenic fungi: *Sclerotinia sclerotiorum*, *Phytophthora nicotianae*, *Sclerotium cepivorum*, *Fusarium oxysporum*

f. sp. *dianthi*, and *Fusarium proliferatum*. The most sensitive fungus was *Ph. nicotianae*, followed by *S. sclerotiorum*, *S. cepivorum*, *F. proliferatum*, and *F. oxysporum* f. sp. *dianthi*.

Growth Promoters

Public concern over the use of antibiotics in livestock production has increased in recent years because of their possible contribution to the emergence of antibiotic-resistant bacteria, and their transmission from livestock to humans. Essential oils contain secondary metabolites with antimicrobial properties, which make them potential alternatives to antibiotics in order to manipulate microbial activity in the rumen (Benchaar et al., 2008). Indeed, essential oils can modulate rumen fermentation by interacting with microbial cell membranes and inhibiting the growth of some gram-positive and gram-negative bacteria. In this respect, the addition of certain plant extracts to the rumen inhibits deamination and methanogenesis, resulting in lower ammonia N, methane, and acetate, and higher propionate and butyrate concentrations (Calsamiglia et al., 2007). Castillejos et al. (2008) noted that rosemary oil in vitro modified rumen fermentation at 500 mg/L of the culture fluid, increasing the proportion of propionate and decreasing that of acetate and the acetate to propionate ratio.

Mathlouthi et al. (2012) conducted a study to characterize the in vitro antimicrobial activities of three essential oils (oregano, rosemary, and a blend of essential oils) against pathogenic and nonpathogenic bacteria, and to evaluate their effects on broiler chicken performance. The results obtained show that the suppression of antibiotic growth promoters from the feed decreased the zootechnical performance of broiler chickens. The addition of rosemary, oregano, and a blend of essential oils counteracted these effects. These three essential oil preparations had the same efficiency in broiler chickens, regardless of their different antimicrobial activities.

Yesilbag et al. (2011) conducted a study to evaluate the effects of dietary supplementation with rosemary volatile oil (100, 150, or 200 mg/kg of plant oil) on performance and meat quality. Their conclusion was that dietary supplementation with rosemary and its volatile oil improved broiler meat quality. This improvement was greater as the dose increased in terms of physical–chemical properties and microbiology, while general sensory acceptance was significantly lower at higher doses. Moreover, growth performance was positively affected by rosemary volatile oil supplementation. Furthermore, Yesilbag et al. (2012) showed that a concentration of rosemary oil (140 mg/kg) in the diet of quail (*Coturnix coturnix Pharaoh*) caused a significant increase in live weight, live weight gain, and carcass yields during the growing and finishing periods. These authors concluded that rosemary oils could be a potential natural growth promoter for quail.

APPLICATIONS IN FOOD MATRICES

Although the use of plant herbs and essential oils in meat and fat products is a common industrial practice, the effects of these additives can be unpredictable, depending on the concentration of the substance and the characteristics of the food products. The decision to use a particular amount of plant extracts for the purpose of food preservation should be made carefully, considering the peculiar characteristic of each matrix.

Meat

Pork

In the search for new field applications of rosemary oil, few articles describe the use of rosemary essential oil as a feed ingredient or as an exogenous additive to the meat matrix. As a feed ingredient, in a preliminary study, Janz et al. (2007) assessed the performance of finisher pigs raised on diets containing 0.05% of rosemary essential oil with the purpose of determining the effect of these diets on pork quality. Meat quality attributes were found to be unchanged by the dietary treatment; these results indicate that a higher level of dietary supplementation may be required in order to trigger observable differences in pork characteristics.

With regard to exogenous applications, Estévez and Cava (2006) observed that the effect of rosemary essential oil on the oxidative stability of frankfurters depended on the level of essential oil added and the origin of the frankfurter (made from Iberian or non-Iberian pork). In the case of non-Iberian pork, 150 ppm of rosemary essential oil showed an antioxidant effect. However, at higher levels (300 and 600 ppm), no effects on lipid oxidation were detected; moreover, the oil significantly enhanced the oxidation of proteins and the release of iron from myoglobin. Contrary to this, in Iberian pigs, rosemary essential oil inhibited the development of lipid and protein oxidation in a concentration-dependent manner. In this case, higher oil concentrations increased the antioxidant stability of the meat. The presence of certain amounts of tocopherols in the frankfurters could have influenced the activity of the essential oil added, possibly leading

to antioxidant or prooxidant effects, albeit the different fatty acid composition and the oxidative status of the frankfurters might also have had an effect.

Lipid and protein oxidation in meat products is of principal concern for the preservation of meat quality during storage. Whereas the influence of lipid oxidation on quality traits such as odor and taste is well recognized, the impact of protein oxidation on meat quality requires further study. The oxidative damage of proteins leads to textural alterations, which affects the gelation, emulsification, viscosity, solubility, and rehydration properties of meat proteins. The use of natural preservatives to control oxidation in meat products is promising. Nieto et al. (2013) studied the effect of two levels (0.05% and 0.4%) of rosemary essential oil on the protein oxidation occurring in pork patties during storage. The oxidative stability of the meat proteins was evaluated as the loss of thiols and the formation of myosin cross-links. Protein thiols were lost during storage to yield myosin disulfide cross-links. Essential oils of rosemary were found to retard the loss of thiols otherwise resulting in myosin cross-links.

Beef

Studies conducted by Nowak et al. (2012) on the use of rosemary oil in beef preservation revealed the scarce incidence of the oil on the microbial quality of meat stored in a modified atmosphere (80% O_2/20% CO_2). Addition of the essential oil to the modified atmosphere at an MIC of 2 failed to significantly influence the microbial quality of meat. Furthermore, such a concentration had a highly detrimental effect on the meat organoleptic factors (Nowak et al., 2012).

Lamb

The use of rosemary oil has also been considered for enhancing the quality of lamb. Smeti et al. (2013) showed that a low dose of rosemary essential oils (0.06% in the diet) had no significant effect on retarding color loss, lipid oxidation, or organoleptic properties of Barbarine lamb at the time of consumption. These authors concluded that more research is needed on adequate dosages and methods of incorporating rosemary extract before any effects can be reliably identified.

In agreement with the previously mentioned authors, Vasta et al. (2013) observed that supplementing lamb feed with rosemary essential oils did not produce any detrimental effects on either intramuscular fatty acid composition or the volatile compound profile of the meat. The sesquiterpenes β-caryophyllene and copaene were detected only in the meat of the supplemented lambs and were absent from the meat of the control animals; longifolene was considered to be a marker of rosemary supplementation.

Poultry

Combined natural antimicrobial treatments (ethylenediaminetetraacetate (EDTA), lysozyme and rosemary oil) have been examined in semi-cooked coated chicken meat stored in vacuum packages at 4 °C by Ntzimani et al. (2010). Based on both microbiological (total viable count) and sensory (taste attribute) analyses, these treatments extended the shelf-life by 7–8 days, as compared to the control samples. The presence of rosemary oil (0.2% v/w) in cooked samples produced a distinct yet acceptable (and even pleasant) odor and taste, which was well received by the panelists.

New trends in the food industry include the production of healthier food, enriched with highly polyunsaturated fatty acids. This could be to the detriment of lipid oxidation. The increased lipid oxidation resulting from the incorporation of n-3 polyunsaturated fatty acids (PUFAs) in the feed was blocked by the addition of oregano and rosemary essential oils to the diet (Basmacioglu et al., 2004). A combination of these essential oils had a greater effect in preventing the lipid oxidation of broiler meat enriched with n-3 PUFAs than when they were used individually or when alpha-tocopheryl acetate was used alone. The combination of these essential oils at 150 mg/kg proved to be as effective as alpha-tocopheryl in retaining the sensory qualities of breast meat after 15 days in storage, and it was more effective than alpha-tocopherol when administered at doses of 300 mg/kg. Taste, odor, and overall acceptance in rosemary group had scores of 5.17, 5.07, and 5.01, respectively, on a scale of 1–6. The scores for taste, odor, and overall acceptance when alpha-tocopheryl was administered were, respectively, 5.09, 4.98, and 5.19 versus 1.89, 1.27, and 2.37 in control group. Individually administered essential oils had bad scores, were worse at high doses (300 mg/kg), and were between 2 and 3.31. These authors concluded that there is a possible synergistic effect between oregano and rosemary oils in reducing the rate of lipid oxidation during storage.

The use of rosemary oil has also been considered in the processing of turkey meat. Vasilatos and Savvaidis (2013) demonstrated that the use of rosemary essential oil under vacuum packaging conditions extended the shelf-life of fresh turkey meat stored in refrigeration (2 °C) by 7–8 days. While meat under control treatment had scores below the acceptability limit (about 5 on a 0–9 scale) on day 12, meat under rosemary treatment had good score in taste (about 8) and acceptable scores (about 6) for odor on day 18.

Fish

Peiretti et al. (2012) investigated the effects of three concentrations (0.2%, 1%, and 3%) of rosemary oil on minced rainbow trout muscle after different periods of storage at 4 °C. The results indicate that application of rosemary oil to minced muscle may be advantageous, as it improved the pH, oxidative stability of the lipids, and fatty acid profile, which in turn significantly prolonged the fish meat shelf-life. Treatment with rosemary oil had a positive effect, leading to low biogenic amine content, especially in terms of putrescine, cadaverine, tyramine, and histamine. Moreover, the presence of the terpenoid fraction (α-pinene, eucalyptol, camphor, β-caryophyllene) of rosemary oil in fish flesh improved the quality of this ready-to-cook fish.

Di Turi et al. (2009) administered rosemary oil (200 ppm) to seabass for 200 days. The limits to the overall sensory acceptability for the consumption of farmed seabass stored on ice were reached on day 13 for the control diet and day 14 for the experimental diet. Therefore, dietary supplementation of rosemary oil had a positive effect on the flesh quality of farmed seabass stored on ice, extending by one day the fish acceptability time.

Makri (2013) noted that rosemary essential oil at a concentration of 500 mg/kg inhibited oxidation (by inhibiting the formation of conjugated diene and triene hydroperoxides and thiobarbituric reactive substances) in minced gilthead sea bream muscle up to the third month of frozen storage at −22 °C.

Processed Food

Olmedo et al. (2013) noted that the addition of rosemary essential oil (0.2 g per 100 g) improved the oxidative and fermentative stability of flavored cheese prepared with a cream cheese base, preventing lipid oxidation and the development of rancid and fermented flavors. On a 150-mm linear scale, rancid and fermented flavors decreased from 15.13 to 13.81 and from 17.09 to 15.86, respectively. Saltiness and bitterness taste increased by one point and sourness by two points. As a consequence, this essential oil prolonged the shelf-life of this product.

The effect of the addition of rosemary essential oils on the generation of volatile compounds in liver pâtés from Iberian and non-Iberian pigs was studied by Estévez et al. (2004). The effect of essential oil was different depending on whether the pâté was made from Iberian or non-Iberian pork, inhibiting lipid oxidation in the former and enhancing oxidative instability in the latter. Using solid-phase microextraction (SPME), 41 volatile terpenes were isolated from pâtés with added rosemary essential oil supplements that might contribute to the aroma characteristics of liver pâtés.

Technological Applications

The main compounds of the essential oils responsible for the flavor and functional properties are volatile and chemically unstable in the presence of oxygen, moisture, and heat. The stability of essential oils can be enhanced through microencapsulation by spray drying, the most commonly used encapsulation technique in the food industry. The effects of partial or total replacement of gum arabic by modified starch, maltodextrin, and inulin on the characteristics of rosemary essential oil microencapsulated by spray drying were evaluated by de Barros Fernandes et al. (2014).

Previously, Pandit and Shelef (1994) had assayed the effectiveness of encapsulated rosemary essential oil against *Listeria monocytogenes* in pork liver sausage. These authors did not reach any conclusive results. While they found that encapsulated rosemary oil was much more effective than standard rosemary essential oil, it was not clear whether the effect was due to the encapsulation or to the greater percentage of oil used.

Bionanocomposite film is an excellent vehicle for incorporating a wide variety of additives. The compatibility of rosemary essential oil with chitosan/montmorillonite nanocomposite has been confirmed in order to produce an active bionanocomposite for food packaging. Chitosan nanocomposites containing rosemary essential oil exhibit antimicrobial properties and greater phenol content (Abdollahi et al., 2012), which makes them very interesting for food preservation.

SUMMARY

- Rosemary essential oil is a commonly used potent natural food additive, listed by the EFSA and FDA.
- The *Rosmarinus* genus is primarily found in the western Mediterranean region.
- Rosemary, as a spontaneous shrub, exhibits great intraspecific chemical variability.
- Rosemary has been used since ancient times for medicinal, culinary, and ornamental purposes.
- The biological activities described for rosemary essential oil vary according to the chemotype of the plant studied.

- In food science, rosemary is one of the most important additives in terms of essential oil applications, due to its antimicrobial, antimycotic, and antioxidant properties.
- The potent antibacterial activity of rosemary essential oil against numerous foodborne pathogens has been related to the synergistic and/or cumulative effect of its volatile components.
- The administration (both exogenous and endogenous) of essential oil as a food preservative has been successfully tested with different types of meat (pork, beef, lamb, poultry), fish, processed foods, and even as a part of the biopolymer films for food packaging.

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Chapter 78

Rose Pepper (*Schinus molle* L.) Oils

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INTRODUCTION

The investigation of natural products in agriculture is a response to the strong negative environmental impact caused by the massive use of synthetic pesticides (pollution of ecosystems), as well as their effects on the human population (proliferation of diseases, including cancer). The situation has led to greater awareness and, thus, changes in management strategies in primary production. Consequently, there has been an increased interest in alternatives to traditional chemical products, which can be considered as environmentally friendly and safe for humans. In turn, different sources of bioactive compounds from natural resources have been investigated, including plant-derived volatile compounds. These compounds usually have bioactivity against harmful organisms (e.g., plant pathogens), are of low toxicity to mammals, have no neurotoxic effects, and have low persistence in the environment, while also being biodegradable (Céspedes et al., 2006).

Moreover, improvement in the production of foods originating in the bovine and apiculture industries has led to the extensive use of synthetic antiparasitic drugs, such as those destined to eradicate mites and insects. The long-term use of these substances brings about resistance phenomena (Ducornez et al., 2005; Romeo et al., 2010), environmental harm, and consequently, a negative impact on human health (Gregorc, 2012).

Aromatic plants and their essential oils have been used for centuries to protect stored products or to repel pests in human dwellings. However, unlike other plant products, plant essential oils have a long history of use by mankind beyond the control of pests, particularly as fragrances, flavors, seasonings, or spices as well as the medicinal uses. However, the use of essential oils as commercial insecticides has only been improved in recent years. Varroa (*Varroa destructor*) in apiculture, the horn fly (*Haematobia irritans*), and ticks (*Rhipicephalus microplus*) in cattle production are examples of plagues whose control has not been satisfactorily resolved (Milani, 1995).

Thus, it is necessary to carry out research using novel molecules acting by different mechanisms and that are environmentally harmless, such as those from natural origin that can be a source of antiparasitic agents or as lead compounds to synthesize active molecules. Both the essential oils and their fractions offer an interesting alternative to synthetic pesticides (Imdorf et al., 1999; Ardeshir et al., 2002; Torres et al., 2012).

This chapter provides evidence on the effects of the aguaribay (*Schinus molle* L.) essential oil on the parasitic species above mentioned. *Schinus molle* is a native species from the Latin American flora. The application of this natural product may control the transmission of parasitic diseases and endemicity (Lapissonde et al., 2012).

Bovine production and apiculture in the Santa Fe Province, an area that in the most important livestock region in Argentina, is commercially important. In both cases, the health problems produced by ectoparasites have a highly negative economic impact. According to a livestock survey carried out in 2012 in the Santa Fe Province, there are around 6,667,000 heads of bovines that are affected by horn fly and ticks. This situation has provoked economic losses, which in 2011 reached US$380 million. This problem was mainly due to the inefficient administration of antiparasitic drugs (Pesoa, 2012).

Ticks are considered to be one of the external parasites that cause great economic losses in world livestock production. The mite-borne diseases, babesiosis and anaplasmosis, cause damage to the agricultural economy—not only by the decrease in the production of meat, milk, and leather, but also by bovine weakness, which often involves death. In addition, there are also expenses produced by treatments with synthetic products, which do not permanently solve the problem and may favor the emergence of resistant strains and environmental contamination.

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In Australia, economic losses caused by ticks have been estimated at more than US$100 million per year, and $1 billion dollars in Central and South America. Furthermore, it has been shown that the poisoning of animals subjected to successive baths with chemical acaricides, and the physical stress from bath treatments, predisposes cattle to other diseases (Martins, 2006).

Direct damage produced by ticks on cattle and, consequently, economic losses due to blood bites, can be classified as follows:

- Each *R. microplus* female is responsible for a loss of 8.9 mL of milk/day and 1 g of body weight/day.
- Damages to animal leather having many bites, which consequently becomes deteriorated, diminishing its value by up to 50% (Manzano-Román et al., 2012).

In the apiculture sector, according to a survey conducted by the Apiculture Chain from the Santa Fe Production Administration, around 395,000 apiaries have been found to be infested with *Varroa*. In addition, there has been an increase in the degree of environment harm and health problems in the population. One hundred and sixty individuals have become intoxicated with pesticides in Santa Fe in 2008. *Varroa destructor* is a parasitic mite on honey bees (*Apis mellifera*) that causes major economic losses in the beekeeping world. A high degree of infestation usually leads to weakening of the hive, and sometimes even can destroy it.

Currently, *V. destructor* is controlled by products such as synthetic pyrethroids and phosphorated derivatives, which must be applied at a time that the hive is not producing honey (autumn and winter). The repeated use of synthetic parasiticides has generated populations of resistant parasites. In addition, the use of higher doses than those recommended result in the contamination of bee products (honey and wax).

Different experiments have been conducted in apiaries using *S. molle* essential oil. The acaricidal effect on the varroa mite using aguaribay (*S. molle* L.) essential oil has been evaluated through applications in experimental hives for 15 years without finding parasite resistance. Beekeepers working with these hives have found that honey production from hives treated in spring with the essential oil of *S. molle* doubled their production when compared to treatment with traditional acaricides (Guala et al., 2014). In addition, the honey yield normally produced by a beehive (15–20 kg) increased to 35–40 kg with continuous treatment with *S. molle* essential oil, but with the same cost of using synthetic products. In addition, the essential oil of *S. molle* L. as an antibacterial against *Staphylococcus aureus* and *Escherichia coli* has proven to be promising (Deveci et al., 2012; Mendonça Rocha et al., 2012).

This essential oil has also been demonstrated to be useful as a substitute for synthetic antioxidants in creams and canned foods, mainly due to its optimum solubility and because it does not change the organoleptic characteristics of foods (Guala et al., 2009). Only a slight change in color has been observed in bases whose basic color was white. Toxicity studies performed in rats have demonstrated the absence of acute effects in the animals (Centro de Medicina Comparada, 2012).

These results not only indicate the safe use of this essential oil but, in the case of Argentina, they might constitute novel options for the livestock industry, with an aim toward full-scale production of this essential oil. *Schinus molle* cultivation will allow regional economic development, leading to profitable enterprises in garden centers to generate seedlings, their subsequent implantation in the field, and industrial processing by steam distillation.

BOTANICAL ASPECTS

There are over 30 South American *Schinus* species (Anacardiaceae). They are known as *pimentero*, *molle*, *pimienta del diablo*, *terebinta, aguaribay*, *molle*, *falso pimentero*, *baie rose*, *rose pepper*, and *pepper tree* depending on the region. From a taxonomic point of view, there are two closely related species in America: *S. molle* L. and *Schinus areira* L. (synonymous: *S. molle* L. var. *areira* (L) DC). These species are highly referenced in the literature (Viturro et al., 2010).

Linneo differentiated these two species, while De Candolle considered *S. areira* as a variety of *S. molle*, naming it *S. molle* L. var. *areira*. Upon studying the Argentine Anacardiaceae, Argentine taxonomist Juan de Dios Muñoz considered them as two different species (de Dios Muñoz, 1990; Zuloaga and Morrone, 1999). *Schinus molle* distribution comprises Brazil, Paraguay, Uruguay, and the northeast region of Argentina, whereas the cultivation of *S. areira* has spread to the major subtropical regions of the globe. *Schinus areira* leaflets are alternate, normally shorter (20–25 mm long) and thinner (3–8.5 mm thick) than those of *S. molle*. The number of leaflets in *S. areira* ranges from 14 to 35, seldom 51; they are generally alternate, linearly lanceolate, slightly crescent-shaped and apiculate, nonsegmented, and unevenly dentate. *Schinus molle* has from 5 to 51 leaflets, generally opposite, lanceolate, unevenly serrated, and acuminate (de Dios Muñoz, 1990). A third close species, *Schinus terebinthifolius* Raddi (syn. *S. aroeira* Velloso) can be easily be differentiated from *S. areira* and *S. molle* by a macroscopic observation, due to the presence of pubescent leaflets (Viturro et al., 2010).

Aguaribay (*S. molle* L.) is a large tree (8–10 m high), with perennial foliage that loses one third of its leaves per year. The top of this tree is made up of flexible hanging branches that are easily cracked by the wind; its bark is a light brown

color and has a wrinkled and rough surface. This tree has evergreen composite alternate and imparipinnate leaves that are 10–30 cm long, with five to nine pairs of lanceolate, acute, and serrate leaflets. It has yellowish flowers arranged in wide terminal axillary panicles. The fruit is a greenish drupe that, when mature, becomes pink or light brown, depending on the area where it grows. The drupe has a diameter of 0.4–0.6 cm with a globular ectocarp, a pulpy and resinous mesocarp, and a bony endocarp, with compressed seeds and flat cotyledons (Barkley, 1957; Cabrera, 1976; Dimitri, 1988). The essential oil is contained in the flowers, fruits, and leaves; it has a characteristic aromatic, irritating odor. In certain periods of the year, mainly during the pre-blooming period, the stem and branches produce a brownish resin that has high adhesive properties.

USAGE AND APPLICATIONS

Among the different traditional uses of *S. molle* L., it has purgative, diuretic, disinfectant, and tranquilizer properties, depending on the parts employed. It can also be used as mouthwash. Its bark can be used to prepare ointments for dermatological use. Infusions prepared with the leaves have been widely used in popular Peruvian medicine as a blood pressure-lowering drug and against veterinary infections. In Mexico, an emulsion of its gum is used to heal ocular ailments and bronchitis. In Argentina, the leaves and its resin are employed in popular medicine as emmenagogue and to treat bronchitis, respectively (Viturro et al., 2010). Other activities have also been reported, such as a repellent and insecticide against *Triatoma infestans*, vector of Chagas disease (Ferrero et al., 2006), antimicrobial (Dikshit et al., 1986; Atti dos Santos, 2009, 2010), pediculicide (Gutiérrez et al., 2009), and allelopathic (Zahed et al., 2010) properties.

USAGE AND APPLICATIONS IN FOOD SCIENCE

The essential oil of *S. molle* L. is being used to control the proliferation of ectoparasites in livestock and apiculture. It also has antimicrobial and antioxidant properties when used in foods. Its parasiticide properties ensure the absence of contamination of meat and milk in livestock production and the safety of honey, wax, and propolis in apiculture. It should be noted that contamination of these products may occur when synthetic products, which are difficult to eliminate, are applied in excess. The *S. molle*–derived products are applied externally and their effects are elicited upon contact of the parasite with the oil. These products do not leave any contaminating residue and are safe to handle.

This essential oil also is a promising antibacterial agent because it has proven to be an inhibitor of the growth of *S. aureus* and *E. coli*. As an antioxidant, the tests performed so far are promising. The oil can be used in canned products and creams as a surrogate of synthetic antioxidants due to its excellent solubility. In addition, the product's organoleptic properties are not affected by the oil.

The proposed uses are based on the assays presented in the following section. In all cases, the essential oil of *S. molle* L. has the chemical composition presented in Table 1.

In Vivo and In Vitro Assays Performed on Ticks (*Rhipicephalus microplus*)

This section presents assays that were used to compare the efficiency of a 5% emulsion in water of the essential oil to kill ticks upon successive applications on bovines (pour-on). The emulsion of the essential oil and water was kept stable by constant agitation upon its return to the storing tank. However, several emulsifying agents were tested to assess their use and safety. The *S. molle* essential oil tested was obtained from aired ground fruits, leaves, and small stems harvested between March and April. In vivo field studies were performed as follows: 20 Brangus heifers (female bovines that have never given birth, normally 3 years old or older) weighing 160–180 kg each were used. The preliminary observation allowed estimating the presence of 12–15 adult ticks in the hind quarters and groin of the animals. Four groups (A–D) of five animals each were used. They were placed in separate corrals and given the same food. A 5% (w/w) emulsion in water of the essential oil of *S. molle* was prepared. The oil was applied on a daily basis during different periods of time depending on the group. About 50 mL were aspersed on the ticks of each animal, according to the following schedule:

- Group A: only one application at the beginning of the experiment (day 0)
- Group B: two applications on days 0 and 4
- Group C: applications were performed on days 0, 4, and 8
- Group D: animals remained as untreated controls

The applications were done by locking each animal in a pen, with counting done visually. A tick was considered dead when it was not found on the animal. Two ticks were taken at the beginning of the experiment from each animal to perform a subsequent in vitro test. Observations were performed in each animal, with the number of ticks counted and the differences

TABLE 1 Chemical Composition of *Schinus molle* L. Essential Oil

Compound	(%)
Alpha-thujene	0.7
Alpha-pinene	3.2
Beta-pinene	4.0
Sabinene	41.9
Myrcene	2.7
Alpha-phellandrene	0.1
Alpha-terpinene	0.9
p-Cymene	1.0
Limonene	2.0
Beta-phellandrene	0.2
Gamma-terpinene	1.8
Sabinene hydrate-*cis*	0.3
Terpinolene	0.4
trans-Sabinene hydrate-	0.2
cis-*p*-Menth-2-en-1-ol	0.2
trans-*p*-Menth-2-en-1-ol	0.1
Terpinen-4-ol	7.1
Alpha-copaene	0.4
Beta-elemene	1.6
Beta-caryophyllene	2.8
Aromadendrene	0.2
Alpha-humulene	0.4
Allo-aromadendrene	0.9
Germacrene D	4.2
Beta-selinene	0.5
Bicyclogermacrene	4.5
Gamma-cadinene	2.2
Delta-cadinene	1.8
Spathulenol	2.2
Caryophyllene oxide	0.4
Alpha-muurolol	2.1
Alpha-cadinol	1.3
Minor compounds (<0.05%)	6.7

Data are the percentage composition of the essential oil of *Schinus molle* used in the experiments carried out in apiaries and ticks. Compounds are listed in order of elution in the nonpolar column (unpublished data).

calculated. Counts were performed on days 1, 4, 8, and 12. Results were expressed as the number of total dead ticks. The mortality percentage was 24.6% in group A, 43.3% in group B, 35.3% in group C, and 6.3% in group D.

In vitro assays were performed to compare the efficiency of the emulsion over time. The experiment was as follows: four groups ($n = 10$ each) were employed. Ticks were placed into Petri dishes. Groups 1, 2, and 3 were treated with 20 mL of the 5% emulsion and group 4 was left untreated. The number of dead ticks was recorded after 24, 36, and 48 h. Results were expressed as the total number of deaths obtained from the beginning of the treatment. Mortality percentages were 100% in group 1 after 36 h, 80% in group 2 after 48 h, 100% in group 3 after 36 h, and 10% in group 4 (control) after 48 h.

The death efficiency percentage of a 10% emulsion of the *S. molle* essential oil was compared with a 0.1% solution of cipermethrin. The experiments were performed on 12 Braford cows weighing 260–300 kg each. The preliminary observation allowed estimation of an average of 15–20 ticks in different evolutionary stages, adults and teleogins, situated on the hindquarters and groins of the animals. Cows were divided into two groups (group A and group B) and kept in separate corrals. Animals belonging to both groups were equally fed. A 10% (w/w) emulsion in water of the essential oil was prepared, as well as a 0.1% v/v cipermethrin solution. A 10% concentration was employed to ensure an effective parasiticide effect.

Both preparations were applied once by aspersing about 50–60 mL on each animal on adult ticks and teleogins (group A was treated with the emulsion and group B was treated with cipermethrin). The administration of the products and tick counting was done by locking each animal in a pen. A visual observation was carried out and ticks were considered dead when they were not found on the animal. Observations were performed over a period of 12 days. Results were expressed as the total number of dead ticks recorded from the beginning of the experiment. Mortality percentages were 36% in group A and 47% in group B, both recorded after 10 days. A 10% error was estimated due to the tick's migration on the animal.

The product was used at a concentration of 5% since previous experiments had demonstrated that concentrations ranging from 3% to 8% showed parasiticide effects. Also, the mortality percentage obtained was considerable when applied sequentially every 7–8 days. The 10% emulsion had a rapid knockdown effect, which tends to decrease over time. The 0.1% cipermethrin solution had a more rapid and higher knockdown effect than the essential oil. However, this effect wears out over time. To obtain a longer permanence of the essential oil on the animal, different formulations should be tested in the future, such as microencapsulation, inclusion in chitosans, and multiple emulsions.

In vitro experiments corroborated the results obtained with in vivo assays. A 10% error was estimated considering migratory phenomena and/or changes in the tick's lifecycle that might occur on the animal. The application of this product must be done by aspersion with the animals kept in a pen. This methodology allowed recovery of 40% of the product that is spilled on the floor due to a "dripping effect"; thus, the product is collected in a basin so that the essential oil can be recovered with the initial purity degree and can be used in subsequent applications. The profit obtained with this procedure is highly dependent on the influence that the environmental policies and regulations of each country may have. However, the methodology constitutes an attractive alternative, considering the negative impact that synthetic products have on the biomass.

In Vivo Experiments Carried Out in Apiaries with the Oily Solution of the Essential Oil of *Shinus molle* Against Varroa (*Varroa destructor*)

The aim of these studies was to assess the mortality efficiency of a 2% oily solution of the essential oil of *S. molle* on *Varroa*. The essential oil was prepared at a concentration of 2% (w/w) in edible oil and the applications were performed directly in apiaries inside Langstroth type hives.

The application was done with different materials soaked in the oily solution: frames grooved in the upper border, gray cardboard strips, and polyurethane small pillows. As control, hives with polyurethane small pillows soaked in edible oil were used, as well as hives where no material was incorporated.

Treatments were performed during November and January (summer season in the Southern hemisphere) when no synthetic products can be applied due to the toxicity risk of the residues remaining in the honey, propolis, wax, or royal jelly. Five hives were employed for each treatment. The infestation percentage with varroas was determined, considering that a percentage infestation of 8–10% or greater represents a life risk to the hive. Counts were performed periodically, as shown in Tables 2–3, with day 0 considered to be the day when the product was applied for the first time.

The experiments reported here demonstrate that it is possible to employ ecological products in the treatment against ectoparasites of bovines and bees. Furthermore, the absence of restrictions for the consumption of *S. molle*-derived products leads to greater safety in those agroindustries. A clear example is that of the treatment of residual waters employed in the sanitary baths to control ticks, where cipermethrin must be eliminated. The latter procedure has a cost of US$28 per 1000 L of water to be purified; in the case of the essential oil, waters can be readily disposed of in the effluent collectors.

TABLE 2 Varroa Infestation Control on Hives Using *S. molle* Essential Oil

Test 1								
Treatments	**Infestation Percentage**							
Days	0	9	15	25	49	68	89	105
Frames grooved	0.49	0.86	0.41	0.72	0.96	0.42	2.08	6.97
Cardboard strips	0.88	0.76	0.97	0.35	1.73	1.02	1.75	4.16
Pads	0.00	0.39	0.00	0.96	1.08	1.48	1.79	3.52
Edible oil pads	0.57	0.00	1.70	1.70	1.12	2.24	1.53	4.04
Control	0.00	0.38	0.33	0.92	0.77	2.15	1.02	4.45
Test 2								
Treatments	**Infestation Percentage**							
Days	0	14	28	42	56	70	81	
Frames grooved	1.38	0.88	0.47	0.82	0.99	0.92	1.93	
Cardboard strips	0.88	0.76	0.81	0.81	1.43	1.07	2.55	
Pads	1.20	0.44	0.46	0.46	0.72	0.48	1.34	
Edible oil pads	1.37	1.90	1.96	1.96	1.15	3.32	2.42	
Control	0.42	0.38	1.85	1.88	0.47	2.55	1.12	

TABLE 3 Comparative Test Using an Oily Solution of *Schinus molle* L. Essential Oil with Two Synthetic Commercial Products

Product	**Infestation Percentage**							
Days	0	9	15	25	49	68	89	105
Essential oil solution	0.49	0.86	0.41	0.72	0.96	0.42	2.08	5.17
Amitraz[a]	0.88	0.76	0.97	0.35	1.73	1.02	1.75	4.16
Oxalic acid	0.00	0.39	0.00	0.96	1.08	1.48	1.79	3.52

[a]*N,N′-[(Methylimino)dimethylidyne]di-2,4-xylidine.*

SUMMARY POINTS

- The essential oil of *S. molle* L. is being employed as a co-adjuvant in several food derivates both directly and indirectly: as an antiparasitic substance in cattle or as an antimicrobial and antioxidant agent.
- The activity against ticks (*Rhipicephalus microplus*) using a 10% (w/w) emulsion of *S. molle* essential oil in edible oil was compared with a 0.1% (w/w) cipermethrin solution. Mortality percentages after 10 days were 36% and 47%, respectively.
- To obtain a longer permanence of the essential oil on the animals, different formulations should be tested (e.g., microencapsulation, inclusion in chitosans, preparation of multiple emulsions).

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Chapter 79

Rose-Scented Geranium (*Pelargonium* sp.) Oils

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List of Abbreviations

FDA Food and Drug Administration
GRAS Generally Recognized As Safe
FEMA Flavor and Extracts Manufactures Association
CIMAP Central Institute of Medicinal and Aromatic Plants
GC Gas chromatography
GC–MS GC-Mass spectrometry
C/G Citronellol/Geraniol
FAO Food and Agriculture Organization

INTRODUCTION

Rose-scented geraniums (*Pelargonium* species) belong to the family Geraniaceae. They are high-value, perennial aromatic shrubs, originating from South Africa, Reunion Madagascar, Egypt, and Morocco. These species are commercially cultivated in Reunion, China, Egypt, Morocco, and India for the production of "geranium oil" (Rajeswara Rao, 2013a). The commercial geranium oil is obtained by steam-distillation of fresh aerial parts of rose-scented *Pelargonium* species and their cultivars and hybrids (Lis-Balchin, 1990). Geranium oil is one of the most widely used fragrance materials and is an indispensable component of the most rose-scented perfumes and soaps. The natural geranium oil is itself a perfume and blends well with other perfumes. It possesses green herbal, fresh, and earthy characteristics and is often used in masculine fragrances. It is nontoxic, a nonirritant, and possesses energy-boosting and soothing properties (Narayana et al., 1986).

Natural geranium oil is a very complex mixture of monoterpenes, sesquiterpenes, and some other low-molecular-weight aromatic compounds (Lis-Balchin, 2002). The chemical composition of geranium oil varies due to intrinsic and extrinsic factors (Rajeswara Rao, 2013a; Lis-Balchin, 2002; Verma et al., 2013). So far, more than 240 compounds have been identified in geranium oils of diverse origins (Rajeswara Rao, 2013a). Nevertheless, the major components of geranium oil (about 60–70%) are three monoterpene alcohols: citronellol, geraniol, and linalool. Some marker constituents—such as isomenthone, menthone, nerol, *cis*-rose oxide, *trans*-rose oxide, α-terpineol, α-pinene, myrcene, and β-phellandrene—can be found in geranium oils from all localities (Lawrence, 1984). The structures of the marker constituents of geranium oil are shown in Figure 1.

The major types of geranium oil available on a commercial basis include Bourbon, Chinese, Algerian, Egyptian, and Moroccan types (Table 1). The rose-scented geranium is a highly adaptable crop and can be cultivated in varied climates, such as tropical, subtropical, temperate, and Mediterranean climates, at altitudes ranging from 120 to >2400 m (Rajeswara Rao, 2013a). In India, mainly two types of geranium have been identified: Algerian/Tunisian and Bourbon/Reunion types. The former cultivar is inferior in comparison to the latter in terms of commercial essential oil quality (Verma et al., 2013). A typical gas chromatogram of rose-scented geranium (*Pelargonium* species) essential oil produced in India is shown in Figure 2.

BOTANICAL ASPECTS

Previously, *Pelargonium* and *Geranium* were placed together under the same genus, *Geranium* of the family Geraniaceae. However, later they were considered to be two separate genera (Sweet, 1820). Nevertheless, this ambiguity established "geranium" as a vernacular name for the *Pelargonium* species, especially for the zonal cultivars (Miller, 2002). Thus, in trade, the name "geranium" is still misapplied for the essential oils derived from *Pelargonium* species.

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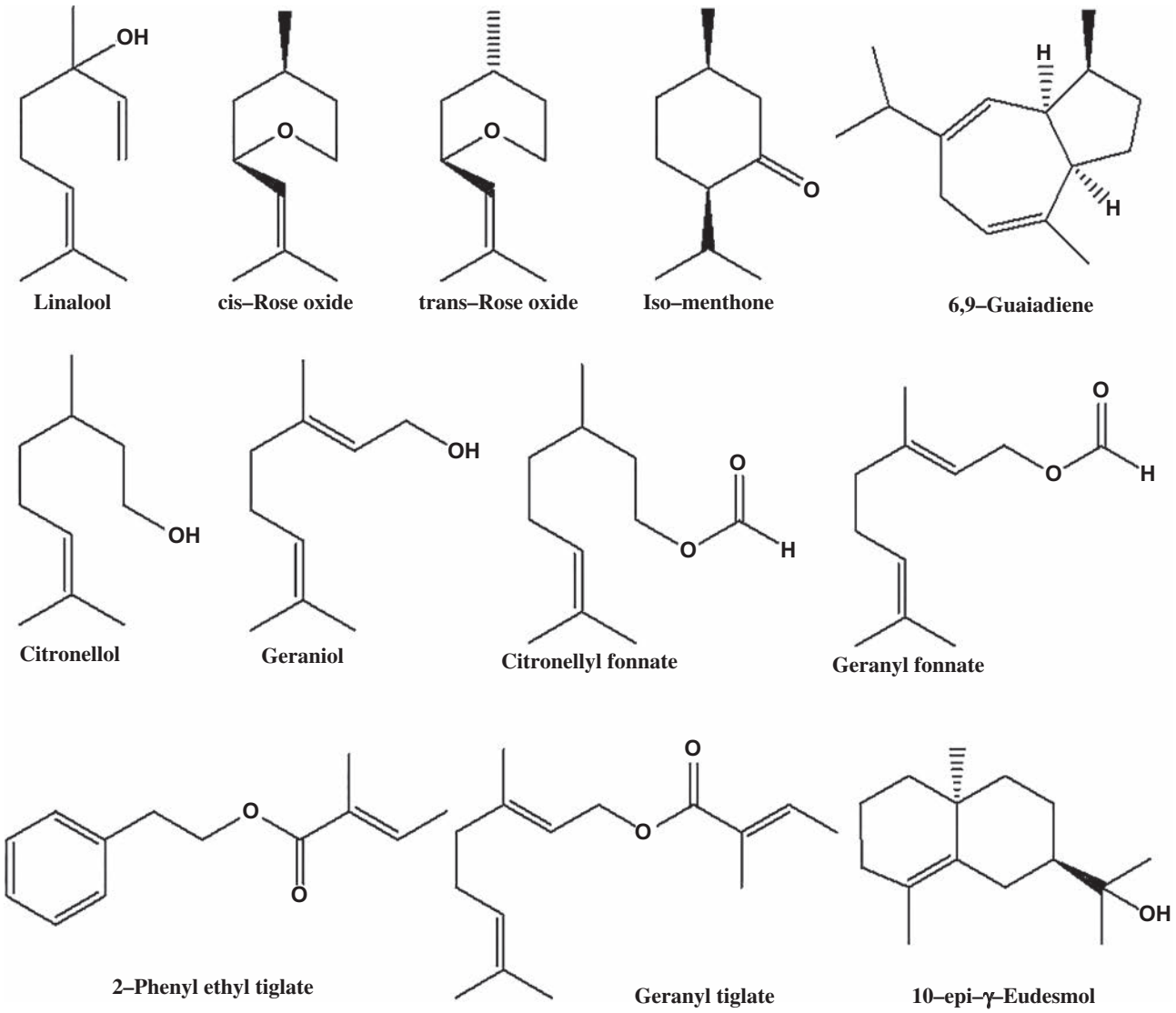

FIGURE 1 Structures of the major constituents of geranium oil.

TABLE 1 Major Constituents of Rose-Scented Geranium Oils of Different Origins

	Lawrence (1984)						Verma et al. (2013)
Compound (%)	Algeria	Egypt	Morocco	Bourbon	China	USA	India
Cis-rose oxide	0.69	1.04	1.31	0.52	2.25	1.09	0.5–1.6
Trans-rose oxide	0.31	0.40	0.56	0.28	1.04	0.74	0.0–1.0
Isomenthone	5.33	6.05	5.63	8.12	4.51	6.30	4.5–6.6
Linalool	6.25	9.90	5.62	13.79	3.79	1.89	2.9–9.2
Citronellyl formate	9.40	7.43	7.64	12.39	17.45	24.40	4.4–9.2
Geranyl formate	6.49	3.89	4.33	5.92	2.20	4.97	3.8–6.2
Citronellol	27.87	32.10	18.59	23.24	44.39	29.23	15.2–31.3
Geraniol	24.97	19.70	18.59	20.67	6.40	7.41	14.1–34.6
10-*epi*-γ-eudesmol	5.41	4.62	5.20	1.04	2.03	2.77	4.7–6.7
Geranyl tiglate	1.56	1.44	1.54	0.45	1.64	1.40	2.0–2.5

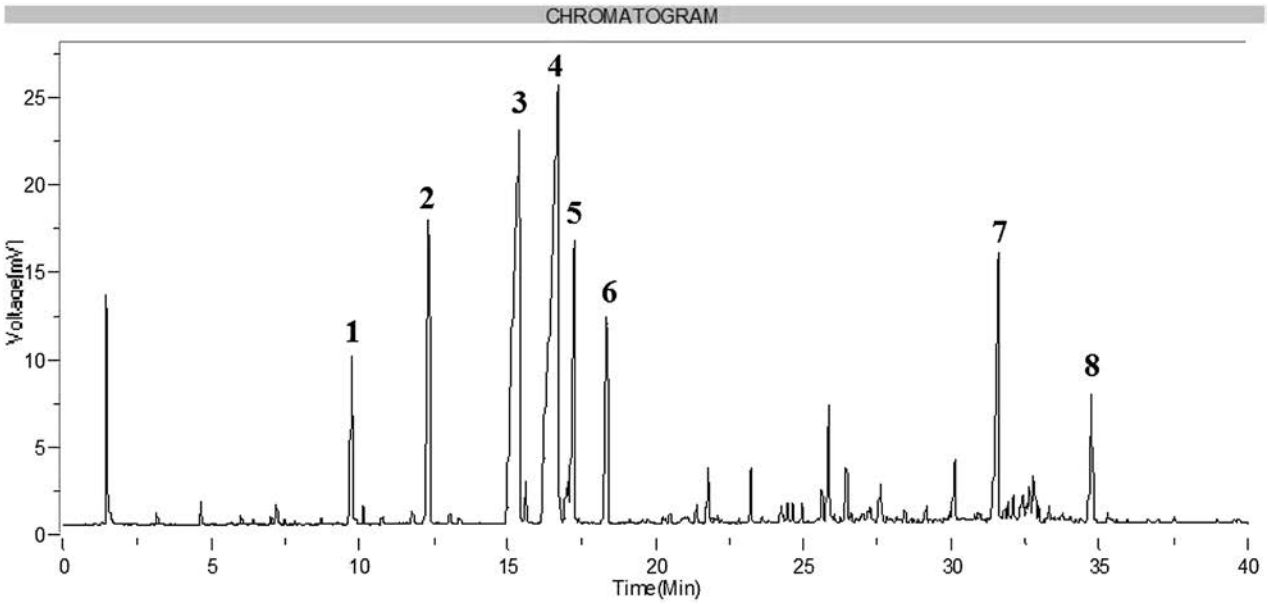

FIGURE 2 A typical gas chromatographic profile of rose-scented geranium (*Pelargonium* species) essential oil cultivated in India. Peaks: **1**: Linalool; **2**: Isomenthone; **3**: Citronellol; **4**: Geraniol; **5**: Citronellyl formate; **6**: Geranyl formate; **7**: 10-epi-γ-Eudesmol; **8**: Geranyl tiglate.

FIGURE 3 A close-up image of leaves (left) and the flower of *Pelargonium* species cultivated in India.

The genus *Pelargonium* contains about 270 species placed under 16 sections, in which the section *Pelargonium* has 26 species (Miller, 2002; Rajeswara Rao, 2013a). Most of the *Pelargonium* species (approximately 80%) occur in South Africa, mainly in the winter rainfall region of the southwestern Cape (Van der Walt and Vorster, 1988).

The genus *Pelargonium* comprises scented and unscented species. The odor of the leaves of various scented *Pelargonium* species range from pleasantly fruity or floral to rather oppressively balsamic. Commercial geranium oil is obtained from the shoot biomass of several *Pelargonium* cultivars, the most fragrant being that of *Pelargonium* cv. *rose*, which grows on the islands of Reunion; its probable parents are *Pelargonium radens* and *Pelargonium capitatum* (Demarne and Van der Walt, 1989).

Ambiguity prevails over the true botanical names of the commercial geranium oil yielding cultivars. It is generally believed that all present-day cultivars, which are being cultivated for isolation of essential oil rich in geraniol and/or citronellol, are hybrids and are referred to as *Pelargonium* species. However, another botanical name, *Pelargonium graveolens* L′ Her. ex Ait. is also in use due to its close similarity with present-day cultivars. It is suggested that the plant be referred to as *Pelargonium* species until the taxonomic complexity of these cultivars is resolved (Rajeswara Rao, 2013a).

A close-up image of the leaves and flowers of prevalent Indian rose-scented geranium (*Pelargonium* species) cultivar is shown in Figure 3. A field view of this crop is shown in Figure 4.

FIGURE 4 A field view of rose-scented geranium (*Pelargonium* species) cultivated in India.

USAGE AND APPLICATIONS

The *Pelargonium* species are used in traditional medicine by the South African tribes to treat various ailments. They provide relief from diarrhea and dysentery. The *Pelargonium* species are used traditionally for the treatment of other problems, such as wounds, abscesses, fever, colic, nephritis, suppression of urine, colds, sore throats, hemorrhoids, and gonorrhea; they also are used for stimulating lactation and controlling anthelmintic infections (Rajeswara Rao, 2013b).

The essential oil from *Pelargonium* species is known for its variety of applications. This oil has a strong rose-like odor with a minty top note. Rhodinol ex geranium oil has a delightfully sweet, fresh, rosy, uniform, and tenacious aroma and is used extensively in perfumery. It is a very gentle oil, and in general, it can be used by everyone. In aromatherapy, it is used as a relaxant, a sedative for nervous tension, and a treatment for bronchitis, laryngitis, and menopausal problems. It is a cleansing, balancing, soothing, astringent, mild skin tonic, and thus is beneficial in skin care, as well as for relieving anxiety, fear, fatigue, depression, and elevating moods (Rajeswara Rao, 2013b). It promotes women's health and is also useful in eczema, athlete's foot, and respiratory tract health. Furthermore, its spicy, exotic, floral scent also makes it a suitable aphrodisiac agent (Jalali-Heravi et al., 2006).

Due to their substantial antibacterial activity, *Pelargonium* essential oils and extracts can be used as effective novel food and cosmetic antimicrobial agents (Lis-Balchin et al., 1998a). The major chemical constituents of *Pelargonium graveolens* essential oils—citronellol, citronellyl formate, geraniol, and citronellyl acetate—possess marginal antitumor activity (Fang et al., 1989). Rath et al. (2005) assessed the antifungal activity of geranium oil and its six major constituents (citronellol, linalool, geraniol, isomenthone, geranyl formate, and citronellyl formate) against *Candida albicans* and *Cryptococcus neoformans*, which cause common fungal infections such as candidiasis and cryptococcosis in humans. Citronellol was found to have higher antifungal activity, followed by geraniol, isomenthone, geranyl formate, and citronellyl formate.

Due to its pleasant smell and profound biological activities, geranium oil is extensively used in various cosmetic preparations, such as soaps, creams, toothpastes, ointments, perfumes, and other pharmaceutical formulations. Owing to the limited production and higher prices of rose products, *P. graveolens* is used as a substitute for the expensive attar of roses (Van der Walt and Vorster, 1988). Terpeneless geranium oil is a perfume by itself, blends well with floral bouquets, and finds application as a base for synthetic rose compounds (Rajeswara Rao, 2013a).

Geranium oil and its components (mainly citronellol and geraniol) are well known for their insecticidal activity (Dale and Saradamma, 1982). The hydrosol (distillate water rich in hydrophilic fraction of oil) is sprayed on crops to repel pests (Rajeswara Rao, 2013a). The oil is among the most effective essential oils against *Sclerotium rolfsii*, causing collar rot of *Mentha* sp. (Singh et al., 2000). In addition to uses of *Pelargonium* species in biological systems, it also finds applications in nanotechnology (Rajeswara Rao, 2013a).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Due to consumer concern about the safety of products containing synthetic chemicals as preservatives, there has been increasing use of natural essential oils in food flavoring, food preservation, perfumes, cosmetics, and pharmaceuticals. A variety of microorganisms cause food spoilage, which is one of the most important problems for food industry. Many pathogenic microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, and *Campylobacter jejuni*, are reported as causal agents of various foodborne diseases. Moreover, with increasing bacterial resistance to antibiotics, there is considerable interest in investigating the antimicrobial effects of essential oils and different extracts against a wide range of bacteria to develop alternate and safe antimicrobials for food preservation.

Essential oils have been screened for their potential uses as alternative medicines in treating many infectious diseases and as food preservatives, ensuring protection from the toxic effects of oxidants. In particular, the antimicrobial activities of plant essential oils and extracts have become the basis for many applications, including raw and processed food preservatives, pharmaceuticals, and alternative and natural remedies (Lis-Balchin and Deans, 1997). Foodborne diseases and spoilage may be controlled through natural compounds occurring in plants, herbs, and spices, which have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against foodborne pathogens (Deans and Ritchie, 1987). In general, essential oils that contain high amounts of monoterpenes, eugenol, cinnamaldehyde, thymol, and carvacrol are reported to have a wide range of strong antibacterial properties (Lis-Balchin and Deans, 1997).

Geranium oil has been used extensively in perfumery, cosmetics, pharmaceuticals, and the food industry (Narayana et al., 1986). The chemical composition of geranium oil is the main criteria for its application in various industries. The perfumery value of geranium oil is determined by the citronellol-to-geraniol (C/G) ratio. The Bourbon cultivar of Reunion origin possesses almost equal content of citronellol and geraniol (C/G ratio = 1) and it is considered to be the best quality commercial geranium oil; however, a C/G ratio of 0.5 to 2.0 is indicative of better odor quality; therefore, these compositions are acceptable in the geranium oil market (Verma et al., 2013).

Aerial parts of *P. graveolens* are used in folk medicine in Iran and in other parts of the world as a food and tea additive, as well as for relieving of some gastrointestinal, topical, dental, and cardiovascular disorders (Ghannadi et al., 2012). Geranium essential oil has historically been used in the treatment of dysentery, hemorrhoids, inflammation, heavy menstrual flows, and cancer. The French have treated diabetes, diarrhea, gallbladder problems, gastric ulcers, liver problems, sterility, and urinary stones with this oil (Slima et al., 2013). A study also showed a positive influence of geranium oil in the male reproductive system of animals, similar to that of vitamin E (Slima et al., 2013). Moreover, the cutaneous delivery of geranium oil using massage can suppress inflammatory symptoms with neutrophil accumulation and edema (Maruyama et al., 2005).

In addition to fragrance and pharmaceutical applications, geranium oil is also finding extensive applications in food flavor and preservation. The oil has been approved for food use by the U.S. Food and Drug Administration and has been granted "generally recognized as safe" status by the Flavor and Extracts Manufactures Association. The recommended concentrations are below 0.001% in finished products (Dasuki, 2002). The excellent antimicrobial and antioxidant activities and the intense rose-like aroma make it appropriate for the food industry. Geranium oil is used in the food industry for flavoring jams, jellies, cakes and puddings, drinks and beverages, sauces and ice creams. It is also used in dairy products, dehydrated fish, vegetables and fruits, malt extract (e.g., margarine), molasses, mushrooms, and processed foods (Lis-Balchin, 1990). This oil would make a great addition to mouthwash or gargles for sore or infected throats.

The antimicrobial activities of the essential oils of *Pelargonium* species have been evaluated over a range of microorganisms. This oil has a strong antibacterial effect, with low minimal inhibitory concentration values against *S. aureus* (0.72 mg/mL), and also against *Bacillus cereus* (0.36 mg/mL) and *Bacillus subtilis* (0.72 mg/mL) (Rosato et al., 2007). Deans and Ritchie (1987) evaluated 50 commercial essential oils against 25 bacterial genera. Geranium oil showed maximum sensitivity against the dairy product bacterial strains, *Brevibacterium linens*, and the toxin-producing *Yersinia enterocolitica*. Pattnaik et al. (1996) evaluated geranium essential oil against 22 bacterial and 12 fungal strains. Out of these, 12 bacterial and all studied fungal strains were inhibited by geranium oil.

The observed antibacterial properties show that *P. graveolens* has a good potential for use as an antimicrobial agent and natural preservative in different products (Ghannadi et al., 2012). Antifungal activity of *P. graveolens* essential oil was also reported by Chandravadana and Nidry (1994) against a postharvest fruit anthracnose fungus, *Colletotrichum gloesporoides*. Furthermore, the essential oil exhibited higher antifungal activity in comparison to citronellol or geraniol alone.

According to Dorman and Deans (2000), the decreasing order of antibacterial activity of the individual oil components of geranium oil is as follows: linalool (zone of inhibition: 12.5 mm), geranyl acetate (9.4 mm), nerol (8.0 mm), geraniol (7.0 mm), menthone (6.8 mm), β-pinene (6.3 mm), limonene (6.1 mm), and α-pinene (5.8 mm). Lis-Balchin et al. (1998b) investigated the efficacy of *Pelargonium* essential oils in a quiche filling; in this model food system, they showed potent antimicrobial activity that was comparable with that of commercial thyme, clove, geranium, and coriander oils.

Furthermore, Lis-Balchin et al. (2003) conducted a study to evaluate the antimicrobial potential of *Pelargonium* species essential oils and their corresponding hydrosols in a model food system; the results indicated the potential application of certain *Pelargonium* species essential oils as food antimicrobial agents.

Foodborne diseases are a serious public health concern around the world. In the United States, 31 species of pathogens cause 9.4 million cases of foodborne illnesses every year (Scallan et al., 2011). Revathi et al. (2012) evaluated geranium oil against bacterial stains, including *S. aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, and *Proteus mirabilis*, isolated from the household refrigerator. The oil showed good sensitivity against all tested organisms.

According to the Food and Agriculture Organization, one quarter of the world's food crops are affected by mycotoxins annually; as a result, about 5 billion people are at risk of chronic exposure to these aflatoxins through contaminated foods (Shephard, 2003). However, the results obtained from *P. graveolens* essential oil against these kinds of toxins producing microorganisms are really appreciable. The oil was found to be highly effective, showing better fungi-toxicity against *Aspergillus flavus* over the synthetic fungicides tested (Singh et al., 2008).

Moreover, the food industry requires the antioxidative properties of botanical extracts. Food with added natural antioxidants can be referred to as *functional foods* because the consumer is afforded greater health benefits by these products. Cavar and Maksimovi (2012) observed the antioxidant activity of essential oil and its corresponding hydrosols of *P. graveolens* grown in Bosnia, highlighting its possible use in the food industry. The antioxidant activity of the hydrosols is reported to be better than those of essential oils, and even 10 times higher than thymol (Cavar and Maksimovi, 2012). Presently, tocopherols, ascorbic acid, rosemary extracts, lycopene, and some flavonoids are used as natural antioxidants in food products. Furthermore, marked antioxidant activities along with antibacterial activity of the methanolic extracts of *Pelargonium* species and cultivars also suggests that its extracts can be used in the food, cosmetic, or other industries (Lis-Balchin and Deans, 1996). Cases of cardiovascular diseases and cancers are increasing continuously, which are thought to be caused by the involvement of reactive oxygen species (modern theory of free radical biology and medicine). However, these kinds of risks can be reduced by an appropriate dietary pattern including natural antioxidants (Balasundram et al., 2006).

In light of the growing interest in the use of natural products in daily life, the scented *Pelargonium* species can be used for culinary flavoring (Miller, 2002). In the food industry, geranium oil is used for flavoring nonalcoholic drinks, ice creams, candy, baked goods, gelatins, and puddings (Lis-Balchin, 1990). In addition, it is also considered to be safe for flavoring alcoholic drinks and tobacco products. The constituents of geranium oil, citronellol and linalool, are used in the food industry in citrus beverages (Lis-Balchin, 1990). Traditionally, rose-scented geranium leaves are placed in the bottom of the glass when making apple jelly. However, the crushed leaves of rose-, lemon-, and peppermint-scented geraniums give an extract on boiling with water, which is a delightful addition to tea, either hot or iced (Swain, 1974).

Hence, on the basis of research carried out so far on *Pelargonium* species worldwide, it can be ascertained that its essential oil and its components are indispensable in perfumery, pharmacology, and food. In addition, they are endowed with an array of biological activities that have therapeutic potential. The pharmacological properties of the extracts and essential oils of rose-scented geranium substantiates their use in traditional medicines and validates their commercial exploitation in the perfumery, cosmetic, food, and pharmaceutical industries; however, their toxicity profiles must be considered.

SUMMARY POINTS

- *Pelargonium* species naturally occur mainly in South Africa; however, their cultivars have been introduced in different countries due to commercial interests.
- Geranium oil possesses antimicrobial, pesticidal, insect repellent, antioxidant, and anticancer properties.
- *Pelargonium* essential oils, which are rich in citronellol and geraniol, are being used in perfumery, cosmetics, and aromatherapy.
- Apart from the essential oil, its derivatives/value added products—terpeneless oil, concentrate, absolute, and commercial rhodinol—are also widely produced and used in fragrance, flavor, and aromatherapy applications.
- Geranium oil is still underutilized in industrial forays, such as for food flavoring and preservation, in pharmaceuticals, and in plant protection.
- The excellent antimicrobial activities of geranium oil offer an attractive alternative for food flavoring and preservation; however, before using it in food, it is necessary to investigate how geranium oil and its constituents interact with food matrix components.
- Geranium oil can be a potential component in integrated disease management programs for effective control of various plant diseases in the future; however, necessary experiments in this regard have yet to be taken up for the development of the biocontrol agents.

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Chapter 80

Saffron Crocus (*Crocus sativus*) Oils

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List of Abbreviations

cm Centimeter
ft Foot
mm Millimeter
IRI Ischemia-reperfusion Injury

INTRODUCTION

Crocus sativus, commonly known as saffron crocus, is a delicate-looking lavender plant that produces stigmas. When harvested and dried, these stigmas create saffron. Saffron is a spice that has been used by humans since ancient times. It is one of the costliest spices in the world; therefore, its cultivation has great economic significance wherever it takes place. The plant is indigenous to southwest Asia. It is difficult to determine the origin of *C. sativus* plant, but it is presently cultivated in Western Asia, Turkey, Iran, Greece, India, and Spain. Approximately, 60,000 flowers are needed to produce 1 kg of saffron, which sells for about US $10,000 (Hill, 2004). At present, Iran accounts for approximately 90% of the world's production of saffron (Ghorbani, 2008).

In general, saffron tastes bitter (presence of picrocrocin) and has a penetrating hay-like odor (presence of safranal). The presence of crocin (carotenoid pigment) gives a rich golden yellow color to textiles and foods upon treatment with saffron. Saffron oil is prepared from a flower's stigma by solvent extraction; the oil has almost the same characteristics as the spice. Saffron oil contains more than 150 volatile and aroma-yielding compounds. Volatile components with a very strong odor are mainly terpenes, terpene alcohols, and esters (Liakopoulou-kyriakides and kyriakidis, 2002). During harvesting and drying, the heat along with enzymatic action leads to the breakdown of picrocrocin to yield D-glucose and a free safranal (**1**). Safranal, which is responsible for much of saffron's distinctive aroma, is composed of 60–70% of dry saffron's volatile fraction. The compound 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one (**2**) is also responsible for aroma of saffron (Gonda et al., 2012). However, the quantification of safranal is still used as an index of commercial quality of saffron oil. Visible ultraviolet and gas chromatography techniques are used for grading saffron oil (Bononi et al., 2015). It also has many nonvolatile active components—many of which are carotenoids, including zeaxanthin, lycopene, and various α- and β-carotenes. Moreover, saffron's golden yellow-orange color is due to the presence of α-crocin and is composed of more than 10% of dry stigma mass. Crocin is *trans*-crocetin di-(β-D-gentiobiosyl) ester or 8,8-diapo-8,8-carotenoic acid. The two esterified gentiobioses make α-crocin ideal for coloring water-based and non-fatty foods, such as rice dishes (Gonda et al., 2012). Saffron is also rich in vitamin B2 (riboflavin) and vitamin B1 (thiamine) content (Ahmed et al., 2010).

Saffron oil varies in quality and strength depending on several factors, including geographical location, amount of style picked along with the red stigma, and age of the saffron flower. The compositions of the active ingredients of oil change with the source. Heriberto and coworkers analyzed 11 different saffron types obtained from different locations and showed that the percentage of active ingredients differ depending on geographical location (Caballero-Ortega et al., 2007). The structures of some constituents of saffron oil are depicted in Figure 1.

The saffron plant has been used for medicinal purposes for more than 4000 years to treat as many as 90 different health conditions and ailments. The active components present in saffron possess many medicinal properties. The safranal has antimicrobial, antioxidant, anti-inflammatory, and cytotoxic effects, as well as anticonvulsant and antidepressant

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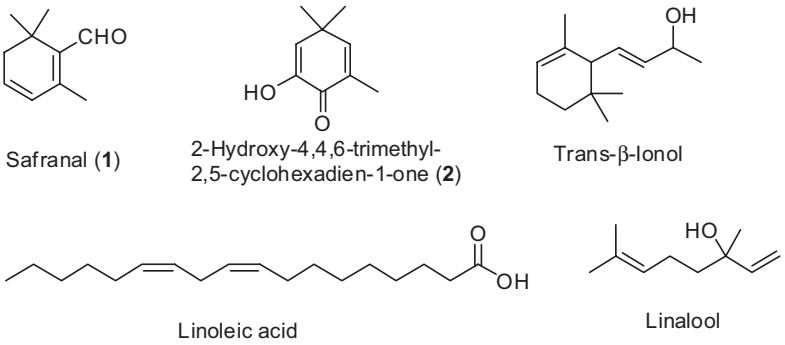

FIGURE 1 Structures of phytochemical components of saffron oil.

properties. The α-crocin has been found to have antioxidant, antidepressant, and anticancer properties. Saffron is widely used in Indian, Persian, European, Arab, and Turkish foods. It is common to find saffron in body oils and fabric dyes or as a flavoring in beverages and foods.

BOTANICAL ASPECTS

Crocus sativus (L.) is a perennial plant classified under the genus *Crocus*. It belongs to the family *Iridaceae* of the order *Asparagales*. It is also known as *kesar* or *zafran* in Hindi and *zafrah* in Arabic and Persian (Katariya et al., 2011). Saffron has been cultivated in various parts of the world for more than 4000 years. The natural ancestor of saffron crocus was known as *Crocus cartwrightianus*, which has its roots in Central Asia. Initially, people cultivated saffron by selecting the specimens with exceptionally long stigmas. In this process, *C. sativus* emerged in the later phase of the Bronze Age in Crete. According to experts, saffron was documented for the first time in an Assyrian botanical reference in the seventh century BC. It has been cultivated in South Asia mainly by Kashmiri and Chinese, anywhere between 2500 and 900 years ago. Currently, almost all saffron is grown in a belt bound by the Mediterranean to the West and the rugged region encompassing Iran and Kashmir to the East (Dalby, 2002, 2003).

The plants undergo estivating during spring. The saffron plant gives rise from 5 to 11 slender and almost vertical green leaves, with each of them growing up to 40 cm or 16 inches in length. The purple buds emerge and give flower during autumn. The vividly colored flowers of saffron appear only in October. The saffron flowers have a wide variety of colors, ranging from a pale pastel shade of lilac to a darker and streaky mauve. When plants are in full blossom, their height averages to 20–30 cm and bears up to four flowers, each with three dark red to reddish brown stigmas (25 and 30 mm long; Ahmed et al., 2010).

Because the purple flowers of saffron are of the sterile triploid variety and are unable to produce fertile seeds for reproduction, this plant has to rely on humans to dig up the corms or underground bulb-like parts, break apart the corms, and replant them. Usually, a corm is able to survive only for one season. One corm is broken up into 10 parts (or "cormlets"), and each of these divisions gives rise to a new plant. The corms of the domesticated saffron are basically petite brown balls or globules growing up to 4.5 cm or 1.8 inches in diameter (Ahmed et al., 2010). *Crocus sativus* plants require strict agroclimatic conditions for their growth; these conditions influence the quality of the spice (Liakopoulou-kyriakides and kyriakidis, 2002). Dry saffron is highly sensitive to changes in pH and rapidly breaks down chemically in the presence of light and oxidizing agents. Therefore, it must be stored in airtight containers to prevent exposure to atmospheric oxygen (Ahmed et al., 2010).

USAGE AND APPLICATIONS

Saffron oil and extracts prepared from flowers are of commercial value because of their pharmacological properties and odor characteristics. Many reviews have been published covering the various aspects of saffron, including cultivation, extraction methods, traditional use, and pharmacological activities (Basker and Negbi, 1983; Liakopoulou-kyriakides and kyriakidis, 2002; Ahmed et al., 2010; Bhargava, 2011; Katariya et al., 2011; Ulbricht et al., 2011; Hosseinzadeh and Nassiri-Asl, 2013; Wright, 2014; Amin and Hosseinzadeh, 2015; Nassiri-Asl and Hosseinzadeh, 2015). Since the early days, the use of saffron oil has been documented in different regions of the world in conventional medicine for the prevention and treatment of various diseases and health conditions. Traditional uses of saffron oil are summarized in Table 1 (Hosseinzadeh and Nassiri-Asl, 2013). The ancient Greek physician Hippocrates was the first to use saffron extracts for medicinal purposes as a purgative and salve to treat pain and other problems, as well as for its aphrodisiac properties in cases of loss of male

TABLE 1 The Traditional Uses of Saffron Oil

System/Effect	Traditional Uses
Analgesic and anti-inflammatory	Earache, toothache, swelling, otitis, anal pain, gout, cancer pain, gingivitis, discomfort of teething infants
Cardiovascular system	Cardiac stimulant, removes blockages of vascular
Eye disease	Painful eye, lacrimation, day blindness, corneal disease and cataract, purulent eye infection, pterygium, poor vision
Central nervous system	Narcotic, antihysteric, central nervous system stimulant, hypnotic, mental disease, sedative, anticonvulsant, neurasthenia
Gastrointestinal system	Stomachic, decreased appetite, treatment of hemorrhoid, prolapse of anus, jaundice and enlargement of the liver, antiflatulent
Genitourinary system	Abortive, treatment of amenorrhea, aphrodisiac, impotency, contraceptive, emmenagogue, stimulate menstruation, prolapse of anus, stop menstrual periods, promote menstruation, use in puerperium period, terminate pregnancy, painful urination, diuretic, kidney stone
Infectious diseases	Antibacterial, antiseptic, antifungal, measles, smallpox, scarlet fever
Respiratory system	Asthma, bronchitis, expectorant, pertussis, dyspnea, pleurisy, antitussive, diphtheria, disability tonsils resulting snoring, respiratory decongestant, expectorant
Skin disease	Treatment of psoriasis, eczema, acne, wounds
Miscellaneous	Immunostimulant, diaphoretic, tissue coloration, anticancer

potency. The spice was often added to love potions in ancient Rome. It was assumed that smelling saffron flowers opens the heart and excites the sexual drive. Saffron oil also has a significant effect on men with erectile dysfunction (Shamsa et al., 2009).

Furthermore, ancient Arabs used saffron as a therapy for problematic labor as well as for curing liver ailments. Saffron flowers worn in a girdle and the spice are said to relieve menstrual cramps. In Iran, pregnant women often wore a ball of saffron near the womb to ensure speedy delivery (Cunningham, 1985). Saffron relieves lumbar pains at the time of menstruation. Similarly, its beneficial effects in the treatment of women's illnesses, such as leukorrhea and anxiety, have been demonstrated. It is also reported to be effective for relieving headaches, especially in menopausal women, and for arthritis (Bhargava, 2011).

The ethanol extracts of flowers and safranal have relaxant effects on smooth muscles. It has frequently been used to treat asthma and cough. Saffron has been clinically proven to be effective in treating psychiatric disorders such as anxiety and depression. Safranal was found to induce a dose-dependent decrease in the incidence of both minimal clonic seizures and generalized tonic-clonic seizures. Saffron petals and hydroalcoholic extracts of the stigmas possess anti-depressant activity, comparable to that of standard drugs imipramine and fluoxetine. The water:methanol (50:50, v/v) extract of stigmas is effective in treating patients with Alzheimer's disease by inhibiting A–beta fibrillogenesis formed by oxidation of the amyloid β–peptide fibrils (Katariya et al., 2011; Georgiadou et al., 2012). In Yemen, the plant is used regularly as a stimulant.

Saffron is one of the herbs containing the highest content of riboflavin and thus is very effective in lowering blood pressure, stimulating the nervous system, and preventing nerve spasms. Imenshahidi and coworkers have established that the aqueous extract of saffron stigma has hypotensive properties due to the presence of crocin and safranal. Saffron oil was also showed to possess calcium antagonistic activity and thus is used as a cardioprotective agent (Imenshahidi et al., 2010).

Saffron extracts are also effectively used in treating hemorrhages, especially when the blood is black, clotted, and viscid (sticky), such as in people suffering from nosebleed. It is used in topical formulations for its antinociceptive and anti-inflammatory activity to treat atopic dermatitis, ichthyosis vulgaris, and other xerotic diseases of mild severity (Katariya et al., 2011).

USAGE AND APPLICATIONS IN FOOD INDUSTRIES

Essential oils have various biological activities, including antimicrobial, antiviral, antioxidant, and anticancer (Bakkali et al., 2008). Saffron oil is extensively used by food industries to color as well as to flavor the foods. It has the potential to be incorporated in food products, drinks, and beverages for its antimicrobial, antioxidant, and flavoring properties to improve the quality.

TABLE 2 Composition of Saffron

No.	Component	Mass %	No.	Component	Mass %
1	Water-soluble components	53	3	Lipids	12.0
1a	Gums	10	3a	Nonvolatile oils	6.0
1b	Pentosans	8.0	3b	Volatile oil	1.0
1c	Pectins	6.0	4	Protein	12.0
1d	Starch	6.0	5	Inorganic matters (ash)	6.0
1e	α-Crocin	2.0	5a	HCl-soluble ash	0.5
1f	Other carotenoids	1.0	6	Water	10.0
2	Fibers (crude)	5.0			

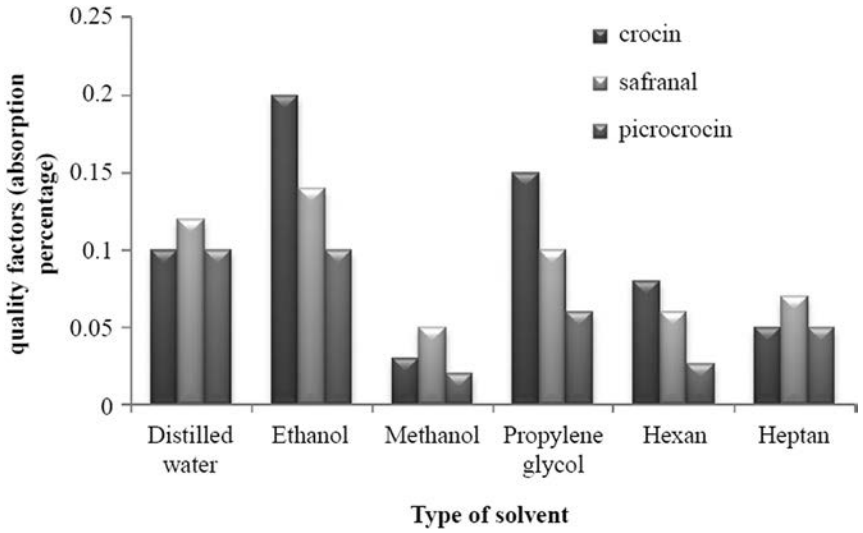

FIGURE 2 Effect of solvent on the quality of saffron extract. *(Reprinted with permission from the publisher Emerald.)*

Saffron from Iran, Spain, and Kashmir is classified into various grades according to the relative amounts of red stigma and yellow styles it contains. There are different grades of Iranian saffron and Spanish saffron depending on the content of stigma. Table 2 summarizes the general composition of saffron (Katariya et al., 2011).

Essential oil extracted from saffron using various solvents is used in food products. Mohseni has worked to improve quality of saffron oil by using different solvents by extraction method, including distilled water (DW), ethanol/DW, methanol/DW, propylene glycol/DW, heptane/DW, and hexane/DW for the extraction of oil. These extracts were analyzed using an ultraviolet spectrophotometer. The author concluded that saffron oil prepared using ethanol contains important constituents and is most suitable as an edible oil, as depicted in Figure 2 (Mohseni, 2014).

Many papers have been published to determine the chemical composition of saffron oil. Yu-Zhu and coworkers analyzed the composition of saffron oil prepared from stigmas and corms, as summarized in Table 3 (Yu-Zhu et al., 2008). The volatile components of saffron oil vary slightly depending on the method of extraction and source of saffron. Shao et al. compared three methods—hydrodistillation, Soxhlet extraction, and supercritical fluid extraction—for the extraction of essential oil from Greek saffron. The chemical composition of saffron oil extracted by these methods is presented in Table 4 (Shao et al., 2014).

Saffron Oil as an Antimicrobial for Food Preservation

The food industry is rapidly changing to provide more preserved foods to the consumer. Consumers prefer food with safe preservatives. Microbial activity is one of the most common causes of spoilage. It is a major concern in the food industry as it causes significant economic losses and can have serious public health consequences. Microorganisms produce

TABLE 3 Chemical Composition of Volatile Compounds Obtained From Extracts of Stigma and Corm of Saffron

Component	Percent	Component	Percent
Stigmas			
β-Isophorone	1.32	2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (safranal)	81.82
β-Linalool	0.27	Eucarvone	0.31
2-Isopropylidene-3-methylhexa-3,5-diena	4.45	6-(2-Butenylidene)-1,5,5-trimethyl-(E,E)-cyclohexene	0.21
α-Isophorone	5.57	6-(2-Butenylidene)-1,5,5-trimethyl-(Z,E)-cyclohexene	0.26
2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one	1.90	α,β-Dihydro-β-ionone	1.19
3,4,5,6-Tetramethyl-2H-pyran-2-one	1.14	Palmitic acid methyl ester	0.13
Corm			
n-Tridecane	1.31	n-Octadecane	1.13
n-Tetradecane	6.17	Hexadecanoic acid	33.32
n-Pentadecane	1.74	Palmitic acid ethyl ester	11.21
Diethyltoluamide	1.32	n-Eicosane	0.97
n-Catane	1.39	Octadecadienoic acid	27.55
n-Heptadecane	2.02	1,3,5-Tribenzoylbenzene	2.78

saccharolytic, proteolytic, pectinolytic, and lipolytic enzymes with metabolic end products that are associated with food spoilage. Thus, microbial activity is considered to be of great importance for the manifestation of spoilage. Microbial food spoilage manifests itself as visible growth and food textural changes.

Salmonella is one pathogen that is found in several spices and has been involved in foodborne outbreaks. Saffron spice, however, does not experience this degradation by pathogens due to its antimicrobial activity. Pintado and coworkers have evaluated the antibacterial potential of saffron using samples from Iran, Greece, and Spain that were artificially contaminated with clinical isolates belonging to five different serovars of *Salmonella*. They found that active constituents of saffron, safranal (8–16 mg/mL), and crocin (64–128 mg/mL) exhibited antibacterial activity, especially against *Salmonella* (Pintado et al., 2011). This is one reason why bacteria do not grow in saffron spices. Another study showed that ethyl acetate extract of saffron stigma was active against bacteria (*Micrococcus luteus*, *Staphylococcus epidermitis*, *Staphylococcus aureus*, and *Escherichia coli*) and fungi (*Candida albicans*, *Aspergillus niger*, and *Cladospourium* sp.) using the cupplate diffusion method. The ethanolic and methanolic extracts of saffron also showed moderate anti-Brucella activity using the disc diffusion method (Motamedi et al., 2010; Hosseinzadeh and Nassiri-Asl, 2013).

Aflatoxin B1 (AFB1) is a carcinogenic metabolite produced by certain *Aspergillus* species. Tzanidi and coworkers investigated the effect of saffron stigmas on the growth of *Aspergillus parasiticus* as well as AFB1 production in yeast extract sucrose (YES) medium. The result showed that the addition of saffron-dried stigmas prevented AFB1 production in samples of YES inoculated with *A. parasiticus* throughout the period of observation (18 days). Therefore, saffron oil might be used and added under specific conditions in the processing of agricultural products to prevent AFB1 production (Tzanidi et al., 2012).

The antibacterial effects of aqueous, ethanol, and methanol extracts prepared from saffron petals have been investigated on *S. aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella typhimurium*, and *E. coli* O157:H7 using agar dilution and a broth microdilution method. The minimum inhibitory concentrations (MIC) of these extracts are shown in Table 5. These studies suggested that the essential oil of saffron can be used in food products (Nosratabadi et al., 2011).

Saffron Oil as an Antioxidant

Atmospheric oxygen is one of the sources for oxidation process. The oxidation of food ingredients degrades the quality of the foods. It produces free radicals, which in turn are responsible for cell damage or death. It leads to rancidity or color changes in the food. Antioxidants are agents that inhibit the oxidation of other molecules. Antioxidants terminate free radical chain reactions by removing free radical intermediates and inhibit other oxidation reactions.

TABLE 4 Chemical Composition of the Volatile Fraction of Saffron Oil Extracted by Hydrodistillation (HD), Soxhlet Extraction (SE), and Supercritical Fluid Extraction (SFE)

No.	Compound	MW	HD	SE	SFE
1	2-Methyl butyric acid	102	–	0.47	0.21
2	3-Methyl butyric acid	102	–	0.62	0.13
3	4,4-Dimethyl-2-cyclopenten-1-one	110	0.37	0.41	0.40
4	Hexanoic acid	116	–	0.12	0.18
5	β-Isophorone	138	0.04	0.11	0.12
6	4,4-Dimethyl-cyclohex-2-en-1-ol	126	0.34	0.18	0.11
7	Linalool	154	5.36	0.12	0.18
8	Nonanal	142	0.16	0.21	0.24
9	Phorone	138	1.42	1.36	1.61
10	α-Isophorone	138	0.14	0.18	0.22
11	Ketoisophorone	152	0.52	0.61	0.64
12	3,5-Dimethyl benzaldehyde	134	0.37	0.32	0.46
13	Safranal	150	30.72	38.17	40.31
14	Decanal	156	0.24	0.10	0.16
15	4-Hydroxy isophorone	154	0.63	0.56	0.66
16	2,4-Decadienal	152	0.47	0.52	0.51
17	Decanoic acid	172	0.12	1.48	1.42
18	3-Hydroxy-5-methyl-2-(1-methylethyl)-2,5-cyclohexadiene-1,4-dione	180	0.13	0.26	0.18
19	β-Ionone	192	0.71	0.68	0.72
20	*trans*-β-Ionol	194	8.68	1.42	1.21
21	Epoxy-β-ionone	208	0.11	0.16	0.14
22	4-Oxo-β-dihydroionone	208	0.23	0.16	0.18
23	3-Oxo-β-ionone	206	0.17	0.15	0.19
24	Clovene	204	0.14	–	–
25	Dodecanoic acid	200	1.17	4.23	3.21
26	1-Pentadecene	210	0.21	–	–
27	Tetradecanal	212	0.71	0.76	0.88
28	Hexadecane	226	0.11	–	–
29	Myristic acid	228	1.32	3.27	4.01
30	Methyl tetradecanoate	242	2.12	2.16	2.71
31	Octadecane	254	0.12	–	–
32	Nonadecane	268	0.67	0.17	0.31
33	Pentadecanoic acid	242	0.32	1.68	1.49
34	Methyl hexadecanoate	270	–	0.13	0.21
35	Hexadecanoic acid	256	0.10	0.34	0.38
36	Heptadecanoic acid	270	0.12	0.56	0.37
37	Oleic acid	282	0.12	0.67	0.82

TABLE 4 Chemical Composition of the Volatile Fraction of Saffron Oil Extracted by Hydrodistillation (HD), Soxhlet Extraction (SE), and Supercritical Fluid Extraction (SFE)—cont'd

No.	Compound	MW	HD	SE	SFE
38	Linoleic acid	280	4.13	8.12	6.69
39	Octadecanoic acid	284	–	0.10	–
40	6,10,14-Trimethylpentadecan-2-one	268	3.16	3.02	3.28
41	Octadecanal	268	0.21	0.17	0.24
42	Eicosane	282	0.56	0.12	–
43	Heneicosane	296	0.17	–	–
44	Docosane	310	0.36	0.33	0.28
45	Hydrocarbons		2.50	0.62	0.59
46	Alcohols		14.38	1.72	1.50
47	Esters		2.12	2.29	2.92
48	Carbonyl compounds		40.51	47.91	51.14
49	Carboxylic acids		7.40	21.66	18.91
50	Total identified		66.91	74.20	75.06

TABLE 5 Minimum Inhibitory Concentrations of the Aqueous, Ethanol, and Methanol Extracts of Saffron Petals Against Bacteria Studied by an Agar Dilution and Microdilution Method

	Minimum Inhibitory Concentration (mg/mL)		
Bacterial Strain	Aqueous Extract	Ethanol Extract	Methanol Extract
Agar dilution method			
Salmonella typhimurium	40	40	40
Escherichia coli O157: H7	40	40	40
Staphylococcus aureus ATCC 6538	40	40	40
Listeria monocytogenes ATCC 19118	40	40	40
Bacillus cereus ATCC 11778	40	40	40
Broth microdilution method			
S. typhimurium	40	<40	40
E. coli O157: H7	40	<40	40
S. aureus ATCC 6538	40	40	40
L. monocytogenes ATCC 19118	40	40	40
B. cereus ATCC 11778	40	40	40

Assimopoulou et al. explored the methanol extract of saffron as well as active constituents, crocin and safranal, for radical scavenging activity by using a DPPH assay. They demonstrated that crocin showed high radical scavenging activity (50% and 65% for 500 and 1000 ppm solution in methanol, respectively), followed by safranal (34% for 500 ppm solution). The high radical scavenging activity is probably due to the ability to donate a hydrogen atom to the DPPH radical. Thus, it justifies the use of saffron oil in functional foods and drinks for antioxidant activity(Assimopoulou et al., 2005).

Serrano-Diaz et al. studied the antioxidant potential of extracts from saffron flowers and their separate parts using four in vitro assays: lipid peroxidation, deoxyribose assay, Rancimat test, and Trolox equivalent antioxidant capacity. All the samples studied were demonstrated to be potential antioxidants. Moreover, the authors established that addition of saffron oil to sunflower oil improves the oxidative stability of sunflower oil in the Rancimat test. Thus, saffron oil could be used as a functional ingredient with subsequent added value (Serrano-Diaz et al., 2012).

The safety and antioxidant activities of saffron oil were evaluated by Ramadan et al. Ethanolic extracts of saffron were found to be harmless, they did not cause any mortalities or signs of toxicity in mice when administered orally at doses up to 5 g/kg by weight for 8 successive weeks. They also did not cause any toxic changes in the liver and kidney (Ramadan et al., 2012).

SUMMARY POINTS

- Saffron is one of the most costly spices in the world.
- The production of 1 kg of saffron requires around 60,000 flowers.
- Dried flower stigmas are used for extraction of essential oil.
- Safranal, crocin, picrocrocin, and carotenoids are the main active ingredients of saffron oil.
- Saffron oil is one of the most commercially important essential oils in the food industry due to its flavor, color, and antimicrobial preservation characteristics.
- Saffron essential oil is widely used in Indian, Persian, European, Arab, and Turkish foods.
- Saffron essential oil is used in beverages, drinks, and food products.

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Chapter 81

Sage (*Salvia officinalis*) Oils

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List of Abbreviations

^{0}C Celsius grat
BC Before Christ
Cm Centimeter
EMA European Medicines Agency
Etc Et cetera
L Linnaeus

INTRODUCTION

Sage is defined as a medicinal herb in Old Latin (*Salvare* means "cure, save"). This plant, which belongs to the Lamiceae family, is grown throughout the world and especially in the Mediterranean, Europe, and North America. Sage, whose homeland is the Mediterranean, is grown in sea view and on calcareous slopes and islands in Turkey. Therefore, it is commonly called *Adaçayı*, which means "island tea" in Turkish. Sage is also called *Maryam goli* in Persian. *Salvia officinalis* has various other names, the most common being sage, common sage, garden sage, golden sage, kitchen sage, true sage, culinary sage, Dalmatian sage, and broadleaf sage.

Sage has the oldest history among medicinal herbs. This plant has been used by Egyptians, Greeks, and Romans since ancient times. It was used in order to increase yield among Egyptians. The importance of sage in ancient times can be understood from Anglo-Saxon writings, where it was written that "why should man die when he has sage?" Today, this plant is utilized in pharmacology and the food industry. Although sage has 900 species throughout the world, only a few species can be used as raw material for drugs. The rest of the species have quite a bad odor and are harmful to human health.

Sage components include etheric essential oil, thujone, cineole, linalool, borneol, salven, pinene and camphor, tannins, triterpene, flavones, isoflavones, and resinous components. There are triterpene and flavones in its essential oil. Sage extract contains 1–60% monoterpene, 0.4–3.5% triterpene, and 1.1% flavonoid (Table 2).

BOTANICAL ASPECTS

Scientific names: *Salvia officinalis* L.

Botany

Salvia officinalis L. (sage) has its origins in the Mediterranean and belongs to the Labiatae family (Table 1). This bushy plant is either annual or perennial. The length of odorous and aromatic sage varies between 40 and 100 cm (Figure 2). The leaves of sage are erect on the edges; they are 1–4 cm diameter, 4–10 cm long, and oval. The edges are sharp, green-grey colored, pilous, and bitter. Aromatic odor excretions are found toward the lower surface of the leaf (Figures 1 and 2). Its violet-like flowers are found in clusters. Each flower group bears 2–10 or rarely 40 flowers. The plants may contain both male and female organs or only female organs. There are two male organs.

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TABLE 1 Scientific Classification of Sage

Kingdom	Plantae	Plants
Division	Magnoliophyta	Angiospermae
Class	Magnoliopsida	Monocotyledons
Family	Lamiaceae	
Genus	*Salvia* L.	
Species	*Salvia officinalis* L.	Sage, garden sage, common sage

TABLE 2 Essential Oil Components in Sage (*Salvia officinalis* L.)

Essential Oil	Up to 3%	Monoterpenoids	α-Thujone	10–60%
			β-Thujone	4–36%
			Camphor	5–20%
			1,8-cineole	1–15%
		Caffeic acid dimer	Rosmarinic acid	Up to 3.3%
			Melitric acid A	
Hydroxycinnamic acid derivates	About 3.5%	Caffeic acid trimers	Methyl melitrate A	
			Sagecoumarin	
			Salvianolic acid K	
		Caffeic acid tetramer	Sagerinic acid	
Phenolic diterpenes				0.35%
Triterpenes		Pentacyclic triterpene acids		0.4–3.5%
		Triterpene alcohols		0.10–0.18%
Flavonoids	About 1.1%	Flavones and their glycosid		

EMA (2010).

FIGURE 1 Leaves and flowers of sage (*Salvia officinalis* L.).

FIGURE 2 Sage (*Salvia officinalis* L.).

Harvesting

Sage is cultivated in a short time period, generally beginning from May and before flowering or at the beginning of flowering. Essential oil derivatives in sage leaf are at the highest levels before flowering. Therefore, leaves are picked in May or June before flowering. Active substances in the leaf (e.g. cineol, camphor, boneol alfa, and beta-thujone) reach their peak at noon. Leaves picked in the harvest are dried in a shady and narrow place. The drying temperature should not exceed 35 °C because herbal essential oil evaporates at high temperatures. The leaves are kept in an airtight container after they are thoroughly dried.

USAGE AND APPLICATIONS

Salvia officinalis (sage) has been used for medical purposes for thousands of years. The plant has been used for many complaints (e.g., insomnia, measles, sea sickness, sexually transmitted diseases, worms) and has a historical background dating back to ancient Greek and Roman times. Sage was used for medicinal purposes against itching in Ebers Papyrus (1500 BC) period. It was also used for labor pain, ulcer, coughing, hoarseness, perspiration, and contractions. It was used for mental clarity and to strengthen memory toward the end of seventeenth century.

Sage is used by people as a diuretic, anti-inflammatory, antimicrobial, antiseptic, expectorant, and for hyperhidrosis. Sage, which was used against plague in the past, is used for various clinical conditions today: sedative, antimicrobial, antioxidant, antitumor, antihypertensive, perspiration, coronary heart disease, chronic bronchitis, asthma, chronic renal failure, cirrhosis, dysmenorrhea, insomnia, infantile colic, and dyspepsia. Other sage components also have antiseptic properties for insect stings and antifungal properties as well. The powder of the sage leaf is applied in insect sting cases (Sensoy, 2007); in addition to this, it is used as an herbal drug (Aríca et al., 2010) in neurologic diseases, such as Alzheimer disease.

Due to its phenolic substances, sage has been the subject of various studies. These studies reported that sage can be used as an antiperspirant, antifungal, antiseptic, antibiotic, astringent, antispasm, estrogenic, hypoglycemic, diuretic, carminative, and tonic agent (Cenic-Milosevic et al., 2013).

Essential oil obtained from *S. officinalis* has medicinal effects against respiratory and digestive syndromes, heart and blood circulation, metabolic conditions, and endocrine diseases (Badiee et al., 2012). Moreover, sage leaves are used in medicine as an effective antiseptic and anti-inflammatory (Bauner et al., 2012); in addition to this, sage has shown anticancer activity.

Salvia officinalis extracts have antioxidant, anti-inflammatory, and antimutagenic activities that have different pharmacological effects. It was reported that sage contributes to the treatment of Alzhemier disease (Mohammad, 2011) and has inflammatory, estrogenic, and sedative effects.

Sage can be helpful against muscle pains and chronic stress or mental tension. In addition, sage can be used as mouthwash for sore throat, infected gingiva, mouth ulcers, and colds. Sage oil has an aroma like camphor, thujone; therefore, it is used in the perfume industry. This plant has sedative effects on sweat glands, which decreases sweat secretion in the hand, foot, armpit, and the whole body (Mohammad, 2011).

Sage treatment had shown promising and beneficial effects for slowing the progress of bone loss, thus indicating its usefulness as a potential therapeutic agent in humans (Abdallah et al., 2010). Moreover, it was reported that sage reduces the injury area and hastens the recovery period of the injury (e.g. skin breaks, muscle tears, or a bone fractures). (Anitha et al., 2013). Sage can be used as tablets or droplets, as tea or essential oil; it can also be used for coloring carpets or rug yarn (Olmez and Kayabasi, 2002).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Sage also has had an important place as a spice since ancient times. Ancient Egyptians, Greeks, and Romans used this plant extensively in their meals, just like today. Sage was used in fresh food in order to extend the life of meat products. Today, sage leaves are used as a spice for flavor in various Mediterranean dishes.

The pilous and whitish leaves of sage are used in tea after they are dried and boiled or to add odor or flavor to meat dishes. Sage is especially recommended for a nice odor and flavor in cooking liver, duck, goose, chicken, and game animal. It is used with potatoes, rested meat, and soused dishes in European cuisine. In Italy, fresh sage leaves are consumed together with bread and butter by villagers in order to protect health. This herb is also used in making Holland cheese and brewing tea. Because sage gives sharp and slightly bitter flavor to food, it is preferred in Italian meat dishes in order to give taste. Fresh sage leaves are added in flaked pastry in order to increase the taste. Moreover, sage is added to biscuits, pastries, cakes, cheeses, and sauces. It is suggested to add sage when cooking food that causes tympanites, such as beans.

Salvia officinalis leaves are used either fresh or dried in Italian meat and chicken dishes, soup, pasta, or potatoes as spice, appetizer, or garnish. Putting sage leaves on sliced potatoes enables crispiness in the oven. Fresh leaves of sage are also used as tea among people and are believed to help treat digestive disorders. Sage added to vinegar is reported to be effective against diabetes, stomach aches, flushing, depression (Karamanos, 2000), excessive sweating (Mohammad, 2011; Karamanos, 2000), and hormonal problems (Sensoy, 2007).

Salvia officinalis has commercial importance in the food industry as a natural antioxidant. Some researchers determined that there is a firm relationship between food and medical natural antioxidant resources, phenolic components, and antioxidant activity quality. In the studies mentioned in this chapter, it was stated that the antioxidant effect of sage is especially dependent on its phenolic components (Table 3). Antioxidants prevent oxidative stress within cells and therefore are used in the treatment of many diseases, such as cancer, cardiovascular diseases, and inflammatory diseases.

Flavonoids from herbal phenolic components, cinnamic acid derivatives, coumarins, tocopherols, and phenolic acids constitute the most important group of natural antioxidants. It is known that these antioxidants prevent materials found in the food and are easily oxidizable from oxidation. Sage is the most important source of phenolic components, which are used as antioxidants. The phenolic content in *S. officinalis* extracts correlates with their antiradical activity, confirming that phenolic compounds are likely to contribute to the radical scavenging activity of these plant extracts (Roman et al., 2009). Pizzale et al. (2002) stated that sage includes carnosic acid, carnosol, and metyl carnosate components and has strong antioxidant activity according to the species.

There has been concern about the potential side effects of synthetic antioxidants. Therefore, natural antioxidants are replacing synthetic antioxidants due to the demand of consumers and the food industry. Therefore, the use of herb extracts as natural antioxidants has an important place in food industry. It was determined in some of these components that the antioxidant capacity is greater than that of synthetic antioxidants. Sage is known for its antioxidant characteristics due to its rich phenolic component.

The antioxidant characteristics of sage are related to rosmarinic acid and carnosic acid. Phenolic components found in the sage are rosmarinic acid, carnosic acid, and salvianolic acid (Sensoy, 2007). In another study, sage was added to the drinking water of rats and mice; as a result, it was observed that sage has antioxidant effectiveness (Lima et al., 2005).

TABLE 3 Antioxidant Capacity, Total Phenolics, and DPPH Radical Scavenging Activity of Sage

Antioxidant Capacity (μmol/100 ppm Extract)	Antiradical Activity (%)	Ascorbic Acid Equivalent of the Extracts Antioxidant Capacity (AOC_{EAA}), (g/L)	Total Phenolics (mg GAE/100 g DW)	DPPH Radical Scavenging Activity, $IC_{50\%}$ (mL/L)	References
–	–	1.2152	27.94	12.64	Atanassova et al. (2011)
327.7 ± 26.1	74.6 ± 6.1	–	–	–	Ozcan and Al Juhaimi (2011)
–	–	–	460.20 ± 4.36	–	Basuny et al. (2011)

In the food industry, the auto-oxidation rate decreases with vacuum-packaging and cooling, as well as packaging under inert gas. An important problem is oil oxidation, which restricts the shelf-life of bakery products. Especially in bread making, the oxidation of polyunsaturated fatty acids, which have high fat content, may result in undesirable odors and tastes with long storage durations. Basuny et al. (2011) stated that essential oil and phenolic components obtained from medicinal herbs serve as antioxidants in bakery products, and it was put forward in the study that oil and phenolic components obtained from sage have inhibitory effects against six bacteria species (Table 5).

Sage has been used successfully in the preservation of food. Sage delays or decreases the formation of microorganisms (Table 5). Therefore, sage can be used to prevent the spoilage of food. In addition, sage extract can be effective in reducing pathogens in meat products. Sage can be used as alternative protective material in foods and in pathogen control. In addition, it can decrease the tissue deformation of sausages stored in cold conditions and has no effect on sensory properties.

Oxidative deformation is observed in foods, including oil, and is an important factor that shortens the shelf life of fresh meat products (Kenawi, 2012). To prevent or delay oxidative deformation, sage is used as an additive material in foods. Salem and Ibrahim (2010) determined 2-thiobarbituric acid reaction materials, microbiological aerobic plate count, and lactic acid bacteria count in sausages made of buffalo meat and dry fermented sausages including sage oil (Table 4). The study found that sage oil can be used in sausages as natural antioxidant.

Medical aromatic herbs and spices have been used for centuries to prolong the shelf life of fresh meat products. In a study in which the effect of sage extract was analyzed for prolonging the shelf life of beef minced with soybean protein and increasing microbial quality, Ahmed and Ismail (2010) found that microbiological quality and shelf life was increased

TABLE 4 Thiobarbituric Acid Reaction Materials, Aerobic Plate Count, and Lactic Acid Bacteria Count of the Dry Fermented Sausage in Sage Oil

Final Product within Acceptable Lipid Oxidation	Samples	Ripening Period (Day)		Reference
		8	15	
Thiobarbituric acid reactive substances (mg malonaldehyde/kg meat)	A_1	1.18	0.96	Salem and Ibrahim (2010)
	A_2	0.81	0.51	
	A_3	0.76	0.46	
Aerobic plate count (log CFU/g)	A_1	6.12	3.93	
	A_2	6.08	3.75	
	A_3	6.00	3.61	
Lactic acid bacteria count (log CFU/g)	A_1	5.22	5.01	
	A_2	5.21	$4.99 < x < 5.01$	
	A_3	5.20	4.99	

A_1, Control sample; A_2, Sample mixed with 0.025% sage oil extract; A_3, Sample mixed with 0.05% sage oil extract.

TABLE 5 Antimicrobial Activity and Microbial Quality of Sage Oil in Some Microorganisms

Microorganisms	Treatment Dose	Product	Store Temperature	Reference
Escherichia coli	200–1000 ppm sage oil	Bakery products	Room temperature	Basuny et al. (2011)
Klebsiella pneumonia				
Pseudomonas aeruginosa				
Protrus vulgaris				
Bacillus subtilis				
Staphylococcus aureus				

when kept for 7 days at 4 °C with 0.3–0.5% sage extract. Fasseas et al. (2007) stated that sage extract decreased the lipid oxidation of meat, but this effect depends on storage duration and temperature.

Lipid oxidation lowers the quality of the food and reduces the shelf life. Ethanol extract reduces or prevents oxidation in meat products. The application of sage ethanolic extract in mayonnaise could be useful to control souring and septicity, as well as to maintain the quality and extend the shelf life of mayonnaise during storage at district temperature (Rasmy et al., 2012). Lopez-Bote et al. (1998) stated that oil oxidation formation was delayed in meat obtained from broilers fed sage extract, stored in cool environment, and then cooked in 70 °C. During cooling, oxidation decreased in beef and pork meat in which sage essential oil was added. Sage essential oil also delayed oil oxidation in frozen meat (Simitzis et al., 2008).

Sage prevents insect formation in grain seeds. Therefore, some people add a few leaves of sage in the storage of legumes. Because insects cause serious damage to the quality of legume seeds (Dal et al., 2001), sage can be used as a repellent against insects in storage. Legume seeds used in agriculture and nutrition are important to maintain the insect damage. Thus, legume seeds are stored in long-term intact and protected from insect damage. Long-term storage of legume seeds is an important activity in the sense of physical and biological quality. Therefore, it was found that sage can be used for storing seeds (Altíndal and Altíndal, 2011). Various studies have focused on the fact that *S. officinalis* oil can be used for the biological control of pests. Sage oil and extract spread more rapidly compared to synthetic components and do not harm specific helpful insects; therefore, it is accepted as an alternative method in biological control (Larocque et al., 1999).

Sage may be added to feed as an antimicrobial. In various studies carried out on the antimicrobial activity of sage, it was stated that sage has important antioxidant capacity (Nostro et al., 2004; Bakkali et al., 2008). As a result, sage can be used as an antioxidant resource instead of vitamin E. Moreover, cheese made from goats that are fed with sage in the mountains can be rather tasty.

Herbal extracts such as sage have considerable antimicrobial effect. In some studies, it was reported that the use of herbal extracts in the feed of laying hens had antioxidant and cholesterol-lowering effects. Therefore, these extracts may be used as a feed supplement (Kahraman, 2009).

Sage, which naturally grows in Turkey, is used in honey production. The color of this sage honey is light yellow. The flavor and odor of this honey is good and crystallization of this honey is slow, but it differs from the characteristic aroma of other honey types. Moreover, the honey that is made most commonly in America is made from sage. Honey made of sage is known as white honey; it is whitish or clear and tasty. Approximately 6.5 kg/da honey can be obtained from one decare of sage plants. For this reason, cultivation of sage is of great importance to beekeeping.

SUMMARY POINTS

- Sage has been an important medical and aromatic herb since ancient times.
- Sage can be used as a natural antioxidant in the food and drug industry instead of synthetic antioxidants.
- Sage can be used as a flavoring in food, in prolonging the shelf life of food, and in storing materials such as food and seeds.
- Sage is used as an antibacterial and antifungal.
- Sage is important in the production of honey.
- Sage can be used against pests (e.g., insects).
- The essential oil and essence of sage can be used in the medicine and cosmetic industries.

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Chapter 82

Sandalwood (*Santalum album*) Oils

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List of Abbreviations

EO Essential oil
SA *Santalum album*
SW Sandalwood
SWO Sandalwood oil

INTRODUCTION

Sandalwood (SW) is an evergreen tree that is native to southern Asia. SW is known as Indian or white SW. It is indigenous to the highlands of southern India, mainly Coorg, Chennai, and Mysore. It generally occurs at altitudes of 2000–3000 feet and attains a height of 60–65 feet. The plant is distributed in the Western Ghats and a few other mountain ranges, such as the Kalrayan and Shevaroy Hills. The tree thrives in well-drained, loamy soil preferably on slopes of hills exposed to the sun. The tree cultivates best between altitudes of 600–1050 m with a minimum of 20–25 inches rainfall per year. The ideal temperature for its growth is between 12° and 30 °C (Kulkarni and Srimathi, 1982). The finest wood grows in driest region particularly on red or stony ground while on rocky ground the tree often remains small but gives the highest yield of oil (Kirtikar and Basu, 1933). The economic and cultural value of SW comes from the oil contained in the heartwood, and the oil content varies widely between species and even within species. The oil is a pale yellow to yellow viscous liquid which is highly rated for its sweet, fragrant, persistent, spicy, warm, woody, and animalic nature (Arun kumar et al., 2012). The essential oil (EO) of SW is in high demand by the food industries due to its persistent aroma with the fixative property. The heartwood of SW was priced at 1,200,000 of Indian rupees per ton (907.18 kg) and oil was priced at 22,000 Indian rupees per kg but the prices are highly depended on the quality (Jain et al., 2003).

BOTANICAL ASPECTS

Plant Profile

Kingdom: Plantae—Plants
Subkingdom: *Tracheobionta*—Vascular plants
Superdivision: *Spermatophyta*—Seed plants
Division: *Magnoliophyta*—Flowering plants
Class: *Magnoliopsida*—Dicotyledons
Subclass: *Rosidae*
Order: *Santalales*
Family: *Santalaceae*—Sandal wood family
Genus: *Santalum Linn*—sandalwood
Species: *Santalum album*—Sandalwood

SW is a commercially and culturally important plant species belonging to the family Santalaceae and the genus *Santalum*. Its EO is known as East Indian sandalwood oil (SWO). There are around 18 species of SW belonging to the genus *Santalum* which are *Santalum freycinetianum*, *Santalum haleakalae*, *Santalum ellipticum*, *Santalum peniculatum*, *Santalum pyrularium*, *Santalum involutum*, *Santalum boninense*, *Santalum insulare*, *Santalum austrocaledonicum*,

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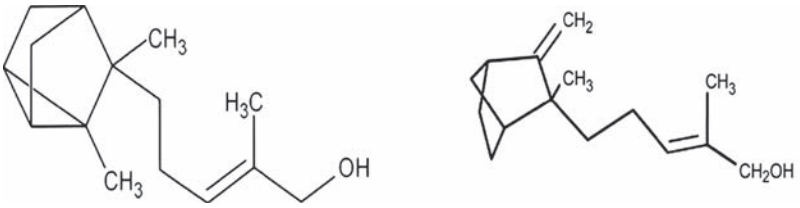
FIGURE 1 SW tree. *Photos taken by author from Bangalore University, Bangalore, Karnataka, India.*

Santalum yasi, *Santalum macgregorii*, *Santalum accuminatum*, *Santalum murrayanum*, *Santalum obtusifolium*, *Santalum lanceolatum*, *Santalum fernandezianum*, *Santalum salicifolium*, and *Santalum spicatum* (Subasinghe, 2013). *Santalum album* is the most valuable natural tree in the world plant kingdom that originated in India and hence the tree is known as Indian SW. Its importance in world market is due to its commercial high-quality EO. The generic name is derived from the Greek "*santalon*" meaning sandalwood, and the species name from the Latin "*albus*" meaning white, in allusion to the bark. It grows to a medium height of 30 feet. After 15–20 years the oil collects in the heartwood and the color of the wood becomes more yellow. It is considered at its peak at 60 years old. Bark varies in color, texture, and thickness. Leaves are ovate, lanceolate, elliptic, and linear in shape. They are both small and large in shape (Figure 1). The SW flowers range from light yellow to purple in color (Figure 2). The plant shows self incompatibility and the flowers are strictly adapted to cross-pollination by insects (Bhaskar, 1992). Young fruits are green, oval to spherical, variable in size, turning red to deep purple or black on maturity (Kulkarni and Srimathi, 1982). The trees blooms flower and bear fruits twice a year (during March–May and September–December), 36% once a year (September–December), and 4% throughout the year (Ananthapadmanabha et al., 1991). Seeds show polymorphic traits in size and shape (Annapurna et al., 2005). Three plant phenotypes have been recorded on the basis of the following features given in parentheses: (1) Thindlu (dark brown bark with irregular flakes and dark brown heartwood); (2) Chikkaballapur (small, bluish green leaves similar to spiked plant with large sapwood); and (3) Robust (compact crown with lush green foliage, thick sapwood and fast growth compared with the other two types) (Srimathi et al., 1983).

The oil is extracted from the heartwood plant by steam distillation which is estimated average ranges from 3% to 6%. The main constituent of SWO is santalol which is a mixture of two isomers, α-santalol and β-santalol. The characteristic odor and medicinal properties of SWO are mainly due to the santalols (Figure 3). All constituents present in SWO and some of the manufacturers and export industries in India are tabulated in Tables 1 and 2, respectively.

The trees are found in India, Nepal, Bangladesh, Sri Lanka, Australia, Indonesia, Hawaii, and other Pacific Islands. The populations are more concentrated in the southern region of India, especially Karnataka, Tamil Nadu, and Kerala. The plant is also grows in Gujarat, MP, UP, Manipur, etc. (Figure 4). A tract of sandal forests ranges from Kolhapur (Maharashtra) to Bellom (Mysore), and on to Chittoor (Andhra Pradesh), that forms the northern fringe of this region, Chittoor to Erode, and

FIGURE 2 SW leaves with berries. *Photos taken by author from Bangalore University, Bangalore, Karnataka, India.*



Alfa- santalol **Beta- santalol**

FIGURE 3 Structures of the constituents present in sandalwood essential oil. *Prepared by author by Integrated Scientific Information System (ISIS, published by MDL Information Systems) draw software.*

TABLE 1 Constituents Present in Sandalwood Plant (*Santalum album*)

Constituents Present	*Santalum album*
α-Santalene	0.5%
cis-α-Bergamotene	0.7%
Epi-β-santalene	1.1%
β-Santalene	0.3%
α-Santalol	48.7%
β-Santalol	32.6%
β-Bisabolol	0.5%
Z-α-*trans*-bergamotol	2.4%

Prepared by author (with the help of different literatures listed in reference section of the original manuscript).

TABLE 2 Some of the Important Manufacturer and Exporter Companies for Sandalwood Essential Oil in India

Name of the Company	Situated	Products
Government SW EO factory	Mysore	Manufacturer and exporter of SW EO
Karnataka Aromas	Bangalore	Exporter of pure SW EO
KFDC Ltd	Kerala	Manufacturer and exporter of SW EO
Suman Perfumers	UP	Manufacturer and exporter of SW EO
Ardor International Pvt. Ltd	Gujarat	Exporter of SW EO
Maplewood Trading Pvt. Ltd	Mumbai	Manufacturer and exporter of SW EO
Fragrances Manufacturer	Bangalore	Manufacturer and exporter of SW EO
Falcon	Bangalore	Manufacturer and exporter of SW EO

Prepared by author.

Mettuplayam (Tamil Nadu), that forms the eastern and southern boundaries further from Mettupalayam through Kerala to Mercara and Mangalore (Mysore) (Jain et al., 2007).

USAGE AND APPLICATIONS

The EO from the SW is used as medicine. SWO is been widely used in folk medicine for treatment of common colds, bronchitis, skin disorders, heart ailments, general weakness, fever, infection of the urinary tract, inflammation of the mouth and pharynx, liver and gallbladder complaints, and other maladies. In traditional medicine, SWO is used for the treatment of headache and stomach ache. In India, SW EO, emulsion, or paste of SW is used in the treatment of inflammatory and eruptive skin diseases (Dwivedi and Abu-Ghazaleh, 1997).

SWO has various medicinal applications including antiseptic, antispasmodic, astringent, antiphlogistic, carminative, disinfectant, diuretic, hypotensive, and memory enhancing properties (Desai and Hirenath, 1991; Burdock and Carabin, 2008). SWO balances the circulatory, digestive, respiratory, and nervous systems. Both SW paste and its EO are very effective as antiinflammatory agents (Li et al., 2013). They have a nice cooling effect and give relief from all types of inflammation in the brain, digestive system, nervous system, circulatory system, excretory system, etc. (Okugawa et al., 1995) resulting from infections, fever, side effects of antibiotics, poisoning, insect bites, wounds, etc. SWO gives relief from inflammations resulting from fever and viral infections due to its anti phlogistic properties (Benencia and Courreges, 1999). Being a relaxant and sedative in nature, this oil works well against spasm too. It relaxes nerves, muscles, and blood vessels and hence ends spasm or contraction. Thus it is helpful in treating problems associated with spasm, such as cramps, aches, coughs, etc. It also increases frequency and quantity of urination. This helps greatly in treating infections and inflammations in the urinary system. Its fragrance keeps away microbes and small insects and that is why it is extensively used in incense sticks, sprays, fumigants, and evaporators to disinfect whole surroundings. The SWO is taken with milk or with water to reduce blood pressure (Sindhu et al., 2010).

Externally, SWO or SW paste (made from mixing water with SW powder) is used for calming and cooling effects on the body and mind due to its emollient activity. It helps relieve fever and burns, and stops excessive sweating. The EO is used to heal skin diseases such as infectious sores, ulcers, acne, and rashes (Arun kumar et al., 2012). When applied to skin, it protects wounds, sores, boils, pimples, etc. from infection due to its antiseptic nature. SW powder helps smooth and cool the skin, and can be made into a paste, lotion, or soap for cleansing, calming, and hydrating sensitive or aging skin. It is used extensively in the production of incenses. This oil can be mixed with bathing water or other lotions or oils to apply on skin and wounds and ensure their protection from infections due to its disinfectant nature (Sindhu et al., 2010). Its antiseptic property is also helps to treat rashes, spots, blackheads, and other skin eruptions. Its germicidal quality inhibits the growth of bacteria. The EO is used as deodorant due to its strong odor (Brocke et al., 2008). It is also acts as skin moisturizer. SW paste tones up the skin. Due to its astringent nature, it is use for especially for oily skin (Desai, 1991).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Aroma is responsible for about 90% of our taste. Smell is the only sense that connects directly to the limbic lobe of the brain—the center of taste, emotion, and memory. EOs are 50–70 times more therapeutically potent due to more

FIGURE 4 Cultivation of sandalwood in different states of India. *Prepared by author.*

concentration than herbal extracts. In general EOs contains healing nutrients, oxygenating molecules, amino acid precursors, coenzyme A factors, trace minerals, enzymes, vitamins, hormones, etc. hence they are used in cooking and baking items. There is a vast number of food products are available in the market where addition of EO as a natural preservative increases their shelf life from months to years. These oils are also used for enhancing flavor in salad dressings, soups, stews, sauces, and even in soft drinks. EOs are added toward the end of cooking as high heat will destroy the therapeutic

benefits. Therapeutic grade EOs are generally safe for human consumption in small amounts as per approval given by the Food and Drug Administration (FDA). EOs are used extensively as generally recognized as safe flavoring agents in foods, beverages, and confectioneries according to strict good manufacturing practice and hence recommended quantities of therapeutic graded EOs are used to add flavor and fragrance to many foods and soft drinks.

SW EO is in demand and used by the food industries in India. In food and beverages, white SW is used in major categories of food products, including alcoholic and nonalcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, and puddings as a flavor enhancer with reported average maximum use levels generally below 0.001% after approval given by the FDA (Burdock and Carabin, 2008). SWO is used in the food industry as a flavor ingredient with a daily consumption of 0.0074 mg/kg (Burdock and Carabin, 2008). SWO blends well with various oils like bergamot, geranium, lavender, myrrh, and rose oil and is used for food preservation. SW powder mixed with milk or made into pills is beneficial in the treatment of dysuria. Its efficacy can be increased by the addition of ajwain water or infusion of ginger. The EO of SW is safe for internal uses due to its antiseptic nature. The EO of SW is also safe when taken with milk, to cure internal infections, such as in throat, esophagus, stomach, intestines, excretory tracts, etc. due to its disinfectant activity. The development of food preservation processes are became the need to extend the shelf life of foods. Application of EO vapors in active packaging and food preservation is a method that allows foods to be stored for longer. Meats, fish, fowl, cheese, fruit, and vegetables were among such foods. SW EO is helpful in packaging the foods like cucumbers, peppers, corned beef, herring, and eggs for longer periods. SW EO vapor is used to cover preservation of several foods and packaging of dry food items from spoilage of microorganisms due to its antimicrobial activity. The use of naturally occurring antimicrobial compounds for food preservation is receiving attention due to awareness about natural food products and a growing concern of microbial resistance toward conventional preservatives worldwide. Encapsulating antimicrobial SW EO in food products helps to protect it from heat and they also release it into products at a controlled rate to deliver effective inhibitory concentrations over extended periods, thereby extending shelf life. SW EO is also used in the food industry for flavor stabilization. SW EO acts as a good masking agent for unpleasant tastes and smells in the food products like meat and other meat products due to the presence of long-lasting strong sweet, woody, and spicy fragrance. SW EO helps to inhibit oxidative deterioration in meat products due to its antioxidant activity (Misra and Dey, 2012).

SW EO is also a very good health tonic for growing children. It helps to maintain harmony of functions in body systems as it is soothing on the stomach and digestive system, circulatory system, and nervous system. The EO of SW, being relaxant in nature, it is mixed with food and drinks that induce relaxation in the intestines and abdominal muscles, thereby facilitating removal of gases. Hence, it is acts as carminative. Chewing gums and all kinds of mouth refreshing confectioneries especially mints are have great market potential to become more attractive and freshness of EO plays a vital role in this. All confectioneries generally contain sweeteners, food grade acid, cooling agents especially mint, and flavoring agents especially EO of herbals plants. Chewing gum and bubble gum have a short-term impact on oral hygiene. SW EO is used in chewing gum and bubble gum to enhance flavor and taste. These products mask foul odors in the mouth by killing bacteria that are linked to bad breath, due to the antibacterial activity of the EO. SW EO has astringent properties that induce contraction in gums, strengthening the hold of gums on teeth. SW EO is used to give intense flavors to products like candies, frosting, and butter cream fillings (Hire and Dhale, 2012).

There are a number of EOs available to add flavor to baked products and SW EO plays a vital role with its strong sweet fragrance. The EO of SW is used as an additional ingredient in baking and also is used to add moisture and texture to backed products like cakes, cookies, puddings, etc. EOs are use in custard powder to enhance the flavor which is extensively used in preparation of bakery products like ice cream, curds, vegetable soups and cakes. SW EO is used in custard powder as one to two drops provides purity, keeps it contamination free, provides a rich flavour, and extends shelf-life of the product. SW EO is also used in bakeries as a bread improver powder, widely used in the preparation of delicious and aromatic food products like biscuits, creams, etc. SW EO provides freshness and a rich flavor. Packaging of baked products is also carried out with SW EO vapor. The antifungal activity of SW EO helps to minimize fungal growth of baked products over longer storage (Chourasia and Rao, 1987). Baked products include cookies, namkeen, dry cake, vegetable patties, biscuits, etc. Cacao absolute is perfect in creams that are blended with SW EO and other EOs. Hence the blended cacao creams are used in flavored biscuit preparations. The creams are spread in between two biscuits to make a sandwich. After approval from the FDA, SWO was used in the preparation of different kinds of candies and chocolates. Chocolate is becoming one of the most popular food types and flavors in the world and is also used in cold and hot beverages, to produce chocolate milk and hot chocolate. The main essential ingredient is cacao and the seeds of the cacao tree have an intense bitter taste. Hence, it is fermented to develop the flavor during chocolate preparation. Blended cacao in chocolate helps to decrease depression, improves general health, and also stimulates sex hormones. Hence, cacao is blended with SW EO and other EOs and then used in chocolate preparations. These kinds of chocolates reduce anxiety and calm the mind due to a high percentage of α-santalol in the SW EO (Hongratanaworakit et al., 2004).

The demand of alcoholic beverages products like beer and wine, and other alcoholic beverages such as brandy, whiskies, rum, gin, etc. have increased in global market and India is the third largest market for alcoholic beverages in the world. When making a new alcoholic spirit the EOs must be added to alcohol dropwise, dissolved, and other additives are added for consumption of the alcoholic beverages. This method is followed for enhancing flavor in alcoholic preparations, such as when adding natural EO from SW to vodkas and liqueurs like brandy as a primary or supplemental flavor. The sweet, powerful, and lasting odors makes SWO useful in the alcohol beverage industry. SW EO is also used in nonalcoholic beverages like fruit and vegetable juices and tonic preparations. In nonalcoholic beverages, flavored cream can be prepared by adding two drops of SW EO to drinks to keet the nonalcoholic drink fresh and delicious. The development of the soft drink industries in India is of great importance because it is a major consumer of EOs, especially of SW EO. The EO of SW is used for preparing soft drinks and help in preservation without spoiling the appearance due to microbial effect of the beverage or causing unpleasant taste in soda, pop, fizzy drink, etc.

Soda water, carbonated water, fizzy water, and other seltzer waters are also the focus of consumers worldwide because of the water being effervescent. Carbonated water is the defining ingredient of carbonated soft drinks. Sparkling water with the natural flavor of EOs provides calorie-free refreshment. Both carbonated water and mineral water are usually calorie free. Flavored carbonated water is often consumed plain or mixed with fruit juice. It is also mixed with alcoholic beverages to make cocktails, such as whisky, soda, or campari soda. Flavored carbonated water prepared with SW EO is also commercially available. Therapeutic grade SW EO is added in mineral water in dropwise to enhance the flavor of the water and to make freshness of the mind (Heubeger et al., 2006) and prevent dental erosion (Parry et al., 2001). SW EO is a preferred EO to add into water because of its sweet pleasant taste and it is also acts as an antioxidant. One of the benefits of adding therapeutic grade SW EO in water is to eliminate toxins, petroleum residues, metals, and other foreign substances without any side effects. SW EO is also added in soda water to increase metabolism and soothe digestive issues.

SUMMARY POINTS

- Indian SW is said to be in danger of extinction species now and hence is very costly.
- Wood and EO obtained from the heartwood of SW are the main useful parts for commercial purpose.
- Steam distillation is the preferred process for the extraction of EO from dried heartwood of SW plant material from the consumer's point of view.
- SW contains 3–6% volatile oil (mainly the sesquiterpenols like α- and β-santalol), resin, and tannins.
- The EO of SW is beneficially made use of in aromatherapy, perfumery, cosmetics, and soap production.
- Therapeutic grade EO from SW is used in flavor food supplements, beverages, candy, baked products, puddings, and other soft drinks in a very low concentration at below 0.001%.
- SW EO is used in alcoholic and nonalcoholic beverages in liquid as well as vapor form for enhancing flavor and as a preservative for storage of the nonalcoholic beverages, respectively.
- α-Santalol is the main chemical present in the SW EO. It helps body freshness, reduces anxiety, and provides energy by keeping brain cool.

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Chapter 83

Shirazi thyme (*Zataria multiflora* Boiss) Oils

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List of Abbreviations

A. flavus *Aspergillus flavus*
A. niger *Aspergillus niger*
A. parasiticus *Aspergillus parasiticus*
A. solani *Alternaria solani*
B. cereus *Bacillus cereus*
B. subtilis *Bacillus subtilis*
C. botulinum *Clostridium botulinum*
C. maculatus *Callosobruchus maculatus*
E. faecalis *Enterococcus faecalis*
E. coli *Escherichia coli*
EO Essential oil
F. gramineum *Fusarium gramineum*
L. monocytogenes *Listeria monocytogenes*
MBC Minimum bactericidal concentration
MIC Minimum inhibitory concentration
P. aeruginosa *Pseudomonas aeruginosa*
P. citrinum *Pinicillium citrinum*
P. interpunctella *Plodia interpunctella*
S. aureus *Staphylococcus aureus*
S. enterica* subsp. *Enterica *Salmonella enterica* subsp. *Enterica*
S. enteritidis *Salmonella enteritidis*
S. flexneri *Shigella flexneri*
S. marcescens *Serratia marcescens*
S. typhi *Salmonella typhi*
S. typhimurium *Salmonella typhimurium*
TDH Thermostable direct haemolysin
Vibrio parahaemolyticus *V. parahaemolyticus*
X.s citri* pv. *Citri *Xanthomonas citri* pv. *Citri*

INTRODUCTION

During the past decades, natural medicine has received considerable attention due to increased consumer concerns regarding the adverse side effects of synthetic chemical compounds used in treating disease and improving health status. In addition, there is a growing demand for natural food products via replacing synthetic chemical additives with naturally occurring alternatives to extend shelf life and/or increase the safety of foods. These natural compounds are derived from animal, plant, and microbial origins. Since ancient times, aromatic plants have been traditionally used for different purposes including medicine and food throughout the world. Essential oils are the best known derivative of these plants, composed of complex natural mixtures of volatile secondary metabolites, mainly terpenes. They are obtained from different parts of plants including flowers, leaves, seeds, bark, and tubers by hydrodistillation, steam distillation, or expression methods. They have a wide-spectrum of biological effects make them appropriate to be used for novel targets. The aim of this chapter

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is to provide an overview on *Zataria multiflora* Boiss. from standpoints of botanical aspects and the application and usage issues of its essential oil (EO) in traditional and modern medicine and more importantly its potential applications in food science.

BOTANICAL ASPECTS

Zataria multiflora Boiss. also called *Zataria bracteata* Boiss. and *Z. multiflora* var. elatior Boiss. is a member of the Lamiaceae family. Labiatae is the other name for this family. In Iran, the Lamiaceae family is well identified by 46 genera and 410 species and subspecies. The largest genus of this family is *Salvia* which has about 800 species discovered worldwide. *Zataria* is another important genus of this family. The plant genus name is derived from the Arabic word "*Za' atar*", a generic name for some Middle Eastern herbs like Thyme, Oregano, and Savory. *Zataria multiflora* Boiss. is a thyme-like plant known as "*Avishan-e-Shirazi*" in Iran. This name comes from Avishan meaning thyme, and Shiraz, a state of Iran. Geographically, it grows wild only in central and southern Iran (Shiraz, Isfahan, Yazd, Dezfol, Kerman, and Khorasan), Pakistan, and Afghanistan (Sajed et al., 2013). In order to recognize *Z. multiflora* Boiss., the ovate, orbicular, densely gland-dotted leaves, and the dense white hairy circular buds on the leaf axils should be considered. It is an aromatic bush, 40–80 cm height, and its young branches are white with dense glandular, spreading, pilose indumentums while mature ones are woody and without any leaves. The flowering stems do not have any branches. The leaves (5–10 × 5–10 mm) look orbicular ovate to orbicular. The flowers of *Z. multiflora* Boiss. are very small and often male sterile, white, and subsessile. The aerial parts are collected, depending on flowering time, in late May up to early November. The leaves and flowers of this plant are used for medicinal purposes. The aerial branch of this plant contains at least 0.6% EO, fatty acids, oleanolic acid, β-sitosterol, and betolin. In spite of high variation in chemical composition of the EO from different ecotypes of this plant, it has been well documented that oxygenated monoterpenic compounds, mainly thymol and carvacrol, are major components of this EO; these two compounds constituted 30–86% of total EO composition evaluated in different experiments. Carvacrol is a monoterpenic phenol isomer of thymol that presents as the main component in the dried plant, where thymol was found to be the most abundant constituent in fresh plant. Other reported main compounds include ϱ-cymene, γ-terpinene, caryophylene, linalool, and α-pinene.

USAGE AND APPLICATIONS

Zataria multiflora Boiss. plant and its derivatives have been long used in folk medicine and healthcare. It is traditionally consumed in forms of decoction, infusion, or vapor as a remedy for headache, common cold, migraine, bone pain, joint pain, pregnancy pain, diarrhea, vomiting, fever, bloating, dyspepsia, and also as a vermifuge, stimulant, diaphoretic, and diuretic in folk medicine (Gupta and Gupta, 1972; Ghasemi Dehkordi, 2002; Naghibi et al., 2005). There has been a growing interest to address the basis of medicinal applications of its derivatives, especially EO, through modern pharmacological and biological studies. These effects include antidiabetic, antinociceptive, antiinflammatory, and immunostimulatory activities as well as regulation of *MDM2* and *ATM* gene expression and modulation of hematological and immunological parameters (Sajed et al., 2013). There are various commercial pharmaceutical forms of *Z. multiflora* Boiss. EO produced in Iran. These forms include syrup, oral drops, vaginal cream, and soft capsule (Table 1).

USAGE AND APPLICATIONS IN FOOD SCIENCE

For centuries, *Z. multiflora* Boiss. has been traditionally used as a flavoring agent in a vareity of foods without considering its special properties. However, in the past decades there has been increasing interest to demonstrate the biological benefits of using this plant as EO in foods, especially in term of biopreservation. These effects include antibacterial, antifungal, antiinsect, and antioxidant activities. It has been well documented that this EO has a strong antibacterial activity against different spoilage bacteria and food-borne bacterial pathogens investigated in laboratory culture media. However, the reported minimum inhibitory concentration (MIC) values vary among different studies based on the EO composition, bacterial strains, and method of antibacterial evaluation. Sharififar et al. (2007) investigated antibacterial effects of *Z. multiflora* Boiss. EO on some bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Serratia marcescens*, and *Shigella flexneri* and found the MIC values ranged from 2.4 to 44 mg/mL. In a study by Zomorodian et al. (2011), *Z. multiflora* Boiss. EO inhibited the growth of *S. aureus*, *E. faecalis*, enterohemorrhagic *E. coli*, *Pseudomonas aeruginosa*, *S. flexneri*, and *Salmonella enterica* subsp. *enterica* at concentrations ranging from 0.12 to 8 µl/mL. Furthermore, it was shown by Khanjari et al. (2011) that this EO inhibited the growth of *Vibrio parahaemolyticus* in a dose-dependant manner. The antibacterial effects of *Z. multiflora* Boiss. EO have been attributed to the high amount

TABLE 1 Commercial Forms of *Zataria multiflora* Essential Oil Produced in Iran

Product Name	Active Ingredient	Dosage Form	Application
Broncho T.D.	*Zataria multiflora* EO with1.0–1.5 mg thymol in 120 mL of syrup.	Syrup	Cough, bronchitis and laryngitis
Gastrolit	*Zataria multiflora* EO containing at least 4.4–7.0 mg thymol in 1 mL of drop.	Oral drop	Symptoms of irritable bowel syndrome
Gastrolit	*Zataria multiflora* EO with at least 5.5–6.5 mg thymol in each soft capsule.	Soft capsule	Symptoms of irritable bowel syndrome
Leucorex	*Zataria multiflora* EO containing 0.027–0.033% thymol.	Vaginal cream	Candidiasis, trichomoniasis, and infection by *Gardnerella vaginalis*
Thymogel	*Zataria multiflora* and *Foeniculum vulgare* EOs with at least 5.4–6.6 mg thymol and 14–18 mg anethol in each capsule.	Soft capsule	Acute cough
Tussivin	*Zataria multiflora* and *Foeniculum vulgare* EOs containing at least 2.2–3.5 mg thymol and 5.5–8.5 mg anethol in each mL of drop.	Oral drop	Acute cough

Adapted from Sajed et al. (2013).

of phenolic compounds, especially carvarol and thymol. It was shown by bacterial inactivation kinetic assay that this EO was able to kill *E. coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* at minimum bactericidal concentration within 1.5, 1.5, and 0.5 h, respectively, indicating rapid inactivating effect on bacterial cells. In addition, the antibacterial properties of *Z. multiflora* Boiss. EO in combination with other antimicrobials have been well investigated. Misaghi and Basti (2007) reported a synergistic antibacterial effect of this EO and nisin on *Bacillus cereus* that was enhanced in lower pH values (6.5 and 6.0). It has been indicated that combination of *Z. multiflora* Boiss. EO with acetic acid decreased the inhibitory concentration of EO against *C. botulinum*. Moreover, it was reported that combination of this EO and nisin improved antimicrobial activity of the EO against *L. monocytogenes*; the MIC of the EO decreased from 2.4 mg/mL when used alone to 1.2 mg/mL when used in combination with nisin. Furthermore, Basti et al. (2007) developed mathematical models to predict the growth of *Salmonella typhimurium* and *S. aureus* as affected by different concentrations of *Z. multiflora* Boiss. EO in different pH and temperature. A significant interaction was observed between three studied factors. In addition to the inhibitory effect of this EO on growth of bacterial cell, it was demonstrated that this EO prevented enterotoxin production by toxigenic food borne bacteria. Parsaeimehr et al. (2010) showed that this EO inhibited enterotoxin C production by *S. aureus* at concentration of 0.015% during 43 days incubation. They also stated that combination of this EO with nisin reduced the inhibitory concentration to 0.005%. It was shown by Azizkhani et al. (2013) that this EO decreased the expression of enterotoxin A, C, and E genes in *S. aureus* in a dose-dependant manner. Furthermore, it was indicated that *Z. multiflora* Boiss. EO decreased the thermostable direct haemolysin titer produced by *V. parahaemolyticus*.

It has been documented through several experiments that *Zataria multiflora* Boiss. EO could be incorporated to the different edible films or coatings to develop antibacterial packaging. Shakeri et al. (2011) investigated the antibacterial properties of *Z. multiflora* Boiss EO incorporated whey protein based film on some food borne pathogenic bacteria. All tested bacteria were inhibited by addition of 2% EO to whey protein isolate film. Salarbashi et al. (2014) developed a soluble soybean polysaccharide film containing *Z. multiflora* Boiss EO that effectively inhibited *S. aureus*, *B. cereus*, *E. coli* O157:H7, *P. aeruginosa* and *Salmonella typhimurium*. Moreover, Ghasemi et al. (2011) prepared an edible active packaging composed of zein and *Z. multiflora* Boiss. EO that exhibited antibacterial effects on *Salmonella enteritidis*, *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus*.

Several experiments evaluated the potential application of this EO as a natural antibacterial preservative in different foods, including dairy, meat, and fish products to enhance shelf life and/or improve the safety of the products. Noori et al. (2011) found that *Zataria multiflora* Boiss. EO at concentration of 0.03% reduced *E. coli* O157:H7 population in minced beef meat during refrigeration storage. It was indicated by Choobkar et al. (2012) that light salted fillets of silver carp treated through dipping in different concentrations of this EO, showed lower *S. aureus* counts during storage compared to the control. Furthermore, Mohammadi et al. (2011) evaluated the antibacterial effect of *Z. multiflora* Boiss. EO on behavior of *E. coli* O157:H7 during production and storage of white brined cheese and reported that the EO concentration of 200 ppm significantly decreased bacterial count during the experiment. There are some evidences that

combination of *Z. multiflora* Boiss. EO with other preservatives resulted in synergistic effect. In this regards, Ekhtiarzadeh et al. (2012) showed that combination of this EO and nisin enhanced antibacterial activity against *L. monocytogenes* and *Vibrio parahaemolyticus* in salted fish fillet. Similar effects were observed in experiments evaluated the impact of this combination on shelf life of silver carp and rainbow trout fillet. Furthermore, Moosavy et al. (2008) indicated through a factorial design that nisin effectively lowered the inhibitory concentrations of *Z. multiflora* Boiss. EO against *S. aureus* and *S. typhimurium* in barley soup. It was shown that 50 ppm of this EO was able to effectively control the growth of *S. aureus* and *L. monocytogenes* during the manufacture, ripening, and storage of white brined cheese (Abbasifar et al., 2009). The study also indicated that combination of *Z. multiflora* Boiss. EO and starter culture exhibited a synergistic effect on bacterial inhibition.

Antifungal properties are an important attribute of *Z. multiflora* Boiss. EO widely considered by food science researchers. These effects include growth inhibition, repression of spore formation, and prevention of mycotoxin production. It was reported that this EO inhibited the growth and deoxynivalenol production by *Fusarium graminearum*. It was shown that *Alternaria solani*, a plant fungal pathogen influenced both growing plants and fruits especially tomato, was inhibited completely by this EO at concentration of 400 ppm. Furthermore, Fakour et al. (2007) stated that *Z. multiflora* Boiss. EO showed a strong growth inhibition of aflatoxigenic *A. parasiticus*. The growth prevention of *A. flavus* by this EO has been reported. The findings by Gandomi et al. (2009) indicated that *Z. multiflora* Boiss. EO completely inhibited *Aspergillus flavus* growth at 200 ppm in broth medium and induced a sever decrease of aflatoxin production at lower concentrations. It is suggested that the antifungal effects of this EO may be due to the structural abnormalities ranged from vacuolization of cytoplasm to detachment of the cell membrane from the cell wall and shedding the cytoplasm from the cell (Gandomi et al., 2011). Preventing spore production may be an important strategy for preserving agricultural commodities from fungal invasion. In this regards, it has been well documented that *Z. multiflora* Boiss. EO is a good candidate for lowering spore formation by *A. flavus*. Furthermore, Abollahi et al. (2011) indicated that this EO inhibited growth, spore germination, and spore production in *Aspergillus niger*. Moreover, Akrami Mohajeri et al. (2012) stated that this EO was able to control growth and spore production of *Penicillium citrinum*. There is some evidence of the potential usage of *Z. multiflora* Boiss. EO in different food products to control fungal growth and mycotoxin production. Gandomi et al. (2009) showed in a study that this EO effectively inhibited the mycelial growth of *A. flavus* on Iranian ultrafiltered white cheese and decreased aflatoxin production by 71.2% at concentration of 200 ppm. Furthermore, growth inhibition and reduction of citrinin production in Mozzarella cheese are important findings stated by Noori et al. (2012). In a study on the preservative effects of *Z. multiflora* Boiss. EO in sponge cake, the EO reduced fungal growth during storage. The effect of *Z. multiflora* Boiss. on growth of *A. niger* in lime fruits was evaluated. It was revealed that fruit rot was decreased by 85% in citrus fruits treated with 600 ppm of this EO.

The potential application of *Z. multiflora* Boiss. EO as a pesticide for protecting growing plants and stored crop from bacterial and insect invasion is a promising usage of this EO that documented in some experiments. It was reported that this EO induced inhibition of *Xanthomonas citri* pv. *citri*, the causative agent of citrus bacterial canker, using detached-leaf assays and whole seedlings in the greenhouse. Mahmoudvand et al. (2011) evaluated the fumigant toxicity of different EOs including *Z. multiflora* Boiss. against different storage pests. They reported that this EO had a good effect, especially on *Plodia interpunctella*, a major pest of stored products including nuts, beans, processed foods, and dried fruits. Furthermore, it is indicated that this EO was able to kill eggs, larvae, and adults of *Callosobruchus maculatus*, a major insect pest of the grains. Similar findings were observed by Golestani et al. (2012).

The antioxidant capacity is another prominent property of *Z. multiflora* Boiss. EO demonstrated by several researches. Radical scavenging is one of the mechanisms involved in antioxidant activity of this EO. It was reported that *Z. multiflora* Boiss. EO collected from different parts of Iran showed strong antioxidant activity evaluated through 2,2-diphenyl-1-picrylhydrazyl assay. Their results indicated that radical scavenging activity of some EO samples was comparable to butylated hydroxytoluene (BHT); IC_{50} value of 19.7 μg/mL was obtained for this EO compared with 18.1 μg/mL for BHT. Some studies reported the potential application of *Z. multiflora* Boiss. EO as an antioxidant agent directly added to food matrix or incorporated in different coatings and films. The antioxidant preservative effect of this EO in a sponge cake was demonstrated by Kordsardouei et al. (2013). Also, it was shown that this EO increased oxidative stability of soybean oil in an accelerated oxidative condition. Moreover, it was observed that chitosan film incorporated with this EO presented antioxidant activity. In addition, Zakipour Rahimabadi and Divband (2012) evaluated the chemical stability of silver carp fillet treated with *Z. multiflora* Boiss. EO and an edible coating during refrigeration storage. This EO showed protective effects against oxidation at concentration of 0.2%. It was found that the lipid oxidation of Cobia fish fillets treated with 500 ppm of this EO was retarded compared with untreated samples during frozen storage at −18 °C for 6 months (Taheri et al., 2013). Furthermore, it was stated that this EO could be used as a natural antioxidant in vegetable oils; sunflower and linseed oils containing this EO showed more oxidative stability during storage compared with the control.

SUMMARY POINTS

- *Zataria multiflora* Boiss. is a member of the Lamiaceae family.
- Oxygenated monoterpenic compounds mainley thymol and carvacrol are major components of *Zataria multiflora* Boiss EO.
- This EO possesses different pharmacological effects.
- Commercial pharmaceutical forms of this EO include syrup, oral drops, vaginal cream, and soft capsules.
- It is traditionally used as flavoring agent in a variety of foods.
- Antibacterial, antifungal, antioxidant, and antiinsect effects of this EO are well documented through in vitro and food-based experiments.

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Chapter 84

Spiked Ginger Lily (*Hedychium* spp.) Oils

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List of Abbreviations

A *Hedychium venustum*
B *H. spicatum* var. *acuminatum*
C *H. coronarium*
D *H. flavescens*;
E Positive control

BOTANICAL ASPECTS

Hedychium Koenig is a genus of the Zingiberaceae family of flowering plants (Angiosperms). Species of *Hedychium* were named as "Ginger Lily" or "Garland Flowers" by Rumphius in the mid-eighteenth century; however, botanically, he named them as *Gandasulinum*. Later, Koenig correctly named them as *Hedychium* species. The species of *Hedychium* bear beautiful and often aromatic flowers. Owing to the sweet scented flowers, many of them are cultivated and used as popular flowers for ornamenting women's hair in Eastern Asia. Underground tuberous rhizomes or sometimes entire plant of *Hedychium coronarium*, *Hedychium spicatum*, and *Hedychium flavum* yield essential oils used in delicate and high-quality perfumes besides in traditional system of medicine.

Hedychium species, sometimes also called as "Butterfly Lily" are highly evolved plant taxa found distributed in Madagascar (Africa), tropical and warm temperate Asia, preferring mountainous terrains. A total of 87 species of *Hedychium* has been reported worldwide (Plant List, 2010). The north east India, Indo–China and Indo–Malaya regions seem to be the center of diversity in Zingiberaceae with the maximum number of species growing here. China has 28 species of which 18 are endemic (Wu and Larsen, 2000), while India has 34 species (Table 1) of which 16 species are endemic (Plant List, 2010; Srivastava, 2001). Considering large number of endemic species, north east India is one of the hot spots of the *Hedychium* genus. In fact, the state of Meghalaya in north east India harbors 32 species, the highest in the Indian states. This high diversity gradually reduces along the Himalayan Mountains toward the west with Sikkim having 10 species, Uttarakhand seven species, Himachal Pradesh two species, and no reported species in Jammu and Kashmir at the westernmost end (Srivastava, 2001; Kumar, 2001; Uniyal, 2007).

Hedychium spicatum is an exclusively Himalayan species distributed wildly in Himachal Pradesh, Uttarakhand, Nepal, and Sikkim. It is known as "*Shati* and *Kapoor Kachri*" in Ayurveda (the indigenous medicinal system of India) and used for treatment of various ailments (Sharma, 1998). This species is also well documented in ethanobotanical reports of India and used by natives for the treatment of asthma, bronchitis, eye diseases, gastric diseases, and as a tonic and blood purifier (Jain, 1991).

Hedychium spicatum is annual-perennial, rhizomatous, erect herb, pseudostem leafy, and 5–150 cm high. Leaves are broadly ovate-lanceolate, 30–60 cm × 10–20 cm, acuminate, glabrous above, sparsely pubescent beneath. Flowers are fragrant, white with an orange–yellow or red base, in dense, terminal, 15–25 cm long spikes. Floral bracts are large, green, one-flowered. Calyx are membranous, three-lobed, ovate, obtuse, shorter than bracts. Corolla tubes are 5–6.5 cm long, much longer than calyxes, corolla lobes white, linear, spreading. Filament of stamen is red. Labellum is white with two-elliptic lobes and orange and yellow base. Capsule globular is three-valved with an orange–red lining. Seeds are black with red aril (Gaur, 1999). It is common in damp shady places, steep slopes, and grassy localities in 1000–3000 m altitude areas in the Himalayas. Flowering and fruiting can be noticed during July to October months.

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TABLE 1 Species of *Hedychium* Occurring in India

S. N.	Species	S. N.	Species
1	*Hedychium aureum* C.BClarke & H. Mann ex Baker	18	*Hedychium greenii* W.W.Sm.
2	*Hedychium calcaratum* A.S.Rao & D.M.Verma	19	*Hedychium griffithianum* Wall.
3	*Hedychium coccineum* Buch.– Ham. ex Sm.	20	*Hedychium hookeri* C.B. Clarke ex Baker
4	*Hedychium coronarium* J.Konig	21	*Hedychium longipedunculatum* Sastry & D.M.Verma
5	*Hedychium dekianum* A.S.Rao & D.M. Verma	22	*Hedychium luteum* Baker
6	*Hedychium densiflorum* Wall.	23	*Hedychium marginatum* C.BClarke
7	*Hedychium edwardsii* C.E.C.Fisch	24	*Hedychium pynursulaeanum* S.K.Jain & S.C.Srivast.
8	*Hedychium elatum* R.Br.	25	*Hedychium radiatum* A.S.Rao & Hajra
9	*Hedychium ellipticum* Buch.–Ham. ex Sm.	26	*Hedychium robustum* A.S.Rao & Hajra
10	*Hedychium elwesii* Baker	27	*Hedychium rubrum* A./S.Rao & D.M.Verma
11	*Hedychium flavescens* Carey ex Roscoe	28	*Hedychium speciosum* Wall.
12	*Hedychium flavum* Roxb. (=*Hedychium* urophyllum)	29	*Hedychium spicatum* Sm.
13	*Hedychium gardnerianum* Sheppard ex Ker. Gawl.	30	*Hedychium stenopetalum* Lodd.
14	*Hedychium gomezianum* Wall.	31	*Hedychium thyrsiflorme* Sm.
15	*Hedychium gracile* Roxb.	32	*Hedychium venustum* Wight
16	*Hedychium gracillimum* A.S.Rao & D.M.Verma	33	*Hedychium villosum* Wall.
17	*Hedychium gratum* Wall. ex Voigt	34	*Hedychium wardii* C.E.C.Fisch.

INTRODUCTION

With an increasing health awareness, consumers are becoming uncomfortable with the thought of having their food treated to various chemicals to boost its shelf life. The food industry is not concerned enough about the wellbeing of its customers. Too often, profitability and cost effectiveness are prioritized above the health and safety of the consumer. For example, in the meat packing industries, the common antioxidant, butylated hydroxytoluene (BHT), is still in use even though it has long been reported as a cancer promoter (Umemura, and Kodama, 2006). An essential oil is a mixture of volatile and aromatic compounds. These aromatic compounds have a tendency to create a hostile environment for pathogens, and can be characterized by the presence of particular aroma compounds terpenoids they contain.

Terpenoids are volatile organic compounds that are odoriferous constituents of essential oils. They contain carbon, hydrogen, and\or oxygen and are not essentially aromatic in character. A common feature of terpenes is that the carbon skeleton of molecules is built up of isoprene (C_5H_8) units. They are therefore classified into different categories depending on the number of isoprene units present (Table 2). The carbon skeleton of terpenes is divisible into isoprene units which are generally joined in a head to tail fashion, that is, the C_1 position of one unit is linked to C_4 position of another. This is known as the "special isoprene rule" and that is why terpenoids are also known as isoprenoids (Mukherjee, 2006). Terpenoids are derived from the universal C_5 precursor isopentyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate, which is generated from two independent pathways located in separate intracellular compartments. In the cytosol, IPP is derived from the well-known mevalonic acid (MVA) pathway that starts with the condensation of acetyl CoA (Newman and Chappell, 1999). In plastids, IPP is formed from pyruvate and glyceraldehyde 3-phosphate. This MVA-independent pathway is also called MEP pathway after the key intermediate methylerythritol phosphate (MEP) (Lichtenthaler, 1999). Essential oils are very effective as antimicrobial and antifungal agents. But even more interesting, many terpenes are already used as flavorants (such as lemon and coriander flavors) and nutritional supplements (such as vitamin A). Many of the essential oils or chemical components are already used in many industrially manufactured foods, not as preservatives. Many essential oils also contain phenols, which are among the most powerful antioxidants known. Thus, not only are the highly phenol-containing essential oils such as wintergreen or clove oils effective flavorants, antifungals, and antimicrobials, they also posses antioxidant properties which can preserve food and give it unexpected health benefits (Bhat et al., 2011). In the present chapter we discuss the uses of ginger lily essential oil as food preservative.

TABLE 2 Major Classes of Plant Terpenoids

No. of Carbons	Class	Parent Unit	Subgroup	Occurrence
5	Hemiterpenoids	IPP	Tuliposides	Essential oil
10	Monoterpenoids (hydrocarbon and oxygenated)	GPP	Iridoids	Essential oil
15	Sesquiterpenoids (hydrocarbon and oxygenated)	FPP	Abscisic acid	Essential oil, resins
20	Diterpenoids (nonoxygenated and oxygenated)	GGPP	Gibberelins clerodanes	Resins, Bitter heartwood
25	Sesterterpenoids (nonoxygenated and oxygenated)	GFPP	Tiglianes	Resins Bitter heartwood
30	Triterpenoids	Squalene	Phytosterols, phytoecdysterols, cardenolides, cucurbitacins, saponins	Resin heartwood, latex
40	Tetraterpenoids	Phytoene	Carotenoids, xanthophylls	Pigments, green tissue latex fruit
10^3–10^4	Polyterpenoids	GGPP	Rubber, balata gutta	Latex, root

IPP, isopentyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranyl geranyl pyrophosphate; GFPP, geranyl farnesyl pyrophosphate

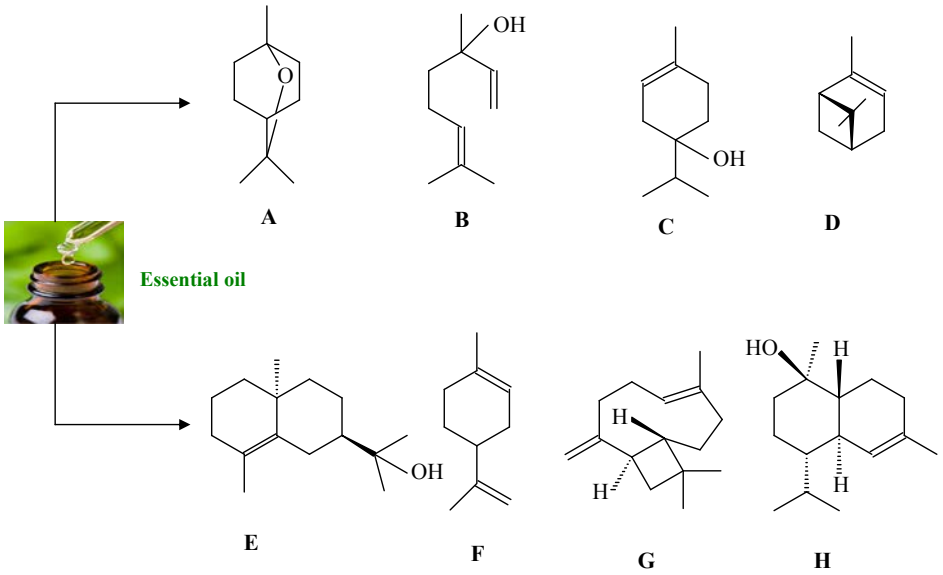
Usage and Applications

Ginger spiked lily (*Hedychium)* belongs to the family Zingiberaceae (spice family) which is well known for its uses worldwide as food flavors, spices, ornamentals, horticulture, cosmetic, cytotoxic agents, and in the indigenous system of medicine (Sharma, 1998). *Hedychium* spp. find applications in the ancient system of medicine and in the indigenous system of traditional foods and medicine. It has been reported that the essential oil of *Hedychium spicatum* completely inhibited the growth of seed-borne pathogens like *Helminthosporium oryzae* (*Cochliobolus miyobeanus*) and *Fusarium moniliforme* (*Gibberella fujikurai*). The essential oils of *H. spicatum* and *H. coronarium* have been reported to possess a short-lived mild tranquilizing action. It results in a depressed condition, response avoidance, and retarded performance like potentiated pentrobarbetone hypnosis and morphine analgesia in rats (Mishra et al., 2003; Dixit and Verma, 1979). Various pharmacological activities like antihelmentic, hepatoprotective, analgesic, antipyretic, antiinflammatory, etc. have been reported in *H. spicatum* and *H.coronarium* (Joshi and Mishra, 2011; Shrimal and Sharma, 1984; Prakash et al., 2005). Ghildiyal et al. (2012) reported antihistaminic and ulcer-protective activities of aqueous and ethanolic extracts of the dried rhizome of *H. spicatum* in guinea pigs, antiinflammatory and analgesic activities in rats, and acute toxicity in mice. It has been reported that neither extract showed any toxic effect, like increased motor activity, salivation, colonic convulsion, coma, or death in mice even at the 2000 mg/kg dose (nearly 10 times the optimal effective dose), indicating the safety of the extracts. The result also confirms the safe indigenous use of this plant in respiratory disorders. Because of its pleasant spicy aroma and taste, 1,8-cineol (eucalyptol) has been reported to be used in flavorings, fragrances, and cosmetics. The cineole-based eucalyptus oil has been reported to be used as a flavoring in various products, including baked goods, confectionery, meat products, and beverages (Harborne and Baxter, 2001).

Usage and Applications in Food Science

Food is the first requirement for human beings to sustain life. Packaged foods are becoming popular practice. To sustain food quality in its natural form, synthetic preservatives, like sodium benzoate and BHT are used. The artificial food preservatives are reported to keep food safe and cause relatively little trouble to the consumer, but it is often hard to know the long-term effects of all the chemicals that are added to foods and some food preservatives have been reported to be carcinogenic. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, and also as protectants against herbivores by reducing their appetite for such plants. They are also reported to attract some insects to favor the dispersion of pollens and seeds, or to repel undesirable ones. Essential oils or some of their components are used in perfumes and make-up products, sanitary products, dentistry, agriculture, as food preservers, additives, and in natural remedies. D-limonene,

FIGURE 1 (a) and (b) *Hedychium spicatum*, (c) *Hedychium coronarium*.



A= 1, 8-cineole, B=Linalool, C= Terpin-4-ol, D =α-pinene, E= 10-epi-γ-eudesmol,F= limonen,G= β-caryophyllene, H= α-cadinol

FIGURE 2 Some major marker compounds in the essential oils of *Hedychium* species.

geranyl acetate, or D-carvone are employed in perfumes, creams, soaps, and flavor additives for food (Masotti et al., 2003; Silva et al., 2003; Hajhashemi et al., 2003; Perry et al., 2003). 1,8-cineole, linalool, caryophyllene oxide, α-cadinol, eudesmol are the major marker compounds of the family Zingiberaceae that includes *Hedychium* spp. (Figure 1) (Prakash et al., 2010; Joshi et al., 2008; Sabulal et al., 2007). The antimicrobial activities of these compounds in their pure form and in essential oils have been reported (Hammer et al., 2010; Hendry et al., 2009). These studies prove that the essential oils containing these active constituents along with other complex mixtures of terpenoids can be the good and eco-friendly natural food preservative (Figure 2). The essential oils of *Hedychium* spp. in particular can be a good and safe source of natural food preservative, as evidenced by the studies reported by us and other workers from different parts of the world.

Chemical Composition

The essential oils of ginger lily (*Hedychium* spp.) are a complex cocktail of mono and sesquiterpenoides, mainly composed of hydrocarbons and oxygenated compounds. In continuation to our research on the family Zingiberaceae, we (Prakash et al., 2010; Joshi et al., 2008) reported a detailed study on essential oil composition of some *Hedychium* spp. and their comparison with earlier reports already existing in the literature. The results obtained and recorded in Tables 3 and 4 revealed that the major marker compounds are 1,8-cineol, linalool, limonene, terpinene-4-ol, etc.

TABLE 3 Comparative of Essential Oil Composition of *Hedychium spicatum* Collected from Different Regions

No.	Compounds	Kovat Indices	Garg et al. (1977)	Dixit et al. (1977)	Nigam et al. (1979)	Bottini et al. (1987)	Sabulal et al. (2007)	Prakash et al. (2010)	Reference
1.	α-Pinene	938	–	1.4	1.8	–	–	1.1	Prakash et al. (2010)
2.	Camphene	963	–	–	–	–	0.3	–	
3.	Sabinene	976	–	–	–	–	0.1	tr	
4.	β-Pinene	980	–	1.4	4.5	–	1.3	1.4	
5.	β-Myrcene	989	–	–	–	–	0.1	tr	
6.	α-Phallendrene	1002	–	–	–	–	0.1	–	
7.	δ-2-Carene	1005	–	–	–	–	0.1	–	
8.	Δ3-Carene	1011	–	1.4	–	–	–	–	
9.	α-Terpinene	1018	–	–	–	–	0.1	–	
10.	*p*-Cymene	1031	–	5.0	9.6	–	0.1	tr	
11.	β-Phallendrene	1031	–	4.5	7.0	–	–	–	
12.	**1,8-Cineole**	**1035**	**56.2**	**37.2**	**27.1**	**29.7**	**44.3**	**17.6**	
13.	γ-Terpinene	1058	–	–	–	–	0.1	–	
14.	*Trans*-linalool oxide	1078	–	–	–	–	0.2	–	
15.	*Cis*-linalool oxide	1087	–	–	–	–	0.2	–	
16.	Limonene	1089	–	1.3	17.0	–	0.5	tr	
17.	Linalool	1098	6.8	18.0	16.6	4.4	25.6	2.6	
18.	Camphor	1141	–	–	–	–	0.4	–	
19.	β-Terpineol	1144	–	–	1.8	–	–	–	
20.	Borneol	1165	–	–	–	–	–	0.1	
21.	Terpineol-4-ol	1176	–	–	–	0.7	1.3	0.2	
22.	α-Terpineol	1189	1.6	–	6.5	1.0	1.9	–	
23.	δ-Terpineol	1171	–	–	–	–	0.3	–	
24.	β-Elemene	1339	–	–	–	–	–	0.2	
25.	α-Cubebene	1351	–	–	–	–	–	0.1	
26.	Furfuryl hexanoate	1367	–	–	–	–	–	0.1	
27.	α-Copaene	1378	–	–	–	–	–	0.5	

Continued

TABLE 3 Comparative of Essential Oil Composition of *Hedychium spicatum* Collected from Different Regions—cont'd

No.	Compounds	Kovat Indices	Garg et al. (1977)	Dixit et al. (1977)	Nigam et al. (1979)	Bottini et al. (1987)	Sabulal et al. (2007)	Prakash et al. (2010)	Reference
28.	111 α-(Z)-Bergamotene	1415	–	–	–	–	0.7	0.1	
29.	(E)-Caryophyllene	1418	0.1	24.1	3.5	–	–	1.5	
30.	(Z)-Caryophyllene	1419	–	–	–	–	0.2	0.1	
31.	(Z)-β-Farnesene	1445	–	–	–	–	–	0.1	
32.	α-Himachalene	1447	–	–	–	–	–	0.1	
33.	Aromadendrene	1454	–	–	–	–	0.1	–	
34.	α-Humulene	1454	–	–	–	–	0.3	2.0	
35.	(E)-β-Farnesene	1458	–	–	–	–	–	tr	
36.	9-*Epi*-β-caryophyllene	1458	–	–	–	–	0.1	–	
37.	Dehydroaromadendrane	1459	–	–	–	–	–	1.0	
38.	γ –Muurolene	1477	–	–	–	–	–	1.5	
39.	*Epi*-cubebol	1492	–	–	–	–	0.2	–	
40.	α-Selinene	1493	–	–	–	–	–	3.2	
41.	α-Murrolene	1499	–	–	–	–	0.2	–	
42.	γ-Cadinene	1513	–	–	–	–	0.3	5.4	
43.	Cubebol	1514	–	–	–	–	–	tr	
44.	β-Himachalene	1516	–	–	–	–	0.1	–	
45.	δ-Cadinene	1524	–	–	–	–	0.9	7.5	
46.	α-Cadinene	1538	–	–	–	–	–	tr	
47.	α-Calacorene	1542	–	–	–	–	–	tr	
48.	(+)-Elemol	1549	–	4.2	–	8.5	–	–	
49.	Hedycaryol	1555	–	–	–	–	2.6	–	
50.	*Cis*-sesquisabinene hydrate	1562	–	–	–	–	0.7	–	
51.	(E)-Nerolidol	1564	–	–	–	–	0.3	0.6	
52.	Germacrene-D-4-ol	1574	–	–	–	–	–	6.8	

53.	Spathuenol	1580	–	–	–	–	0.6	–	
54.	Caryophyllene oxide	1582	–	0.5	–	–	0.5	–	
55.	10-*epi*-γ-Eudesmol	1619	–	–	–	5.1	–	9.7	
56.	1-*epi*-Cubenol	1639	–	–	–	–	0.5	6.9	
57.	β-Eudesmol	1649	–	–	–	12.6	2.2	tr	
58.	α-Eudesmol	1652	–	–	–	4.8	2.3	17.0	
59.	(–)-α-Cadinol	1653	–	–	–	5.3	2.3	–	
60.	γ-Muurolol	1671	–	–	–	–	4.4	–	
61.	Agarospirol	1682	–	–	–	–	0.9	–	
62.	8-epi-β-Bisabolol	1690	–	–	–	–	0.5	–	
63.	Oplopanone	1733	–	–	–	–	–	0.1	
64.	Unknown alcohols		–	–	–	6.2	–	–	
	Total		**64.7**	**99.0**	**95.4**	**78.3**	**97.9**	**87.5**	

tr, trace amount.

TABLE 4 Comparative Chemical Compositions (%) of Essential Oils from Rhizomes of Different *Hedychium* Species Collected from Different Locations

S. N.	Compounds	Kovat Indices	Flame Ionization Detector % of from Different Locations (Collection)							
			Joshi et al. (2008)						Prakash et al. (2010)(NI)	Sabulal et al. (2007)(SI)
			He	Ha	Hc	I	II	III	Hc	
1.	α-Thujene	931	tr	0.2	–	0.1	0.1	5.8	–	–
2.	α-Pinene	938	0.5	2.8	4.0	1.8	2.8	3.7	3.5	–
3.	Camphene	952	–	–	3.1	0.4	0.3	0.1	3.7	0.7
4.	Sabinene	976	22.2	2.0	–	0.1	tr	13.0	–	tr
5.	β-Pinene	980	1.8	3.1	–	4.0	5.3	5.9	–	24.5
6.	*Trans-meta*-mentha-2,8-diene	981	–	–	25.2	–	–	–	12.9	-
7.	β-Myrcene	989	0.1	–	0.1	0.3	0.4	8.7	tr	1.2
8.	α-Phellandrene	1002	tr	–	0.2				0.7	2.7
9.	δ -2-Carene	1005	–	–	–	–	–	–	–	0.4
10.	α-Terpinene	1018	1.8	–	0.2				tr	0.6
11.	*p*-Cymene	1020	0.6	8.2	tr	tr	tr	0.7	tr	0.6
12.	Limonene	1031	0.9	–	–	tr	tr	0.2	20.3	2.6
13.	1,8-Cineole	1035	33.0	0.1	0.1	42.8	45.7	4.3	tr	48.7
14	Z-β-Ocimene	1040	–	–	–	–	–	–	–	0.1
15.	γ-Terpinene	1058	5.3	–	3.6	–	–	–	8.9	1.4
16.	Para-mentha-3,8-diene	1072	–	–	0.7	–	–	–	0.1	–
17.	Terpinolene	1088	0.7	–	0.2	–	–	–	–	–
18.	Linalool	1098	3.3	1.8	21.7	6.4	0.6	2.1	29.3	1.2
19.	*Trans-p*-menth-2-en-1-ol	1135	–	–	–	–	–	–	–	0.1
20.	*Trans*-sabinol	1140	0.4	–	–	–	–	–	–	–
21.	*Trans*-verbenol	1144	0.3	–	–	–	–	–	–	–
22	Terpineol	1144	–	–	–	–	–	–	0.3	0.5
23.	Borneol	1165	–	3.3	0.8	tr	0.1	0.6	2.1	–
24.	**Terpineol-4-ol**	**1176**	**14.3**	**24.8**	**4.1**	**0.9**	**1.1**	**16.5**	**0.5**	**3.1**
25.	α-Terpineol	1189	2.0	2.4	10.9	1.6	1.7	0.1	1.5	7.8

26.	δ-Terpineol	1171	–	–	–	–	–	–	–	0.3
27.	Myertenol	1194	–	–	–	–	–	–	tr	–
28.	Geraniol	1254	–	–	1.5	–	–	–	tr	–
29.	Geranial	1270	–	–	–	–	–	–	tr	–
30.	Bornyl acetate	1285	–	7.6		–	–	–	–	0.2
31.	Carvacrol	1296	–	–	0.5	–	–	–	tr	–
32.	Terpinen-4-yl acetate	1300	–	–	–	–	–	–	–	0.3
33.	α-Copaene	1376	–	–	–	–	–	–	0.1	–
34.	Geranyl acetate	1383	–	–	–	–	–	–	tr	–
35.	(Z)-Caryophyllene	1404	–	–	–	–	–	–	0.1	0.2
36.	α-Gurjunene	1408	–	–	0.2	–	–	–	0.1	–
37.	β-Cedrene	1417	–	–	0.5	–	–	–	0.1	–
38.	(E)-Caryophyllene	1418	–	–	–	–	–	–	0.2	–
39.	β-Caryophyllene	1419	5.6	–	0.3	–	–	–	–	–
40.	(Z)-β-Farnesene	1443	–	–	–	–	–	–	0.1	0.1
41	α-Himachalene	1447	–	–	–	–	–	–	0.2	–
42.	α-Humulene	1454	1.5	–	0.1	0.5	0.4		0.1	
43.	(E)-β-farnesene	1458	0.5	–	0.1	2.0	1.3	–	tr	–
44.	Dehydroaromadendrane	1459	–	–	0.6	–	–	–	0.8	–
45.	γ-Muurolene	1477	–	–	0.2	–	–	–	tr	–
46.	Germacrene D	1480	–	2.1	–	–	–	–	–	–
47.	ar-Curcumene	1483	–	–	0.2	–	–	–	2.7	–
48.	β-Selinene	1485	–	1.0	–	6.8	3.9	1.3	–	
49.	α-selinene	1493	1.3	–	–	0.1	0.3	1.4	–	–
50.	Curzerene	1496	–	–	–	0.5	0 0.2	1.2	–	
51.	β-Curcumene	1512	–	–	tr	1.1	0.6	1.0	1.3	–
52.	δ-Cadinene	1513	–	1.2	0.1	–	–	–	0.3	–
53.	Cubebol	1514	tr	–	–	0.8	0.8	–	–	–
54.	α-Calacorene	1542	–	–	–	–	–	–	tr	–
55.	(Z)-Muurol-5-en-4-a-ol	1554	–	–	–	–	–	–	0.1	–
56.	(E)-Nerolidol	1564	0.3	1.5	tr	–	–	–	tr	–

Continued

TABLE 4 Comparative Chemical Compositions (%) of Essential Oils from Rhizomes of Different *Hedychium* Species Collected from Different Locations—cont'd

S. N.	Compounds	Kovat Indices	Flame Ionization Detector % of from Different Locations (Collection)							
			Joshi et al. (2008)						Prakash et al. (2010)(NI)	Sabulal et al. (2007)(SI)
			He	Ha	Hc	I	II	III	Hc	
57.	Germacrene D-4-ol	1574	–	–	–	2.0	1.1	–	–	–
58.	Spathuenol	1577	–	–	0.5	–	–		0.9	–
59.	Caryophyllene oxide	1582	1.0	–	–	–	–	–	–	–
60.	Humulene epoxide	1607	0.2	–	–	–	–	–	–	–
61.	10-*epi*-ϒ-Eudesmol	1619	tr	0.5	0.7	12.4	12.0	1.2	-	-
62.	β-Acorenol	1634	–	–	–	–	–	–	1.3	–
63.	β-Eudesmolo	1648	0.1	0.5	0.2	–	–	–	1.9	–
64.	α- Eudesmol	1652	–	–	–	–	–	–	0.1	–
65.	α-Cadinol	1654	0.1	–	–	2.9	3.3	1.2	–	–
66.	14-Hydroxy-9-epi-	1664	–	–	–	–	–	–	tr	–
67.	Bisabolone	1737	–	–	–	–	–	–	0.1	–
68.	13,14,15,16-Tetranolabd-(817)-en-12-al	1812	–	–	–	–	–	–	–	0.2
Total			**97.8**	**63.5**	**83.1**	**87.5**	**82.0**	**69.0**	**94.3**	**97.5**

NI, North India; SI, South India; tr, trace amount; He, *Hedychium ellipticum*; Ha, *Hedychium aurantiacum*; Hc, *Hedychium coronarium*; I, Jageshwar collection; II, Shimla collection; III, Bhowali collection; tr, trace.

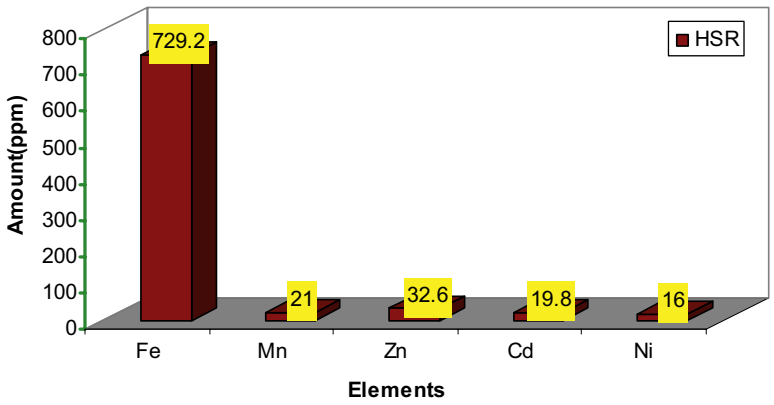


FIGURE 3 Elemental profiling of rhizome of *Hedychium spicatum*. (HSR, *Hedychium spicatum* rhizome.)

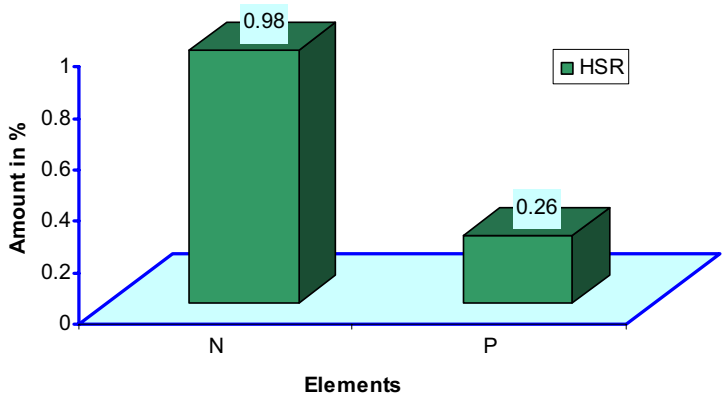


FIGURE 4 Nitrogen and phosphorus content in rhizomes of *Hedychium spicatum*. (HSR, *Hedychium spicatum* rhizome.)

These compounds are reported as antimicrobial in their pure form or because of their presence in the essential oils (Hammer et al., 2010; Hendry et al., 2009). We also analyzed the mineral composition of rhizomes of *H. spicatum* which contains iron (729.2 ppm), manganese (21.0 ppm), zinc (32.6 ppm), nickel (16.0 ppm), and cadmium (19.8 ppm), while nitrogen (0.98%) and phosphorous (0.26%) were also present. Elements like copper, lead, and chromium could not be detected (Figures 3 and 4)

Antioxidant Activity

Natural antioxidants are compounds that protect and save our foods from the oxidative deterioration and contaminations by the attack of pathogens like fungi, bacteria, etc. The antioxidant activity associated with essential oils are due to their electron donating activity, reducing power activity and radical scavenging activity as a result the chain reactions which deteriorate the foods are checked or terminated. The in vitro antioxidant activities of some *Hedychium* spp have already been reported by us (Joshi et al., 2008). Antioxidant activity of *Hedychium* spp. have also been reported by other workers from other part of the world (Arruda et al., 2012; Bhatt et al., 2008; Sravani and Paarakh, 2012).

Antimicrobial Activity

As well as oxidative deterioration, pathogens are a major cause for the contamination and spoilage of food and food materials, which ultimately make the foods unacceptable and causes loss of economy. To overcome this problem, synthetic preservatives are used, which may cause long-term after-effects. Presently, the food industries are interested in using safe and eco-friendly food preservatives and flavoring agents, preferentially natural products over the synthetic derivatives Essential oils from *Hedychium* spp. can be safe and eco-friendly food preservative and flavoring agents

as these oils also possess antimicrobial activities. Four species of *Hedychium*: *Hedychium venustum*, *H. spicatum*, *H. coronarium*, and *Hedychium flavescens* essential oils have been reported to possess antibacterial activity against Gram-positive bacteria like *Staphylococcus aureus*, *Bacillus cereus B. subtilis*, and Gram-negative bacteria like *Serratia marcescens*, *Pseudomanas fluorescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Escherichia coli*, and *Salmonella typhi*. The oils have also been reported to exhibit antifungal activity against *Candida albicans* (MTCC no. 227), *C albicans* (1637), *C. albicans* (3017) ,and *Candida glabrata* (3019) (Sabulal et al., 2007). The presence of 1,8-cineole, linalool, α-pinene, β-pinene, and *E*-nirolidol in the essential oils of 19 *Hedychium* species and cultivars with potent antifungal activity against *Colletotrichum acutatum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, and insecticidal activity against azalea lace bug (*Stephanitis pyrioides*), the yellow fever mosquito (*Aedes aegypti*), and the red imported fire ant (*Solenopsis invicta*) have also been reported (Sakhanokho et al., 2013). Antimicrobial activities of the essential oils of *H. spicatum* and *H. coronarium* are reported by us and recorded in Tables 4–6.

TABLE 5 Minimum Inhibitory Concentrations for Antibacterial activity of Rhizome Essential Oils of *Hedychium coronarium* and *Hedychium spicatum* against Five Pathogenic Bacteria

Essential Oils	MICs (L/mL)					Reference
Bacteria	*Proteus vulgaris*	*Staphylococcus aureus*	*Salmonella typhi*	*Escherichia coli*	*Pseudomonas aeruginosa*	Prakash et al. (2010)
HSREO	0.391	6.25	0.7812	6.25	0.7812	
HCREO	0.1953	12.5	0.7812	3.125	0.1953	

HSREO, *Hedychium spicatum* rhizome essential oil.

TABLE 6 Study of Antibacterial activities of the Rhizome Essential Oils of *Hedychium* Species against Pathogenic Bacteria (Joshi et al., 2008)

Sample	Conc.[µL/disc]	Inhibition Zone [mm] (MIC [mL/mL])					Reference
		Sa	*Sf*	*Pm*	*Em*	*Se*	
Hedychium ellipticum	50	14 (7.8)	11 (31.3)	13 (7.8)	12 (15.6)	14 (15.6)	Joshi et al. (2008)
Hedychium aurantiacum	50	14 (2.0)	12 (15.6)	13 (15.6)	11 (15.6)	14 (15.6)	
Hedychium coronarium	50	14 (7.8)	8 (31.3)	8 (31.3)	16 (15.6)	16 (7.8)	
Hedychium spicatum							
Site I	50	12 (15.6)	n.a.	9 (31.3)	15 (15.6)	16 (7.8)	
Site II	50	15 (7.8)	n.a.	10 (31.3)	12 (15.6)	n.a.	
Site III	50	10 (15.6)	n.a.	10 (31.3)	12 (15.6)	n.a.	
Amikacin†	30	8	22	15	20	24	
Ciprofloxacin†	5	36	3.5	20	34	41	
Ampicillin†	10	19	21	21	20	25	
Gentamicin†	30	22	22	22	19	23	
Tetracycline†	30	11	14	12	26	12	

n.a., not active; MIC, minimum inhibitory concentration; Sa, Staphylococcus aureus; Sf, Shigella flexner; Pm, Pasteurella multocida; Ec, Escherichia coli; Se, Salmonella enterica.
†*Standard drugs (positive controls).*

SUMMARY POINTS

- *Hedychium spicatum* essential oils can be a good source of natural antioxidants to reduce the oxidative deterioration of fats and foods.
- Linalool finds applications in food and flavor as well as its activity against a wide range of microorganisms. This is a major compound present in many *Hedychium* species. Hence the *Hedychium* species can be a natural source of linalool.
- 1, 8-cineole is used in flavorings, fragrances, and cosmetics. Cineole-based oil is used as a flavoring in various products, including baked goods, confectionery, meat products, and beverages.
- Linalool, 1,8-cineol, limonene, caryophyllene, and caryophyllene oxide exhibits antimicrobial activity hence the essential oil from *Hedychium* species may be a good natural food preservative to protect and save the food and food materials from pathogenic microorganisms.
- *Hedychium* species (ginger lily) as whole herb is used as spice and medicine in indigenous system of medicine. Hence the documentation of data obtained on the species will be helpful to make the indigenous people aware about scientific knowledge of food preservation and flavors.
- *Hedychium spicatum* is an endangered species. Because of its high medicinal and food values, its *in vitro* and *in situ* preservation is indispensable, so that the herb can be exploited judiciously for its uses in food, flavor, and traditional medicine.

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Chapter 85

Staranise (*Illicium verum* Hook) Oils

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INTRODUCTION

Essential oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots). They can be obtained by expression, fermentation, enfleurage, or extraction but the method of steam distillation is most commonly used for commercial production of essential oils. An estimated 3000 essential oils are known, of which about 300 are commercially important, destined chiefly for the flavors and fragrance market (Burt, 2004).

Natural products, such as essential oils, are known to possess potential agents for food preservation; in fact their effectiveness against a wide range of microorganisms has been repeatedly demonstrated (Helander et al., 1998; Baratta et al., 1998). Besides this activity, many essential oils have qualified as natural antioxidants (Baratta et al., 1998; Chouksey et al., 2010) and proposed as potential substitutes of synthetic antioxidants in specific sectors of food preservation where their use is not in contrast with their aroma (Giuseppe and Baratta, 2000). Essential oils can also be important to preserve food because they can be used to protect stored grains and legumes, and to repel flying insects at home (Isman, 2000; Chouksey et al., 2010). Essential oils and their constituents have been used extensively as flavor ingredients in a wide variety of food, beverage, and confectionery products and also in numerous applications, including toothpaste (Kim et al., 1995).

Illicium verum Hook. f. (Figure 1) belongs to the Schisandraceae family (The Plant List, 2013) and is commonly known as star anise. Their fruits grow on a medium-sized tree and are frequently used as a spice in the food industry (Huang et al., 2010) and the oil is used as a flavoring in confectionery, liqueurs, and pharmaceutical preparations (De et al., 2002). It is distributed in North America, Atlantic region, and the tropical and subtropical zone of Asia (Yazdani et al., 2009).

The main component in star anise essential oil is trans-anethole (Figure 2), 1-Methoxy-4-(1-propenyl) benzene accounts for 85–90% of the total components (Singh et al., 2006). The other components include terpenes, pinenes, β-phellandrene, α-diterpene, limonene, estragole, safrol, and terpineol, etc. (Tuan and Ilangantileke, 1997).

Previous investigations suggested that *I. verum* essential oil had insecticidal, antimicrobial, and antioxidative properties (Singh et al., 2006; Padmashree et al., 2007; De et al., 2002). The major compound trans-anethole is a volatile constituent that inhibits growth of various food-borne pathogenic microorganisms, including *Salmonella typhimurium*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*, thereby assisting in the preservation of some foods (Karapinar and Aktug, 1987).

The essential oil from star anise fruits is traditionally obtained by steam distillation. However, in this steam distillation process, the relatively high temperature of steam, combined with the hydrolytic influence of water, may cause degradation of the essential oil components (Stahl and Gerard, 1985). To solve this problem, Tuan and Ilangantileke (1997) performed essential oil extraction with liquid CO_2 by supercritical fluid extraction. The authors observed that extraction of essential oil from star anise fruits with this method was better than steam distillation in terms of saving energy, product yield, and product quality (Table 1).

BOTANICAL ASPECTS

Illicium verum Hook. f. is the accepted name for the species of the Schisandraceae family (Mendieta et al., 2012) commonly known as "star anise" and "Chinese anis" (Alonso, 2004). Star anise has many synonyms in different areas. In China, it is called Chinese star anise and *Bajiaohuixiang*. The following are other synonyms (Wang et al., 2011):

- French: Anis de la Chine, Anise etoile, Badiane
- Japan: Dai-uikyo, Hakkaku-uikyo
- German: Stenanis

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FIGURE 1 *Illicium verum. Photo: Dr Marcelo Guerra Santos.*

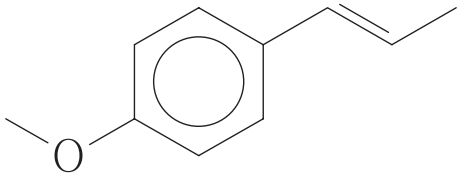

FIGURE 2 Chemical structure of trans-anethole.

TABLE 1 Extraction Yield and Anethole Content

	Steam Distillation	Liquid CO_2 Extraction
Extraction yield (% r.m.)	10.2	11.2
Anethole content in essential oil (%)	92.2	89
Total anethole content (% r.m.)	9.4	10.0

Adapted from Tuan and Ilangantileke (1997); r.m., raw material.

- Italian: Anice stellate
- India: Sonf, Anasphal
- Spanish: Anisestrellado, Badian
- Indonesian: Bungalawang
- Malay: Bungalawang
- Portuguese: Anis estrelado, Badiana da China, Anis da China (Alonso, 2004).

The scientific synonym observed for this plant is *Illicium san-ki* Perr (The Plant List, 2013).

This species is a small tree, 6–8 m in height, gray-brown bark, green branchlets, glabrous; crown conical to globose. All parts of the tree have an agreeable aromatic smell. The leaves are simple, usually clustered at branch-tips. They are entire, oval, tapering toward both ends, acute apex, and cuneate base. Thick and brittle leaf-blade, dark green above, paler beneath, petiole glabrous. Pink-white flowers, solitary and axillary, stout and short pedicel. Six sepals, pink at margin, 16–20 petals, broad-elliptic, smaller than sepals, white outside, red inside, dark red at the middle of the flower. Numerous stamens, shorter than petals, elliptic anther. Eight carpels, forming a conical mass, spreading into a rosette when ripe. Fruit consists of eight follicles, spreading, woody and brown when mature, dehiscent by the ventral side. Solitary seed in each follicle, brown,

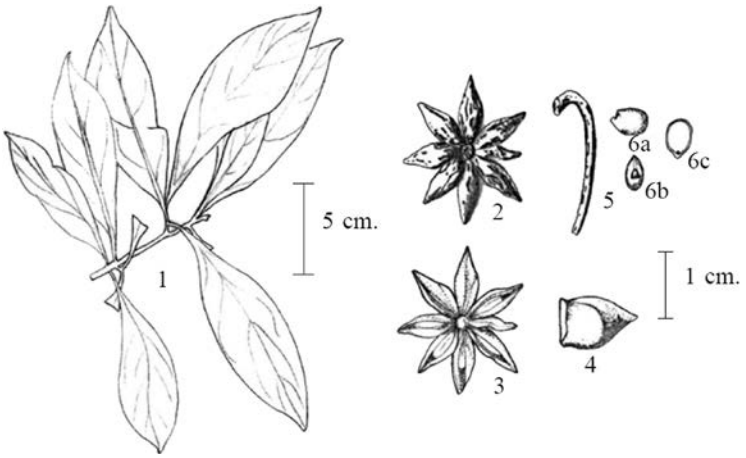

FIGURE 3 Macroscopic characters of *Illicium verum* Hook. f. (1) Twig; (2–3) Entire fruit: star-shape follicle cluster; (4) A single carpel (follicle); (5) Stalk; (6) Seeds: (6a) side view, (6b) seed with hilum, (6c) seed with crustaceous testa, cut vertically. *Adapted from Putiyanan et al. (2010).*

glossy, and glabrous (Putiyanan et al., 2010) (Figure 3). Star anise originates from northern Vietnam and southern China's woods, being cultivated in several regions, mainly Jamaica and some Asian tropical countries (Alonso, 2004).

This plant is propagated by seed and mainly cultivated for perfume, medicines, and as a culinary spice in southern China, as well as in Vietnam. The fruits are harvested before they ripen, then sun dried. The flowers are seen from March to May, and the fruits ripen from September to October (Wang et al., 2011).

USAGE AND APPLICATIONS

Illicium verum has a long history as a medicinal plant in Asian countries, especially in China (Yang et al., 2010). It has morphological relation to another species from the genus *Illicium*, resulting sometimes in serious problems. For example, misidentification between *Illicium anisatum* L. and *Illicium lanceolatum* A. C. Smith (commonly known as Japanese star anise), may cause intoxication (Wang et al., 2011). In September 2003, the United States Food and Drug Administration advised consumers not to consume teas containing star anise. Such teas might be contaminated with the other toxic species of this genus, associated with serious adverse effects including convulsions, emesis, and diarrhea (Upton, 2006). Despite star anise being a well-known spice as well as safe food, some reports observed that star anise might be contaminated with highly toxic Japanese star anise (Lee et al., 2003). It is therefore essential that *I. verum* fruits should be distinguished from the fruits of the toxic species of *Illicium*, particularly in powdered samples of *I. verum*. Various methods have been reported to identify and distinguish between them, such as morphological and chemical analysis by fluorescent microscopy–gas chromatography, thermal desorption–gas chromatography, and high pressure liquid chromatography-electrospray ionization-tandem mass spectrometry (Wang et al., 2011).

Different formulations from *I. verum* include crude drug, powders, and essential oils (Wang et al., 2011). In addition to its culinary use, the star-shaped *I. verum* fruit has been used in traditional medicine (Yazdani et al., 2009) for treatment of stomach aches, vomiting, rheumatic pain, insomnia, skin inflammation (Editorial Committee of Chinese Pharmacopoeia, Wang et al., 2011), and antiseptic (De et al., 2002). These fruits have also been used for treatment of colic, dyspepsia, flatulence, facial paralysis, asthma, and bronchitis (Sung et al., 2012; Chouksey et al., 2010).

The use of the fruit, seed, and essential oil from star anise in folk medicine has encouraged many researchers to investigate medicinal properties of the oil of this species, confirming some of them, including their strong antimicrobial activity (De et al., 2002).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Star anise (*I. verum* Hook f.) (Figure 4) is a well-known spice used in many cultures. Because of its long history of herbal and culinary uses, Chinese star anise has been commonly regarded as being safe and nontoxic (Howes et al., 2009). A closely related species Japanese star anise (*I. anisatum* L, syn., *Illicium japonicum* Sieb, syn., *Illicium religiosum* Siebet-Zucc) has been well documented to cause both neurologic and gastrointestinal toxicities (Ize-Ludlow et al., 2004).

Star anise essential oil produced by the process of steam distillation has a wide range of culinary usage and medicinal properties (Chouksey et al., 2010; De et al., 2002; Singh et al., 2006; Huang et al., 2010). Chinese star anise is one of the flavors used in "Chinese five spices" (Chouksey et al., 2010). It is a spice frequently used in meat and Chinese cooking. This

FIGURE 4 Fruits and seeds from *Illicium verum*. *Photo: Dr Marcelo Guerra Santos.*

oil is extensively used as a flavor ingredient in food as well as in alcoholic and soft drinks (Ize-Ludlow et al., 2004). It is used in baked goods, confections, and liquors (De et al., 2002). It is a common flavoring for medicinal tea, cough mixture, and pastilles (Chouksey et al., 2010).

The essential oil from *I. verum*, rich in *trans*-anethole, is mainly used in the pharmaceutical and food industry. Star anise essential oil exhibited high antioxidant activity (Singh et al., 2006; Padmashree et al., 2007), insecticidal activity (Ho et al., 1995; Chouksey et al., 2010), antimicrobial potential (Singh et al., 2006; Chouksey et al., 2010), and antifungal activity (Yazdani et al., 2009; Huang et al., 2010; Chouksey et al., 2010).

Microorganisms are the most versatile pathogens that are transmitted through soil, water, air, and food and causes diseases in human being and animals. Poorly processed and stored foods can serve as substrates for the growth of pathogenic microorganisms, with a wide range of pathogenic species, harmful to consumer health. Given this reality and seeking new natural food preservation techniques, the addition of essential oils in food becomes a potential biopreservation system as they can prolong the life of food, keeping the quality in relation to their microbiological characteristics (Frutuoso et al., 2013).

Studies have proven antibacterial and antifungal activity of essential oil and extracts of *I. verum* (Huang et al., 2010; De et al., 2002; Dzamic et al., 2009; Chouksey et al., 2010) demonstrated that the crude ethanol extract from the fruits of *I. verum* showed antimicrobial activity against *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, which was determined by agar diffusion methods. Crude hexane and crude dichloromethane extracts showed antibacterial activity against *S. aureus*. The antimicrobial activity of fruit extract is due to presence of anethole. This author also certificated that the *I. verum* fruit extract inhibited the growth of *Aspergillus niger*, one of the most important saprophytic fungi known to be associated with mycotoxin production in agricultural products and foods (Chouksey et al., 2010). Dzamic et al. (2009) demonstrated that *I. verum* essential oil exhibited fungicidal activity against numerous fungi with a minimum inhibitory concentration and minimum fungicidal concentration of 2.5–25 μL/mL: *Alternaria alternate*, *A. niger*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus versicolor*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Cladosporium fulvium*, *Fusarium tricinctum*, *Fusarium sporotrichioides*, *Muco rmucedo*, *Penicillium funiculosum*, *Penicilium ochrochloron*, *Phomo psishelianthi*, *Phoma magdonaldii*, *Trichoderm aviride*, *Trichphyton mentografites*, and *C. albicans*.

In foods, lipid peroxidation and enzymatic hydrolysis cause shelf-life problems. The most limiting factor in determining the shelf life of dehydrated convenience foods is the oxidation of fats and oils, causing off-flavours (Padmashree et al., 2007). There has been increasing interest in the use of natural additives (Singh et al., 2006; Padmashreeet al., 2007; Chouksey et al., 2010) for the stabilization of foodstuffs. Synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and propyl gallate have long been used to control lipid oxidation in foods and they function either by scavenging chain-carrying peroxy radicals or by diminishing the formation of initiating lipid radicals. However, these adverse and toxic effects has led to the search for new substances, mainly of natural origin (Singh et al., 2006).

The utilization of star anise essential oil as an additive is also promising. According to Singh et al. (2006), the essential oil and extract of star anise, rich in *trans*-anethole, exhibited excellent activity for the inhibition of primary and secondary oxidation products in rapeseed oil which were evaluated using peroxide, thiobarbituric acid, p-anisidine, and carbonyl values. The activity of the extract was further confirmed using other antioxidant properties such as ferric thiocyanate method in the linoleic acid system, reducing power and scavenging effect (%) on 2,2-diphenyl-1-picrylhydrazyl radical. Thus, it

makes a strong impact on expanding the investigations of antioxidant constituents present in star anise which are being not only able to protect the oil against oxidation but also to alleviate diseases by preventing oxidative deterioration. In other study, star anise powder, as well as their ethanol/water extract, exhibited strong antioxidant activity (Padmashree et al., 2007). Essential oil from spice exhibited antioxidant activity and the activity did not seem to be concentration-dependent. Thus, both the spice powder and particularly their ethanol/water extract, have great potential as natural antioxidants.

Pesticides of plant origin are gaining increased attention and interest among those concerned with environment friendly, safe, and integrated crop management approaches. In addition, they are playing a vital role in organic food production globally (Rattan, 2010). A number of spices have been reported to have various effects on stored-product insects, including *I. verum* (Ho et al., 1995). According to Ho et al. (1995), the results of their investigation demonstrate that star anise contains bioactive compounds in the nonpolar fraction. In this way, these compounds may be considered a great potential for use as a grain protectant against *Tribolium castaneum* and *Sitophilus zeamais*. The ovicidal action against *T. castaneum* is particularly useful in controlling this insect in grain storage, as the insect populations would be expected to decrease rapidly if star anise extracts could be applied to grain on a commercial scale.

Therefore, *I. verum* species have been used, for a very long time, in a culinary context as a preservative and spice of many foods and it is still an important raw material to the food industry.

SUMMARY POINTS

- *Illicium verum* has a long history as a medicinal plant in Asian countries.
- The fruit consists of eight follicles, spreading, woody and brown when mature, dehiscent by the ventral side.
- Trans-anethole is the major component of essential oil from star anise.
- This essential oil is widely used in food industry.
- Star anise essential oil exhibits high antioxidant, insecticidal, antimicrobial, and antifungal activity.

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Chapter 86

Summer Savory (*Satureja hortensis* L.) Oils

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INTRODUCTION

Food contamination caused by microorganism and oxidation caused by active oxygen species (ROS) are generally responsible for the food, vegetable, and fresh fruit spoilage and destruction during storage. This deterioration and spoilage during storage is a real environmental problem as well as and in the food industry.

To avoid contamination and deterioration of food, using preservatives in foodstuff is essential. Usage of synthetic preservatives in foodstuff, to prevent food contamination and deterioration, is harmful for human health and the environment. Therefore, it is of interest to the food industries to replace the synthetic preservatives with natural, botanical, and safe preservatives. Using essential oils of aromatic plants as a natural and safe preservative with the botanical origin in foods has the ability to prevent food deterioration and food contamination.

Satureja hortensis and its products has been used as a flavoring agent in foods in various countries for many years. In addition, they have various pharmacological activities. Therefore, they could be a suitable candidate to be used as natural and safe preservative for foodstuff.

BOTANICAL ASPECTS

Satureja hortensis L. (Syn. *Satureja postii* Arzn.) is an aromatic plant belongs to the Lamiaceae family has several common names: summer savory, bean herb, garden savory, savory, and winter savory (Anonymous, 2007; Ball and Getliffe, 1972; Davis, 1982).

The plant is a puberulent annual herb and grows to 10–25 cm tall, with a heavily branched stem (10–35 cm), with very short retrorse white hairs. Linear or linear-lanceolate leaves are ± obtuse, emucronate, subpetiolate, crossed opposite with entire-margined (10–30 mm × 1–4 mm). They are rather thick with a ciliate margin and are glandular punctate on surfaces (Figure 1).

Inflorescence is lax and elongate. Verticillasters are approximate congested or distant, with 5–15 congested or lax flowers. Bracts are exceeding the verticillasters. The calyx is tubular campanulate, subbilabiate, 3–4 mm, gland-dotted, and with simple hairs; teeth slightly unequal, three upper about as long as tube and two lower distinctly longer than tube, they are narrow linear to subulate, apiculate, to 3 mm. The corolla does not have a ring of hair. It is lilac, pink, purplish, or white, 4–7 mm. The upper lip is straight and margined; the lower lip has three divisions and is red-spotted at the mouth of the tube. The tube is straight, glandular, and hairy, is exceeding the verticillasters. (Ball and Getliffe, 1972; Davis, 1982; Figure 2).

Nutletes are 1–1.2 mm and broadly oblong. The species is native to south Europe and Turkey but it has been widely introduced and cultivated elsewhere as a culinary herb (Ball and Getliffe, 1972; Davis, 1982; Figure 3).

This plant is widely grown as a pot herb (Ball and Getliffe, 1972). Although *S. hortensis* is cultivated in many areas in the world most cultivation areas of this plant around the world are shown the map in Figure 3.

There are many reports on the composition of the essential oil obtained from *S. hortensis*. The essential oil components of the plants are susceptible to the growing environment of the plant therefore it is expected to have wide variations in the composition of essential oils extracted from the plant grown in different areas as it is for *S. hortensis*. The plant contains 0.2–3% of volatile oil. The main components of the *S. hortensis* essential oil are reported to be carvacrol, *p*-cymene, α-thujune, α-pinene, β-myrcene, β-terpinene, thymol, linalool, and β-caryophyllene (Fathi et al., 2011).

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FIGURE 1 The branched stems of *Satureja hortensis* L. *Note: This figure was published in the internet.*

FIGURE 2 The flowers of *Satureja hortensis* L. *Note: This figure was published in the internet.*

USAGE AND APPLICATIONS

Satureja hortensis is often used as a culinary herb either as a fresh or dried herb, but it also has marked medicinal benefits, especially upon the whole digestive system and has been used as a medicinal herb in folk medicine. Traditionally it has been used for healing and to relieve pain from bone and muscle as well as treating nausea, diarrhea, and cramps. It has also been used as a stimulant, stomachic, carminative, expectorant, aphrodisiac, and anthelmintic. It is used to stimulate the uterus and nervous system (Fathi et al., 2011; Volak and Stodola, 1998). This plant has mild antiseptic and astringent properties

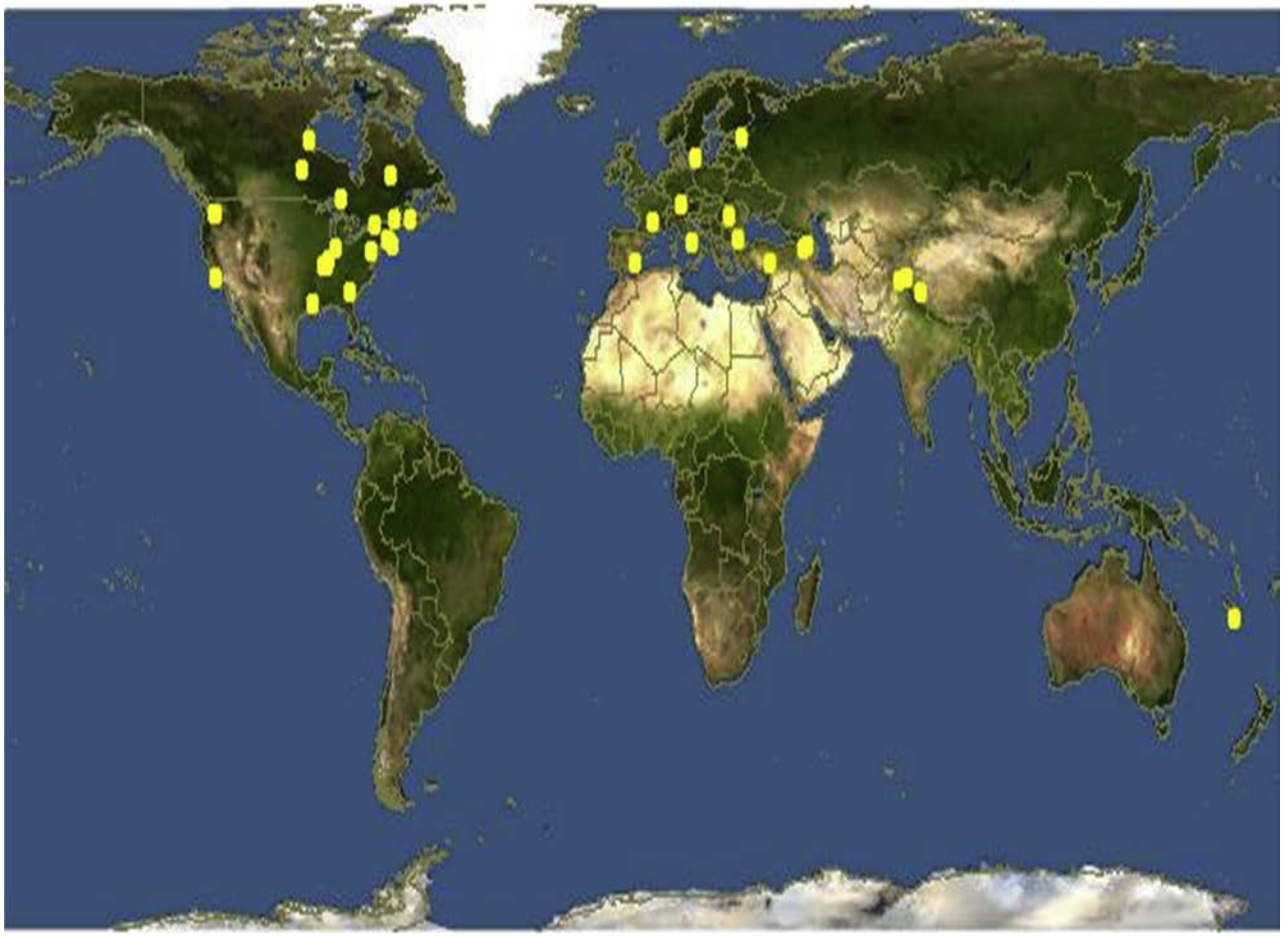

FIGURE 3 Map of most areas in which *Satureja hortensis* L. is cultivated. The cultivated areas are shown by yellow (white in print version) dots. *Note: This figure was published in the internet.*

and has antiviral effects (Anonymous, 2007; Brown, 2002). The oil extracted from this plant has antibacterial and antifungal properties (Small and Deutsch, 2001). The volatile oil of this plant is used in lotions for treating the scalp in the early stages of baldness. Also, the plant is used to prepare an ointment in order to relieve arthritic joints when used externally.

Because *S. hortensis* is an aromatic plant with a strong and pleasant spicy aroma, the plant and the essential oil obtained from this plant have been widely used in food and the food industry for flavoring foods in different areas of the world. In fact, in the Roman civilizations and ancient Greece, summer savory was a popular seasoning herb. The English name of the plant, savory, refers to its pleasant and spicy taste (savoury taste) (Volak and Stodola, 1998). Because of its pleasant flavor and its ability to aid bean digestion it has been used to season bean dishes widely (Small and Deutsch, 2001). It is also one of the ingredients of *herbes de Provence* (*Rosmarinus officinalis*, *Thymus vulgare*, and *Origanum* spp. are the other ingredients) (Brown, 2002). It is added to bouquets garnis, fines herbes mixtures, and as a replacement for parsley in some cases (Small and Deutsch, 2001). This plant is extensively used in the perfume industries as the fragrance of the essential oil has a particular quality to it.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Various contaminations caused by microorganism are generally responsible for the spoilages and destruction of food, vegetables, and fresh fruits. The rate of destruction increases rapidly when these foodstuff are in a humid environment, with high moisture content and high temperature. This is a major dilemma for both the environment and the food industry.

Chemical spoilage, mainly caused by oxidation that produces ROS during lipid peroxidation, may lead to deterioration of food, especially stored food products containing oils and fats. This destruction is the main reason for loss of food nutritional quality, color, and flavor. The presence of the ROS can damage the cells and provide serious problems for the

people consuming these food products. The risk of oxidation of food components and also the contamination problem of food products by microorganisms during storage leads to the obligation of using preservatives in food industry to prevent the food contamination and deterioration.

To avoid starting the oxidation reaction and to delay its progress, oxidation of molecules should be inhibited by using antioxidant compounds. In order to prevent these oxidation processes, synthetic preservatives are generally used. Using synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone is not safe and long-term usage of these compounds may lead to some health problems like carcinogenicity and other toxicities.

Therefore, to avoid these health problems and respond to the consumer awareness about safety and toxicity risks of chemical preservatives, there is a strong need to replace synthetic compounds with natural compounds from safe botanical sources. Aromatic herbs and their essential oils are natural and safe compounds from botanical sources that have strong antioxidant and antimicrobial properties and can replace synthetic preservatives in the food industry to avoid food contamination and delay the destruction of food, vegetables, and fresh fruits.

The main reason for attempting to replace the synthetic antimicrobial agents by essential oils of the plants is because essential oils have a natural safe botanical source which provides more safety for the people consuming the food product and a low risk for developing resistance against pathogenic microorganisms.

Due to the strong and pleasant spicy aroma of S. *hortensis* and the potent antifungal and antibacterial effects of essential oil obtained from this plant, it can be widely used in the food industry as a natural and safe food additive and food preservative to avoid food spoilage and offer aroma and flavor to foods. Also, the essential oil of S. *hortensis* has been used extensively the in perfume industry to provide a better quality of fragrances (Dikbasa et al., 2012). There are various studies providing information about the use of S. *hortensis* essential oil as natural agent in food industry.

As Antimicrobial for Food Preservation

The antimicrobial activities of the essential oil of *S. hortensis* were investigated in Turkey. In this study, the plant was collected from the eastern Anatolia region of Turkey at full flowering stage. The essential oil of the plant was extracted from aerial parts of the plants.

As the resistance of isolated strains of *Bacillus cereus* from various food sources have been studied before, Dikbasa et al. studied the effect of *S. hortensis* essential oil on *B. cereus* strains isolated from different food sources. The antimicrobial activity of *S. hortensis* volatile oil was tested against 77 *B. cereus* strains isolated from food sources (raw milk, chicken, cereal, and meat). Results showed that all the tested strains were highly susceptible against the volatile oil obtained from *S. hortensis*. It was shown that the *S. hortensis* volatile oil is more effective than penicillin when tested on the isolated *B. cereus* strains from all four sources of food. Therefore, this research showed the *S. hortensis* essential oil has the ability to act as potent and broad spectrum antibacterial and antifungal compounds. Due to this broad spectrum activity of this oil, it can be suggested for use as a natural and safe protective agent to be added to different food products to avoid contamination and spoilage (Dikbasa et al., 2012).

Mahboubi and Kazempour studied the chemical composition and antimicrobial activity of *S. hortensis* essential oil in vitro against 20 different kinds of microorganisms. The antimicrobial activity of the essential oil extracted from aerial parts of *S. hortensis* against 21 different microorganisms was evaluated using the micro broth dilution assay. In this study, the values of minimum lethal concentration ranged from 0.06 to 16 and the minimum inhibitory concentrations ranged from 0.06 to 8, as determined for the volatile oil of savory. The savory essential oil showed strong antimicrobial effects against the tested microorganisms. Different tested microorganism strains exhibited differences in their sensitivity to these compounds. Some of the main components of the *S. hortensis* essential oil are thymol, carvacrol, γ-terpinene, and *p*-cymene. They, especially thymol, play a major role in showing strong antimicrobial activity for the oil. It has been elucidated that the minor compounds of the volatile oil also can have an important role in the antimicrobial effects of the oil.

Regarding the various side effects of some drugs, antibiotic resistance increases the need to use natural compound as antimicrobials. Therefore, as a safe and natural component, plants and their volatile oils that are highly effective against a broad spectrum of microorganisms can replace the synthetic compounds (Mahboubi and Kazempour, 2011).

Dilek et al. investigated the antibacterial and antifungal activity of major constituents and the essential oils obtained from the aerial parts of *S. hortensis* collected from two different areas of Turkey against different microorganisms. The microorganisms tested were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Candida albicans*, *Penicillium clavigerum*, *Mucor hiemalis*, and *Absidia glauca*. The major components of the oil of both *S. hortensis* were thymol (40.6% and 43.4%). Results indicated that both essential oils and their major compound obtained from these plants were able to inhibit all tested microorganisms (Dilek et al., 2005; Table 1).

TABLE 1 Major Components of *Satureja hortensis* L. Essential Oil

	Güllüce et al. (2003)	Mahboubi and Kazempour (2011)	Djenane et al. (2013)
Compounds	(%)	(%)	(%)
p-Cymene	9.3	19.6	12.04
γ-Terpinene	22.6	16.0	11.43
Thymol	29.0	28.2	–
Carvacrol acetate	–	–	9.57
Carvacrol	26.5	11.0	46.10
1,8-Cineole	–	–	37.82

The antimicrobial activity of the essential oil of *S. hortensis* was evaluated using 23 bacteria and 15 fungi and yeast species in Turkey. The essential oil of this plant showed great antimicrobial activity against all the tested bacteria and fungi (Güllüce et al., 2003).

Saharkhiz et al. investigated the antimicrobial activity of the essential oil obtained from *S. hortensis* at floral budding, full flowering, immature fruit, and ripened fruit stages. Results indicated strong antibacterial activities against the tested bacteria when the microdilution method was used. They suggested that the wide range of antimicrobial activities of the essential oils obtained from different parts of this plant could have potential candidates for usage in the preparation of infective antimicrobial agents (Saharkhiz et al., 2011).

In this research, the antibacterial activity of hydrosols obtained from summer savory against two of nontoxigenic and toxigenic *E. coli* O157:H7 strains inoculated to two different kinds of fruit, namely fresh-cut cucumbers and tomato. In this work, Sagdic et al. compared the obtained results with a water wash as control. While the water wash was not sufficient to eliminate the bacteria in these fruits, the hydrosols obtained from summer savory completely inhibited both *E. coli* O157:H7 strains. These results suggested that hydrosols obtained from summer savory have the potential to be used as natural food sanitizers in order to provide microbial safety while causing no important problem on treated products or human health (Sagdic et al., 2013).

The antifungal activity of summer savory essential oil was evaluated against *Penicillium digitatum* and *Rhizopus stolonifer*, two important postharvest fungi. The essential oil of this plant was shown to be effective as an antifungal agent. Therefore, it was suggested that the oil obtained from summer savory has the potential to use in fruits and vegetables as an antifungal agent against their fungi (Abdollahi et al., 2011).

The major losses of harvested vegetables and fruit during storage and transportation are due to postharvest diseases. The postharvest pathogenic fungi are responsible for considerable losses of harvested fruits. In the present study, the antifungal potential of the essential oil of *S. hortensis* against *R. stolonifer*, *P. digitatum*, *Aspergillus niger*, and *Botrytis cinerea*, four different postharvest pathogenic fungi on strawberry fruit were examined. The aim of this study was to increase the shelf life of strawberry fruit using this plant oil. The in vivo test volatile oil obtained from *S. hortensis* showed inhibitory effects against all four pathogens. This antifungal activity of the oil suggests the possibility of using it as an antifungal agent on postharvest strawberry fruit in order to reduce postharvest diseases and increase the shelf-life of this fruit (Nabigol, 2011).

One of the most important problems of fruit decay which causes major crop losses of foods and vegetables during storage and shipment is postharvest decay caused by fungi. Relatively high temperature and humidity during marketing and storage of foods and vegetables helps to increase the rate of fungal disease development. These conditions can also affect the rate of grape and strawberry decay during storage and marketing. Different concentrations of essential oil extracted from aerial parts of wild *S. hortensis* collected in Turkey were examined for its effectiveness in reducing grape and strawberry decay during storage at three different temperatures. It was shown that at low temperatures, the essential oil of *S. hortensis* significantly reduced the rate of decay of grape and strawberry fruits during storage. Therefore, this essential oil can be potential fungicides and may be used to protect grape and strawberry from postharvest decay (Dikbas et al., 2011).

The antifungal activity of different concentrations of *S. hortensis* essential oil was investigated against *Botrytis cinerea* on kiwi fruits. Essential oil-treated and inoculated kiwi fruit were stored for 90 days. The results showed while total soluble solids content (TSS), titratable acidity (TA), and vitamin C content of kiwi fruit treated with the essential oil decreased, the weight loss and the firmness of kiwifruits was not affected. However, the kiwi fruit treated with the essential oil of *S. hortensis* showed off-flavor when they were compared with the control sample. This research suggests future work regarding the application of this oil to be used as an agent to control postharvest pathogens (Shirzad et al., 2011a).

Alizadeh-Salteh et al. investigated the effect of savory essential oil antifungal activity on mycelia growth inhibition of *R. stolonifer*. It is known that *R. stolonifer* is the cause of *Rhizopus* rot in stone fruits like peach. This is a postharvest disease, which causes major destruction in these kinds of fruits. Results of this study indicated that antisepticizing with *S. hortensis* essential oil inhibited the growth of *R. stolonifer* completely. Therefore, it may be used as an antifungal agent to increase the shelf life of the fruits when they are stored (Alizadeh-Salteh et al., 2010).

Part of food and feed contamination is due to the toxigenic with the *Aspergillus* species. These species are known to be act as potent hepatocarcinogens in humans. Alizadeh et al. examined the antifungal activity of the essential oils obtained from aerial part and seeds of *S. hortensis* by means of hydrodistillation against some of these toxigenic *Aspergillus* species using different concentration of the oils. Results of the study indicated that the growth of the *Aspergillus* species tested was inhibited. Therefore, it was concluded that the essential oils obtained from *S. hortensis* may be used as an antifungal agent to prevent food contamination (Alizadeh et al., 2010).

In the dairy industry, yeasts play an important role in the preparation of fermented products, in ripening certain cheeses, and fermenting dairy products. In addition, they are partly responsible for spoilage of many food products as well as the dairy products. As spices may have different antimicrobial effects on pathogenic and food spoilage yeasts, the antiyeast activity of summer savory in packaged and unpackaged butters was studied by Sagdic et al. Results of the study indicated that the summer savory may be used as natural antimicrobial agents to prevent the spoilage yeasts in butter and other food products (Sagdic et al., 2010).

One of the major losses of postharvest table grape is caused by *Borytis cinerea*. Abdolahi et al. studied the effect of using summer savory essential oil on controlling decay and preserving quality of tabarzeh table grapes (*Vitis vinifera* L. cv. Tabarzeh). The results indicated that the essential oil obtained from summer savory decreased fungal decay when applied to table grapes after storage for 60 days. The oil had significant effects on the fruit quality parameters and increased the level of maturity index in treated fruits compared with the control fruits. The great antifungal effects of essential oil extracted from summer savory shown in this study suggest that this oil can be used as a safe and natural antifungal agent to preserve table grapes during storage (Abdolahi et al., 2010).

Antibacterial activity of essential oil obtained from *S. hortensis* collected from region Kabyle in Algeria was examined against *Salmonella enterica* serotype Enteritidis CECT 4300 using two different methods, agar diffusion and microdilution. The results of the study showed that the essential oil possess significant antisalmonella activity. The strength of the antibacterial effect of the oil was proportional to the amount of the oil added to the tested product. It was concluded that essential oil of *S. hortensis* may be a promising natural antibacterial agent to be used for liquid whole eggs preservation (Djenane et al., 2011).

As an Antioxidant

Oxidation processes in food products during the storage produce ROS which leads to chemical spoilage of foodstuffs. These deteriorations are the main reason for loss of food nutritional quality, color, and flavor. To prevent and delay oxidation, the reaction should be inhibited using antioxidant compounds. Essential oils are a natural safe botanical source that have strong antioxidant properties and can replace the synthetic preservatives in the food industry to avoid food contamination and delay the deterioration of food, vegetables, and fresh fruits. *Satureja hortensis* essential oil has strong antioxidant activity and can be used in food industry as a natural and effective antioxidant agent.

Satureja hortensis essential oil was used as natural a antioxidant in the oil of mayonnaise in mayonnaise formulated with linseed oil (containing omega-3 fatty acids). The antioxidant activity of the oil was investigated by Vahidyan et al. and was compared with a synthetic antioxidant, butylated hydroxyanisole. The results showed a better antioxidant effect for *S. hortensis* essential oil. It was concluded that a natural antioxidant of *S. hortensis* essential oil can be used in mayonnaise linseed oil (containing omega-3 fatty acids) in order to improve the quality of this mayonnaise formulation. Replacing the synthetic antioxidant with this natural antioxidant in this food product, which is currently one of the most popular sauces in the world, helps to increase the level of public health (Vahidyan et al., 2012).

Fathi et al. examined the antioxidative effect of *S. hortensis* volatile oil and its application in safflower oil. The application of antioxidant activity of the essential oil obtained from the aerial parts of *S. hortensis* was evaluated as a natural preservative in safflower oil using various methods such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), β-carotene bleaching, 2,2-diphenyl-1-picrylhydrazyl, ferric thiocyanate, and peroxide conjugated dienes and thiobarbituric acid values. The results of this study indicated that the antioxidant activity of the *S. hortensis* volatile oil was able to slow the rate of the oxidation of safflower oil and its activity is comparable to the antioxidant effects of synthetic antioxidants. The antioxidant activity of this oil was increased by increasing the concentration. They concluded from their results as well as results of previous research that *S. hortensis* volatile oil shows antioxidant activity in safflower oil. Therefore, it might be possible to use the volatile oil of this plat as a natural antioxidants compound with botanic source in order to replace the harmful synthetic preservatives (Fathi et al., 2013).

Shojaee-Aliabadi et al. characterized the physical, optical, mechanical, barrier, and antioxidant properties of biodegradable composite κ-carrageenan films when incorporated with *S. hortensis* volatile oil. In addition, the antimicrobial activities of these films were evaluated against five pathogens. The films incorporating *S. hortensis* essential oil resulted in good antioxidant activity and the five tested microorganisms were inhibited by these films. These results suggested the possible use of *S. hortensis* volatile oil as a natural antibacterial agent in the food industry in order to be used in microbial sensitive and/or highly oxidative food product packaging (Shojaee-Aliabadi et al., 2013).

In this study, different concentrations of essential oil of summer savory were sprayed on kiwi fruits (*Actinidia deliciosa*) in order to find a natural alternative for preservation of kiwi fruits during the postharvest stage and replace the currently used synthetic agrochemicals. Results indicated that while TSS, TA, and vitamin C content of kiwi fruits decreased during storage (0–1 °C) for 90 days when treated with the essential oil, the weight loss and the firmness of kiwi fruits did not significantly affected (Shirzad et al., 2011b).

The effect of spice feeding on sensory properties and some chemical of chickens fed with the aromatic plant *S. hortensis* was investigated in this study by Yetim et al. Chicken breast and thigh samples from spice feeding chickens were compared with those fed with a basal diet for 40 days. Results indicated that the spice-fed chicken samples showed better stability during storage with no undesirable change to sensory properties (Yetim et al., 2011).

Undesirable odors and flavors of oil in food products is caused by autoxidation during storage and/or heating which deteriorate the edible oil in foods. The antioxidant effects of *S. hortensis* essential oil was evaluated in comparison with BHT, a synthetic antioxidant, to examine the soybean oil oxidative stability. It was concluded that soybean oil amended with *S. hortensis* essential oil showed fairly good antioxidant effect compared with BHT. Control samples were refined, bleached, deodorized soybean oil with no additives (Fathi et al., 2011).

SUMMARY POINTS

- *Satureja hortensis* is a culinary herb, traditionally used as a medicinal plant in folk medicine.
- *Satureja hortensis* has been extensively used in food and the food industry for flavoring.
- *Satureja hortensis* has been use as in the perfume industries.
- *Satureja hortensis* and its product are extensively used as flavoring agents.
- *Satureja hortensis* essential oil has various pharmacological activities.
- *Satureja hortensis* essential oil has antimicrobial activity.
- *Satureja hortensis* essential oil has antioxidant activity.

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Chapter 87

Sweet Fennel (*Ocimum gratissimum*) Oils

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INTRODUCTION

The use of plants for therapeutic purposes is reported in old literature, such as Chinese, Egyptian, Hindu, Greek, and Roman. In Africa, the therapeutic potential of plants was known empirically (Nacoulma-Ouedraogo, 1996). To achieve an improvement in this African medicine, several phytochemical properties have been investigated to provide a scientific justification for the traditional use of medicinal plants.

In spite of modern improvements in food hygiene and food production techniques, food safety is an increasingly important public health issue (WHO, 2002). Therefore, methods for reducing or inhibiting food-borne pathogens are still needed. The industrialized societies appear to experience the trend toward green consumption, desiring fewer synthetic food additives and products with a low impact on the environment (Sacchetti et al., 2005). For this reason, there is a scope for new methods of producing safe foods that have a natural or green image. One such possibility is the use of essential oils from aromatic and medicinal plants that have been known since antiquity to possess biological activity, namely antibacterial, antifungal, and antioxidant properties (Bounatirou et al., 2007). Furthermore, they are also used as insect repellents, and larvicidal and insecticidal agents (Aisien et al., 2004). Some components present in essential oils are classified as generally recognized as safe substances and therefore can be used to prevent postharvest growth of native and contaminant bacteria.

Previous studies have established that essential oil from fresh leaves of *Ocimum gratissimum* L. (family Lamiaceae) have potent antioxidant, anticancer, antiviral, and antimicrobial properties (Adebolu and Salau, 2005; Adjou et al., 2013). It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being *Vriddhutulsi* (Sanskrit), *Ram tulsi* (Hindi), *Nimma tulasi* (Kannada). The foliage is also commonly used fresh in cooked recipes or added at the last moment (Giami et al., 2005).

In other to underline the importance of the use of plant extracts in the replacement of synthetic antimicrobial substances, this chapter emphasizes the botanical aspects, usage, and potential applications of *O.gratissimum* in food science. Through this chapter, we hope to attract the attention of natural product researchers throughout the world to focus on the explored potential of *O. gratissimum*. This chapter looks at the theoretical development of a natural preservative system using the authors' database of medicinal plants as a source of references.

BOTANICAL ASPECTS

The plant *O. gratissimum* is a perennial plant that is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiateae, the genus *Ocimum*, and the species *gratissimum*. It is woody at the base and has an average height of 1–3 m. The leaves are broad and narrowly ovate, usually 5–13 cm long and 3–9 cm wide (Figure 1).

According to available information, such as those published by Prabhu et al. (2009) and the Agroforestry Database (Orwa et al., 2009), *O. gratissimum* is an aromatic, perennial herb, 1–3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips. Leaves opposite; petiole 2–4.5 cm long, slender, pubescent; blade elliptical to ovate, 1.5–16 × 1–8.5 cm, membranaceous, sometimes glandular punctate, base cuneate, entire, margin elsewhere coarsely crenate–serrate, apex acute, puberulent or pubescent. Inflorescence a verticillaster, arranged in a terminal, simple or branched raceme 5–30 cm long; rachis lax, softly pubescent; bracts sessile, ovate, 3–12 × 1–7 mm, acuminate, caducous; pedicel 1–4 mm long, spreading or ascending, slightly curved; flowers in 6–10-flowered verticillasters, small, hermaphrodite; calyx two-lipped, 2–3 mm long, in fruit 5–6 mm, pubescent, upper lip rounded and recurved, reflexed in fruit, lower lip with four narrow, pointed teeth, central pair of teeth minute and much shorter than the upper lip; corolla campanulate, 3.5–5 mm long, two-lipped, greenish white,

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pubescent outside, upper lip truncate, four-fid, lower lip longer, declinate, flat, entire; four stamens, declinate, in two pairs, inserted on the corolla tube, filaments distinctly exserted, upper pair with a bearded tooth at the base; ovary superior, consisting of two carpels, each two-celled, style two-fid. Fruit consists of four dry, one-seeded nutlets enclosed in the persistent calyx (the lower lip closing the mouth of the fruiting calyx); nutlet subglobose, 1.5 mm long, rugose, brown; outer pericarp not becoming mucilaginous in water.

Ocimum gratissimum is a variable polymorphic complex species, often subdivided into subspecies, varieties, and formas, mainly based on differences in chemical content, the morphology of the fruiting calyx, and on different degrees of hairiness, but the variation forms a continuum. Variability is greatest in Africa and India. In Java, two chemotypes exist, the eugenol and the thymol type, respectively, described as *O. gratissimum* L. forma *caryophyllatum* Backer and forma *graveolens* Backer. Forma *caryophyllatum* is characterized by: leaves clove-scented when bruised, upper side short-haired, lower side densely gland-dotted, bracts 4–6 mm long, much longer than wide, lower lip of corolla not flushed with violet; and forma *graveolens* by leaves strongly odoriferous but not clove-scented when bruised, upper surface covered with minute hairs, bracts 2–4 mm long, about as long as wide, lower lip of corolla flushed violet inside.

USAGE AND CLINICAL APPLICATIONS

The use of plants as functional ingredients in foods, drinks, toiletries, and cosmetics is gaining momentum, due to both growing consumer interest in ingredients from natural sources and also because of increasing concerns about potentially harmful synthetic additives. Plant extracts are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multipurpose functional use. Several studies reported that *O. gratissimum* has been used extensively in the traditional system of medicine in many countries. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever, and diarrhea (Effraim et al., 2003). Prabhu et al. (2009) reported that Brazilian tropical forest inhabitants use a decoction of *O. gratissimum* roots as a sedative for children.

Clinical studies indicated that preparations containing 2% *O. gratissimum* oil in cetomacrogol are significantly more active than benzoyl peroxide for the management of acne (Orafidiya et al., 2001). Kumar et al. (2011) reported that the methanolic leaves extract of *O. gratissimum* demonstrated a significant antinociceptive activity at doses of 50, 100, and 200 mg/kg body weight. Holetz et al. (2003) indicated that *Herpetomonas samuelpessoai*, a nonpathogenic trypanosomatid, exposed to 100 µg/mL of essential oil in chemically defined medium at 28 °C for 72 h presented considerable ultrastructural alteration, mainly at the mitochondrial level. Furthermore, cells cultivated in the presence of 100 µg/mL of essential oil showed a decrease of activity of the succinate cytochrome *c* reductase enzyme, a typical mitochondrion marker, compared with untreated cells.

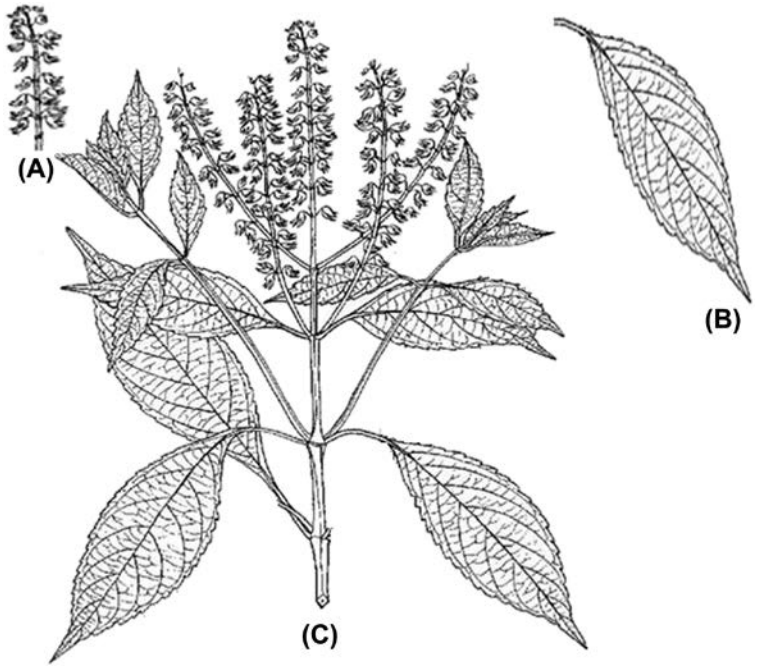

FIGURE 1 Flowering tops of *Ocimum gratissimum* (A); leaves of *O. gratissimum* (B); whole plant of *O. gratissimum* (C).

USAGE AND APPLICATIONS IN FOOD SCIENCE

Many areas of food science are concerned about the use of essential oils. In food products, essential oils have been used in bakery (Nielsen and Rios, 2000), cheese (Vazquez et al., 2001), meat (Quintavalla and Vicini, 2002), and fruit (Lanciotti et al., 2004), among others. The advantage of essential oils is their bioactivity in the vapor phase, a characteristic that makes them useful as possible fumigants for stored commodity protection.

In the available literature, there is very little about the use of sweet fennel oil in food science. In these, the antifungal and antiaflatoxinogenic properties of essential oil from the fresh leaves of *O. gratissimum* were reported. Indeed, an experiment was conducted by Adjou et al. (2013) in order to evaluate the effect of essential oil from fresh leaves of sweet fennel (*O. gratissimum*) on mycoflora and *Aspergillus* section Flavi populations in stored peanuts. Results from this study revealed that essential oil exhibited pronounced antifungal activity against the growth of *Aspergillus flavus* and *Aspergillus parasiticus*. The minimal inhibitory concentration (MIC) of the essential oil was found to be 7.5 µL/mL for *A. flavus* and *A. parasiticus* (Tables 1 and 2) and 5.5 µL/mL for *Aspergillus ochraceus* and *Fusarium. oxysporum* (Table 3). The minimum fungicidal concentration was recorded to be 8.0 µL/mL for *A. flavus* and *A. parasiticus*, 6.5 µL/mL for *A. ochraceus*, and

TABLE 1 *Aspergillus flavus* Colony Diameters Recorded (mm) with Essential Oil of *Ocimum gratissimum*

	Essential Oil of *Ocimum gratissimum*						
Day	5.0 µL/mL	5.5 µL/mL	6.0 µL/mL	6.5 µL/mL	7.0 µL/mL	7.5 µL/mL	8.0 µL/mL
1	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
2	9.1 ± 0.04^{b}	7.5 ± 0.06^{b}	7.2 ± 0.01^{b}	6.6 ± 0.01^{b}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
3	18.6 ± 0.04^{c}	12.4 ± 0.01^{c}	10.5 ± 0.09^{c}	7.3 ± 0.08^{c}	6.9 ± 0.02^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
4	32.8 ± 0.02^{d}	27.3 ± 0.08^{d}	21.6 ± 0.02^{d}	11.3 ± 0.07^{d}	10.2 ± 0.07^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
5	50.7 ± 0.06^{e}	41.3 ± 0.09^{e}	33.7 ± 0.06^{e}	19.1 ± 0.04^{e}	11.6 ± 0.09^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
6	68.6 ± 0.08^{f}	52.1 ± 0.04^{f}	46.3 ± 0.01^{f}	28.2 ± 0.05^{f}	14.4 ± 0.06^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
7	74.2 ± 0.05^{g}	61.5 ± 0.08^{g}	54.8 ± 0.06^{g}	35.8 ± 0.02^{g}	16.0 ± 0.04^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
8	84.8 ± 0.04^{h}	79.3 ± 0.02^{h}	64.9 ± 0.07^{h}	43.7 ± 0.06^{h}	21.6 ± 0.03^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}

Values (mm) are mean (n = 3) ± SD. Letters "a to h" are comparison indices. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests. Diameter of 6 mm is the inoculation point.
Adjou et al. (2013).

TABLE 2 *Aspergillus parasiticus* Colony Diameters Recorded (mm) with Essential Oil of *Ocimum gratissimum*

	Essential Oil of *Ocimum gratissimum*						
Day	5.0 µL/mL	5.5 µL/mL	6.0 µL/mL	6.5 µL/mL	7.0 µL/mL	7.5 µL/mL	8.0 µL/mL
1	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
2	11.4 ± 0.06^{b}	10.7 ± 0.08^{b}	9.1 ± 0.04^{b}	9.4 ± 0.02^{b}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
3	33.7 ± 0.09^{c}	21.6 ± 0.02^{c}	22.5 ± 0.04^{c}	13.9 ± 0.06^{c}	9.5 ± 0.04^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
4	41.7 ± 0.06^{d}	36.5 ± 0.06^{d}	34.2 ± 0.06^{d}	21.4 ± 0.08^{d}	15.6 ± 0.01^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
5	57.5 ± 0.09^{e}	54.2 ± 0.09^{e}	48.6 ± 0.07^{e}	29.7 ± 0.04^{e}	19.3 ± 0.04^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
6	69.8 ± 0.03^{f}	66.1 ± 0.01^{f}	54.3 ± 0.07^{f}	33.8 ± 0.02^{f}	21.6 ± 0.02^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
7	79.3 ± 0.01^{g}	73.8 ± 0.05^{g}	68.4 ± 0.04^{g}	46.3 ± 0.08^{g}	23.7 ± 0.04^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}
8	89.3 ± 0.07^{h}	81.7 ± 0.05^{h}	72.9 ± 0.07^{h}	51.2 ± 0.04^{h}	38.8 ± 0.07^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}

Values (mm) are mean (n = 3) ± SD. Letters "a to h" are comparison indices. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests. Diameter of 6 mm is the inoculation point.
Adjou et al. (2013).

TABLE 3 Colony Diameters Recorded (mm) of *Aspergillus ochraceus* Isolated from Peanut Exposed to the Essential Oil of *Ocimum gratissimum*

	Essential Oil of *Ocimum gratissimum*						
Day	5.0 µL/mL	5.5 µL/mL	6.0 µL/mL	6.5 µL/mL	7.0 µL/mL	7.5 µL/mL	8.0 µL/mL
1	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]
2	8.2 ± 0.05[b]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]
3	14.5 ± 0.01[c]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]
4	17.3 ± 0.04[d]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]
5	25.4 ± 0.06[e]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]
6	30.6 ± 0.03[f]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]
7	41.6 ± 0.04[g]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]
8	45.3 ± 0.07[h]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]	6.0 ± 0.00[a]

Values (mm) are mean (n = 3) ± SD. Letters "a to h" are comparison indices. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests. Diameter of 6 mm is the inoculation point.
Adjou et al. (2013).

TABLE 4 Percentage Inhibition (PI) of Mycelial Growth

Concentration of Essential Oil	Percentage Inhibition			
	Strains Isolated from Peanut			
	Aspergillus flavus	*Aspergillus parasiticus*	*Aspergillus ochraceus*	*Fusarium oxysporum*
5.0 µL/mL	5.77 ± 0.7[a]	0.77 ± 0.5[a]	49.66 ± 0.2[a]	66.44 ± 0.5[a]
5.5 µL/mL	11.88 ± 0.9[b]	9.22 ± 1.1[b]	100 ± 0.00[b]	100 ± 0.00[b]
6.0 µL/mL	27.88 ± 0.3[c]	19.0 ± 0.3[c]	100 ± 0.00[b]	100 ± 0.00[b]
6.5 µL/mL	51.44 ± 0.5[d]	43.11 ± 0.3[d]	100 ± 0.00[b]	100 ± 0.00[b]
7.0 µL/mL	76.0 ± 0.2[e]	56.88 ± 0.7[e]	100 ± 0.00[b]	100 ± 0.00[b]
7.5 µl/ml	100 ± 0.00[f]	100 ± 0.00[f]	100 ± 0.00[b]	100 ± 0.00[b]
8.0 µL/mL	100 ± 0.00[f]	100 ± 0.00[f]	100 ± 0.00[b]	100 ± 0.00[b]

Values are mean (n = 3) ± SD. Letters "a to f" are comparison indices. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.
Adjou et al. (2013).

6.0 µL/mL for *F. oxysporum*. The radial growth of strains was totally inhibited by the essential oil. Percentage of growth inhibition (PI) evaluated by direct method was significantly affected by incubation time and essential oil concentration. Mycelia growth was considerably reduced with increasing concentration of essential oil while their growth increased with incubation time. The essential oil was more active on the mycelia growth of *A. ochraceus and F. oxysporum* than *A. parasiticus* and *A. flavus*. The PI of the essential oil was 76% and 56.9%, respectively, on *A. flavus and A. parasiticus* at 7.0 µL/mL, while the radial growth of *A. ochraceus* and *F. oxysporum* was totally inhibited (PI = 100%) by the oil at lower concentration (5.5 µL/mL) after 8 days of incubation (Table 4). The essential oil of sweet fennel, with fungal growth and mycotoxin inhibitory properties, offers a novel approach to the management of storage, thus opening the possibility to prevent mold contamination in stored peanuts.

Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetable crops cultivated all over the world for its fleshy fruits. It is recognized as one of the important commercial and dietary vegetable crops (Bauer et al., 2004). However, a proportion of this vegetable is rendered unsaleable on farms and in markets due to physical, chemical, and microbiological defects (Amadioha and Uchendu, 2003). Better control measures to prevent spoilage of tomatoes are necessary

to avoid contamination by mycoflora. The use of synthetic antimicrobial to control tomato spoilage molds and pathogen bacteria has been discouraged due to their carcinogenicity, teratogenicity, high and acute residual toxicity, and long-term degradation (Barkat and Bouguerra, 2012). Alternative natural additives are therefore needed in order to guarantee food safety. Houinsou et al. (2012) investigated the antimicrobial activities of essential oil extracted from leaves of sweet fennel against pathogenic and adulterated microorganisms associated to tomato. Results from this study indicated that oil tested has pronounced antimicrobial activity against strains of fungi (*Aspergillus niger*, *F. oxysporum, Fusarium graminearum*, and *Fusarium poae*) and bacteria (*Escherichia coli, Staphylococcus aureus*) isolated from tomatoes. According to these different results, the essential oil of *O. gratissimum* can be used as a good biopreservative of tomatoes to reduce postharvest losses and consequently increase its relative availability for our population.

Moreover, traditional cheese, locally known as *wagashi* in Benin, is an important source of animal protein. However, methods used during production stocking or selling lead to contamination by toxinogenic or pathogenic microorganisms, especially fungi. This contamination may contribute to the loss of quality and safety. In fact, fungal growth may result in several kinds of cheese spoilage: off-flavors, toxins, mycolytic, and enzymes. Furthermore, fungi produce allergenic compounds and toxic metabolites which may penetrate the cheese and affect the consumer's health. Moreover, fungi have been reported by Aïssi et al. (2009) to cause extensive deterioration of *wagashi* which affects its preservation and may lead to occurrence of mycotoxins. Better control measures to prevent spoilage of *wagashi* is necessary to avoid its contamination by mycoflora. Indeed, Sessou et al. (2012) have investigated the efficacy of the essential oil of sweet fennel on fungi isolated from *wagashi*, such as *A. flavus, Aspergillus tamarii*, *F. poae, Fusarium verticillioides*, *Penicillium citrinum*, and *Penicillium griseofulvum* isolated from *wagashi* produced in Benin. The results showed that the percentages of mycelial growth inhibition are significantly influenced by incubation time and essential oil concentrations. Mycelia growth was reduced with increasing concentration of oil. Essential oil of *O. gratissimum* had significant fungistatic activity against all the fungal species isolated with MIC values ranged from 800 to 1000 mg/L. *Penicillium* species and *F. poae* were the most sensible to this essential oil with MIC estimated to 800 mg/L. According to results obtained from this study, the sweet fennel oil could be used as natural antimicrobial agent in against molds responsible for biodeterioration of *wagashi*.

The effectiveness of the essential oil was also investigated in the field of conservation of fishery product. In fact, fish and fish products have an important place in the diet of the people of West Africa. However, the conservation of fish is very difficult due to the lack of adequate conservation system. Smoking is one of primary methods used for preserving fish in West Africa and is still made in the traditional way. The smoked mackerel (*Trachurus trachurus*) is a widely consumed in some African countries because of its availability and accessibility. However, despite many efforts made in the conservation of this fish through smoking, the problem still remains because of the perishable nature of smoked fish due to microbial growth. Thus, the problems mainly related to the preservation during a long period of smoked fish, forcing traders to perform further periodic smoking of the fish already smoked, especially in cases of poor sales. The nutritional quality of these fish smoked in several times is altered and the exposure to smoke for a long time, often leads to the presence of toxic chemical residues in flesh of the fish. The restrictions imposed by the food industry and regulatory agencies on the use of some synthetic food additives have led to a renewed interest in searching for alternatives, such as natural antimicrobial compounds, particularly those from plants. Thus, Degnon et al. (2013) evaluated the quality of smoked mackerel throughout the processing chain, to identify potential sources of microbial contamination and investigate the bioactivity potential of the essential oil extracted from leaves of sweet fennel as antifungal agent in the postsmoking preservation of mackerel (*Trachurus trachurus*) in Benin. Application tests are designed to assess, in a semicontrolled environment, the effect of the essential oil in protecting smoked fish against of microbial alterations. For the incorporation of essential oil on the smoked fish, two methods were used: incorporation using adsorption methods and incorporation by injection method. The injection of the essential oil was performed using sterile syringes at four body parts of smoked fish. An injection in the gill area, another injection at each of the two face sides of the fish, and, finally, a third injection point at the tail of the fish. Periodic microbial analyses were performed in other to evaluate the quality of smoked fish during the storage. Results of microbial count on smoked fish preserved with essential oil of sweet fennel indicated that after five days of storage, no visible growth of bacteria or fungi was detected in samples preserved by adding the essential oil using adjunction method at concentration of 0.25 mL/70 g. However, with the injection method, only 1 mL/70 g totally inhibited the bacteria and fungi growth. In opposition, major signs of tampering were noticed in control smoked fish after 5 days. The preservation of smoked fish by incorporation essential oil increased the shelf life of the product.

The bioefficacy of the sweet fennel oil may be due to the presence of some highly fungitoxic components in the oil such as terpenoids. Indeed, *O. gratissimum* oil has chemical compositions characterized by terpenes (*p*-cymene and γ-terpinene) and terpenoids (thymol) as main chemical groups (Table 5) (Sahouo et al., 2003; Lemos et al., 2005; Anand et al., 2011; Adjou et al., 2013). Other compounds such as eugenol and *cis*-ocimene were detected. Terpenoids are a large group of antimicrobial compounds that are active against abroad spectrum of microorganisms (Dorman and Deans, 2000).

TABLE 5 Main Components Identified as Constituents of Essential Oil of *Ocimum gratissimum* from Different Countries

Compounds	Benin[a] (%)	India[b] (%)	Ivory Coast[c] (%)	Brazil[d] (%)
α-Thujene	8.2	–	1.3	–
α-Pinene	1.2	0.83	0.8	–
Camphene	0.3	0.73	–	–
β-Pinene	0.7	–	–	–
Myrcene	6.4	0.20	1	0.34
α-Phellendrene	0.5	0.46	–	–
α-Terpinene	4.2	–	–	–
p-Cymene	17.6	0.06	–	2.11
Limonene	2.5	0.05	0.3	–
1,8-Cineole	2.1	0.09	–	–
(E)-β-Ocimene	0.3	0.97	–	0.49
cis–Ocimene	–	23.97	–	–
γ-Terpinene	20.0	–	2.3	–
p-Cymenene	2.2	–	–	0.26
Terpinolene	0.1	–	–	–
Linalol	0.2	0.23	–	–
Borneol	0.2	–	–	–
Terpinen-4-ol	1.2	0.33	–	0.24
p-Cymen-8-ol	0.2	–	–	–
α-Terpineol	0.1	–	2.2	–
Thymol methylether	0.3	–	–	–
Thymol	26.9	–	70.8	9.80
Carvacrol	0.7	–	1.1	0.28
α-Copaene	0.1	–	–	0.11
β-Caryophyllene	1.2	1.86	–	3.03
Eugenol	–	53.89	–	57.82
Trans-α-bergamotene	0.1	–	–	–
α-Humulene	0.2	–	–	0.31
Germacrene D	0.1	10.36	–	0.79
(Z)-α-Bisabolene		–	–	17.19
β-selinene	0.4	–	–	–
α-selinene	0.2	–	–	0.45
δ-cadinene	0.1	–	–	–
Caryophyllene oxide	0.2/	–	1.6	0.82
Total	**99.0**	**94.03**	**81.4**	**94.04**

[a] *Adjou et al. (2013)*.
[b] *Anand et al. (2011)*.
[c] *Sahouo et al. (2003)*.
[d] *Lemos et al. (2005)*.

Their antimicrobial activities are linked to their functional groups and it has also been reported that the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are important for antimicrobial activity (Dorman and Deans, 2000). The most active monoterpenoids identified so far are carvacrol and thymol (Hyldgaard et al., 2012). Moreover, the antimicrobial activity of carvacrol, thymol, linalool, and menthol was evaluated against *Listeria monocytogenes*, *Enterobacter aerogenes*, *E. coli*, and *Pseudomonas aeruginosa*. The most active compound was carvacrol followed by thymol with their highest MIC being 300 and 800 μg/mL, respectively (Bassole et al., 2010). These results confirm the high antimicrobial activity of a broad collection of terpenoids especially of thymol which is the major component of the essential oil of *O. gratissimum*.

Despite the strong antimicrobial and antioxidant potential of essential oils, there is much evidence that their large application in the food industry as antimicrobial or antioxidant agents is not very easy, due to the fact that food composition and structure have a significant effect on the dynamic and final outcomes of an interaction. Naturally present ingredients can favor or inhibit the interactive cultures. In fact, a high fat content appears to markedly reduce the action of essential in meat products. It is generally supposed that the high levels of fat and/or protein in foodstuffs protect the bacteria from the action of essential oil in some way. This approach was also reported by Rasooli (2007). Indeed, in fish, just as in meat products, a high fat content appears to reduce the effectiveness of antibacterial oil. For example, some essential oils, at 0.5 μL/g, are more effective against the spoilage organism *Photobacterium phosphoreum* in cod fillets than in salmon, which is a fatty fish (Mejlholm and Dalgaard, 2002). Another approach is that the lower water content of food compared with laboratory media may hamper the progress of antibacterial agents to the target site in the bacterial cell (Smith-Palmer et al., 2001).

Due to the fact that essential oils are volatile, the organoleptic impact on treated food must also be evaluated. Individual oil components, many of them approved food flavorings, also impart a certain flavor to foods. For example, in fish, carvacrol is known to produce a "warmly pungent" aroma: citral is "lemon-like" and geraniol "rose-like" (Kim et al., 1995). Treatment of fresh kiwi fruit and honeydew melon with carvacrol or cinnamic acid has also been found to delay spoilage without causing adverse organoleptic changes (Roller and Seedhar, 2002).

CONCLUSION

This chapter highlights the potential use of sweet fennel oils in foods science, and also justifies and encourages the preservation of food with natural additives due to greater consumer awareness and concern regarding synthetic chemical additives. Nevertheless, in relation to the composition of foods, further investigations are necessary to identify the conditions that maximize their activity without detrimental effects on the organoleptic properties of the product. In the same approach, synergistic effects could be exploited so as to maximize the antibacterial activity of oils and to minimize the concentrations required to achieve a particular antibacterial effect. Interactions between oils and their components and other food ingredients and food additives need to be investigated. Antagonism between oils and food ingredients is undesirable and research is needed so it can be avoided in practical applications. Further elucidation of the mechanisms of antimicrobial actions of oils would provide insights that may prove useful for technological applications.

SUMMARY POINTS

- Restrictions imposed by the food industry and regulatory agencies on the use of some synthetic food additives have led to renewed interest in searching for alternatives, such as natural antimicrobial compounds, particularly those from plants.
- Essential oils as well as derived compounds possess a wide range of activities of which the antimicrobial activity is most studied.
- Plants belonging to the Lamiaceae family like sweet fennel (*Ocimum gratissimum* Linn.) have retained attention because of their variable use as popular medicines to treat diseases.
- Based on the antimicrobial and fungicidal potential of essential oil of *Ocimum gratissimum* L., its use in the food industry as a preservative is becoming more attractive.

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Chapter 88

Sweet Flag (*Acorus calamus*) Oils

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Abbreviations

EO Essential oil
LC_{50} Median lethal concentration
MIC Minimum inhibitory concentration
MRSA Methicillin-resistant *Staphylococcus aureus*

INTRODUCTION

Sweet flag, *Acorus calamus*, belonging to the family Araceae, in the genus *Acorus*, is a semiaquatic plant of aquatic habitats throughout tropical to subtropical climates. It is a perennial monocot with a creeping and much branched aromatic rhizome, known for its medicinal, insecticidal, and pharmacological applications (Balakumbahan et al., 2010). The scented leaves and rhizomes also make sweet flag oils valued in the perfume industry and food flavoring. Many active constituents and essential oils are extracted from the leaves and rhizomes of sweet flag, which play an important role in food science as they often possess insecticidal, antibacterial, and antifungal properties (Motley, 1994). Therefore, it remains potential for sweet flag oils to be developed as natural insecticides for food preservation. However, the application of sweet flag oil as a food preservative requires detailed knowledge about their properties, for example, the range of target organisms and the mode of action. The purpose of this chapter is to provide an overview of current knowledge about the insecticidal and antimicrobial properties and the mode of action of sweet flag oils, to identify research avenues that can facilitate implementation of sweet flag oils as natural preservative in food.

BOTANICAL ASPECTS

Scientific names: *Acorus calamus* Linn, *Chang pu* (China).

Species

There are three species distinguished by chromosome number: a diploid form, an infertile triploid form, and a tetraploid form. The different cytotypes show a great morphological variability and also large differences in the chemical composition of the essential oils from the rhizomes and leaves, which differ depending on species. *Acorus calamus* var. *americanus* for diploids distributes from North America to Siberia. The sterile triploid form, *A. calamus* var. *vulgaris*, is a hybrid between the diploid and tetraploid cytotypes, and grows throughout Europe, India, and the Himalayan region. The tetraploid variety, *A. calamus* var. *angustatus*, is found in eastern and tropical southern Asia (Motley, 1994; Balakumbahan et al., 2010).

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Botany

Sweet flag is a perennial aromatic herb, with creeping, branching, and rhizomatous rootstock. Rhizomes with aromatic are about the thickness of a thumb and can grow to over three feet long (Figure 1). They are prostrate, firm, and stout with compactly arranged annular rings with numerous rootlets. Leaves arising from rhizomes are in the tight bundles, are linear, flat sword-like, 25–50 cm long, 1–1.5 cm wide, with waved margins and stout midribs, base of leaf sheathed, clasping to each other (Figure 2). Spathe is green, much elongated, and similar to the leaves in shape. There is a small greenish–brown tapering spadix, about 3–5 cm long, 1 cm or less in diameter. It bears hundreds of tiny yellowish–green flowers that make up a concave-shaped spadix inflorescence (Figure 3). Infructescence is about 1.5–2 cm in diameter and it turns to straw-brown at maturity (Motley, 1994). The small flowers are hermaphrodite with both male and female organs are pollinated by insects. Its flower season lasts from May to July and the seeds ripen from July to August. Fruits are berries, turbinate, prismatic, with pyramidal tops, about 0.2 cm in diameter.

FIGURE 1 The sweet flag rhizome. *Note: This Figure was published online.*

FIGURE 2 Sweet flag showing foliage, inflorescence, rhizome, and roots. *Note: This Figure was published online.*

Habitats

This aquatic plant grows by ponds, lakes, marshes, river edges, moist soils, and shallow water in ditches.

Range

Probably indigenous to India or Arabia, sweet flag is now found across Europe, southern Russia, northern Asia Minor, southern Siberia, China, Indonesia, Japan, Burma, Sri Lanka, Australia, as well as southern Canada and the northern United States.

Propagation

Sweet flag is propagated by rhizomes. The rhizomes are cut into small pieces and planted. Sprouted rhizome pieces are planted at a spacing of 30×30 cm and depth of 4 cm in July–August. The second fortnight of June is the best time for planting.

Harvesting

In December, the lower leaves turn yellow and dry indicating their maturity. The uprooted rhizome is cleaned and cut into a size of 5–7.5 cm length. The cut rhizomes are dried by spreading under the shade so that the amount of oil present in it is not harmed.

CHEMICAL COMPOSITION

The rhizomes and leaves of sweet flag are known to contain 1–4.8% of essential oils. The essential oils extracted from sweet flag consist of phenylpropanes, monoterpenes, thermolabile, sesquiterpenoids, calamen, clamenol, limonene, calameon, asarone, methylisoeugenol, sesquiterpenes, cedrene, limonen, camphor, and alcohols (Wu et al., 1994; Marongiu et al., 2005). The main active compounds in the essential oils are α-asarone, β-asarone, methylisoeugenol, and methyleugenol (Table 1). However, the chemical composition of the essential oils varies with the sweet flag type, and local, climatic, and seasonal factors as well as storage duration of medicinal herbs (Liu et al., 2013).

USAGE AND APPLICATIONS

The curative properties of sweet flag have been honored throughout history, which date back possibly to the time of Moses in the Old Testament of the Bible and in early Greek and Roman medicine. Sweet flag was also used by Hippocrates, the father of Western medicine. Because of the positive medicinal attributes associated with sweet flag, it has surpassed cultural barriers and gains widespread usage (Moerman, 1981).

FIGURE 3 The spathe and spadix of a sweet flag inflorescence. *Note: This Figure was published online.*

TABLE 1 Main Chemical Constituents of the Essential Oil Derived from Sweet Flag Rhizomes (%)

Compound	Composition (%)
α-Asarone	50.09
Methylisoeugenol	14.01
Shyobunone	13.3
Isoshyobunone	13.0
Calamenene	9.75
Methyleugenol	8.59
β-Asarone	3.51
α-Cedrene	3.09
β-Gurjunene	2.5
Camphor	2.42
Linalool	1.07

Note: This table was published in Liu et al. (2013).

In Ayurvedic medicine, sweet flag has been traditionally used medicinally against different diseases like fever, asthma, bronchitis, cough, stomach complaints, and many disorders of central nervous system. Sweet flag, as an ingredient of the Ayurvedic preparation "Brahmi Bati" is valued as a "rejuvenator" for epilepsy, coma, insomnia, hysteria, and mental retardation (Satyavati et al., 1976). In ancient Chinese, sweet flag, which was first mentioned in Shennong Bencao Jing, has been used for at least 2000 years. It is mainly used to aid digestion, reduce swelling, and relieve constipation. Herbal baths of sweet flag have been said to stimulate the circulation as well as giving relief to patients with rheumatism lumbago (Duke and Ayensu, 1985; Perry and Metzger, 1980). In Greek–Arab medicine, sweet flag is used to treat gastritis, anorexia, and rheumatoid arthritis (Barton and Castle, 1877).

Roman, Chinese, and Arabic cultures considered it a strong aphrodisiac for centuries out of its reputed ability to arouse sexual desire, while in North America and New Guinea it was once used to induce abortion. For Penobscot people, the rhizome was thought to protect the wearer from infectious diseases, so they collected pieces of the dried root, strung them together hung up for preservation. Steaming it throughout the home was thought to "kill" sickness, and chewing it was thought to ward off illness while traveling (Dragendorff, 1898).

USAGE AND APPLICATION IN FOOD SCIENCE

Usage and Applications in Food Preservation

It has been estimated that approximately 10–30% of the world's food crops are damaged or destroyed by insect pests during growth, harvest, or storage. Infestations of stored food insect pests currently are controlled mainly by synthetic insecticides. Although synthetic insecticides are effective, their repeated use has disrupted natural biological control systems and led to resurgence of insect species, sometimes resulting in the development of resistance, toxic effect on food, compromise to workers' safety, and pollution of the environment. Furthermore, food-borne infections are a growing public health problem worldwide, calling for more effective health preservation strategies. To produce safer food, it is necessary to exploit natural products as alternatives to conventional synthetic insecticides for more effective, green food preservation (Hyldgaard et al., 2012). For centuries sweet flag has been widely used in the food industry; sweet flag essential oils and their components have attracted widespread interest for their use as potential insecticidal, antimicrobial, and antifeedant agents. The chemicals in the essential oils of sweet flag are secondary metabolites, which play an important role in food preservation.

Mode of Action

The physiologically active form of sweet flag essential oils has effects on stored grain insects, including being attractive to insects, repelling insects, killing insects, inhibiting insect growth or development, sterilizing insects, and detering feeding by insects (antifeedants) (Jacobson, 1982). These categories will be considered separately in this chapter.

Insecticidal Activity

Sweet flag oils or their components have been demonstrated to possess insecticidal activity against the main stored grain insects, for example, *Sitophilus zeamais*, *Rhyzopertha dominica*, *Tribolium castaneum*, *Tribolium confusum*, *Cryptolestes pusillus*, *Oryzaephilus surinamensis*, *Prostephanus truncates*, the tobacco armyworm *Spodoptera litura*, *Callosobruchus phaseoli*, and the book louse (*Liposcelis bostrychophila*) (Yao et al., 2012; Liu et al., 2013). Methyl eugenol, methyl isoeugenol, and asarone are main the active ingredients of sweet flag oils. Methyl eugenol was demonstrated to possess insecticidal activity against maize weevils and red flour beetles, American cockroaches (*Periplaneta americana*) and mold mites (*Tyrophagus putrescentiae*). α-sarone also demonstrated insecticidal activity against grain storage insects, *Sitophilus oryzae*, *Callosobruchus chinensis*, *Lasioderma serricorne*, and agricultural insect pests such as *Nilaparvata lugens* and *Plutella xylostella*. β-asarone also exhibited insecticidal activity against *S. zeamais*, *R. dominica*, *T. castaneum*, and *Callosobruchus maculatus*.

Contact Toxicity

The median lethal concentration (LD_{50}) of sweet flag oils against *S. zeamais* after 96 h of treatment was 27.26 μg/cm^2 (Yao et al., 2012). Sweet flag oils exhibited contact toxicity against *L. bostrychophila* with an LD_{50} value of 100.21 μg/cm^2 while three constituent compounds, α-asarone, methyl eugenol, and methyl isoeugenol had LD_{50} values of 125.73 μg/cm^2, 103.22 μg/cm^2 and 55.32 μg/cm^2, respectively (Liu et al., 2013). (*Z*)-asarone brought about 100.00% mortality to *S. zeamais* at 40.89 μg/cm^2 96 h after treatment (Yao et al., 2008). In a test with female adults of *N. lugens*, *cis*-asarone caused 100%, 83,% and 40% mortality at 1000, 500, and 250 ppm, respectively, whereas 67% mortality was achieved at 1000 ppm of *trans*-asarone. Against third instar larvae of *P. xylostella*, *cis*-asarone gave 83% and 50% mortality at 1000 and 500 ppm, respectively, whereas *trans*-asarone at 1000 ppm showed 30% mortality (Balakumbahan et al., 2010).

Repellency Activity

Sweet flag oils are aromatic in nature and act as insect repellent. Yao et al. (2012) reported that sweet flag oils possessed strong repellency to different stored grain insects. Sixty hours after treatment, the repellency to *R. dominica*, *T. castaneuu*, *T. confusum*, *C. pusillus*, and *O. surinamensis* were all above 90%, while the repellency to *S. zeamais* was above 70%. (*Z*)-asarone showed 84.50% repellency to *S. zeamais* at 314.54 μg/cm^2 and 77.02% at 78.63 μg/cm^2 12 h after treatment (Yao et al., 2008).

Fumigant Toxicity

Fumigation plays a very important role in insect pest control in stored food products. After treatment with sweet flag oil vapor, the number of offspring of *Sitophilus granarius*, *S. oryzae*, *C. chinensis*, *T. confusum*, and *R. dominica* emerging from food was considerably decreased. Methyl eugenol and methyl isoeugenol possessed fumigant toxicity against *L. bostrychophila* adults with LC_{50} values of 92.21 and 143.43 μg/L, respectively, while crude essential oils showed an LC_{50} value of 392.13 μg/L (Liu et al., 2013). β-asarone has distinct fumigant toxicity against *S. zeamais*, *R. dominica*, *T. castaneum*, and *Callosobruchus maculates*. After the pests were treated with β-asarone for 24 h, the LC_{50} values for *S. zeamais*, *R. dominica*, *T. castaneum*, and *C. maculatus* adults were 49.38, 102.96, 124.04, and 1.07 μL/L, respectively (Yao et al., 2009). Sweet flag oils have been shown to possess potential for development as new fumigants and they may have advantages over conventional fumigants in terms of low mammalian toxicity, rapid degradation, and local availability.

Antifeedant and Growth Inhibitory Effects

Koul (1987) found that sweet flag oil spray at 1.0% and 2.0% was effective in inducing significant feeding deterrence and growth inhibition against *Spodoptera litura* larvae by decreasing food intake, weight gain, and larval development. The essential oils of sweet flag decreased food consumption and larval growth in a dose-dependent manner in both neonate (first instar) and second instar larvae. Sweet flag oils significantly inhibited feeding by fourth and fifth instar larvae. Experiments confirmed that sweet flag oils induce antifeedant and growth inhibitory effects in *Peridroma saucia*, the latter possibly being independent of feeding behavior (Koul et al., 1990). Asarones isolated from the sweet flag oils are potent growth inhibitors and antifeedants to the variegated cutworm. *cis*-asarone added to artificial diet significantly inhibited growth and feeding by first, third, and fourth instar larvae, whereas the *trans* isomer produced an antifeedant effect alone (Balakumbahan et al., 2010).

Antigonadal Activity

The toxic effects of vapor of sweet flag oils on the adults and eggs of *C. phaseoli* were studied. Significant reduction of oviposition was found in Yugoslavian and Russian oil vapor at 5 and 10 µL oil per 400 mL jar after 24 h exposure (Rahman and Schmidt, 1999). It was found that β-asarone from sweet flag oils possessed insect chemosterilant properties, causing inhibition both spermatogenesis and ultimately regression of the ovaries of larvae and adults. With its specific antigonadal function, β-asarone may represent a new type of antigonadal agent which may afford a new safe approach toward insect control (Koul et al., 1977; Saxena et al., 1977).

Antibacterial Activity

Food safety is a highly important issue for both consumers and the food industry due to the rising number of cases of food-associated infections. Sweet flag oils have exhibited antibacterial activity (Phongpaichit et al., 2005; Kim et al., 2011). In vitro experiments into the antibacterial activity of sweet flag rhizomes evaluated the zone of inhibition and showed increases with concentration (Devi and Ganjewala, 2009). Among the four types of bacteria tested, high inhibition zone was observed on *Pseudomonas aeruginosa* (1.62 cm) followed by *Staphylococcus aureus* (1.62 cm). *Escherichia coli* and *Bacillus subtilis* showed smaller zone of inhibition (1.34 and 1.04 cm, respectively). Minimum inhibitory concentration (MIC) test showed that the minimum inhibition concentration was 0.25 mg/mL for *P. aeruginosa*, *S. aureus*, *B. subtilis*, and 0.5 mg/mL for *E. coli*. In vitro antibacterial action against methicillin-resistant *S. aureus* and strains of β-lactamase-producing Gram-negative bacteria (*Shigella dysenteriae*, *Shigella sonnei*) occurred with sweet flag oil (Sabitha et al., 2003).

Antifungal Activity

Many fungi such as *Aspergillus* and *Fusarium* are responsible for producing mycotoxins in various foods. The toxic nature of heme peroxidase from sweet flag oils which inhibited hyphal growth was demonstrated against phytopathogens such as *Macrophomina phaseolina*, *Fusarium moniliforme*, and *Trichosporium vesiculosum* (Ghosh, 2006). β-asarone completely inhibited the growth (100%) of all the test fungi (*Macrophomina phaseolina*, *Curvularia lunata*, and *Alternaria alternata*) at 400 µg/L (Begum et al., 2004). Because the antifungal activity of sweet flag oils is more advantageous compare with synthetic fungicides, sweet flag oils can be exploited as a harmless, indigenous source of antifungal agent.

Other Activity

Experiments reveal that sweet flag oils treatment causes injury to both plasmatocytes and granular hemocytes and also affects the hemogram, it can be inferred that cellular defense reactions of *Spodoptera litura* are impaired (Sharma et al., 2008).

Usage and Applications in Food Flavor

Sweet flag represents a renewable source of flavoring substances which is commonly used in food flavoring. Rhizome is used as a flavor for pipe tobacco. When eaten in crystallized form, it is called "German ginger." The dried and powdered rhizome has a spicy flavor and is used as a substitute for ginger, cinnamon, nutmeg, linalool, and limonene. A pinch of the powdered rhizome is used as a flavoring in tea. Sweet flag is also used in perfumes, potpourris, and vinegar (Angier, 1974; Balakumbahan et al., 2010). In Europe, sweet flag is often added to wine, and the root is also one of the possible ingredients of absinthe. The sweet flag oils are used in production of beer and alcoholic beverages such as bitters, liqueurs, vermouths, and at lower level in foods such as frozen desserts, yoghurts, cakes , and confectionery (Motley, 1994).

SAFETY

Sweet flag and products derived from sweet flag (such as essential oils) were banned in 1968 as food additives and medicines by the United States Food and Drug Administration because of cancerous findings in animal studies, using high doses of carcinogenic β-asarone, present in large amounts in Asian plants (tetraploid), but of limited amounts in European plants (triploid). Studies report limited amounts of the toxic derivatives in northern American plants (diploid). Putting the cancer concern in perspective, large doses were fed to test animals in elicit hepatic cancers (Furia and Bellanca, 1971). Other

studies failed to show embryotoxicity or teratogenicity in the embryos of pregnant mice. These test doses were much larger than those found in herbal preparations. It is not clear whether the observed carcinogenic effects in rats would induce similar detrimental effects to the human organism. Sweet flag has been used by ancient cultures and folk medicinal practices for thousands of years without reports of cancer which suggests that using the whole herb is completely safe. Sweet flag oils have been accepted by the European Commission for their intended use as flavorings in food products, which considered no risk to the health of the consumer.

OUTLOOK

Sweet flag has a long history and numerous traditional economic and ethnobotanical applications. Sweet flag oils or their components have been shown that it not only possesses insecticidal properties, but also contain broad-spectrum antibacterial and antifungal properties. Although the food industry primarily uses sweet flag oils as flavorings, sweet flag oils have the potential to be developed into natural insecticides for food preservation. Further research is required to clarify the use of sweet flag oils in the food industry.

Summary Points

- Sweet flag has a long history and medicinal application.
- Sweet flag is used as a flavoring ingredient.
- The chemicals in sweet flag essential oils are secondary metabolites.
- Sweet flag oils possess insecticidal activity.
- Sweet flag oils possess antimicrobial property.
- Sweet flag oils possess antifeedant and growth inhibitory effects.
- Sweet flag oil is a potential food preservative.

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Chapter 89

Sweet Orange (*Citrus sinensis*) Oils

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INTRODUCTION

Citrus fruits are the most common subtropical crops in the world; there is a great amount of variation among citrus species and cultivars as a result of frequent bud mutation, interspecific and intergeneric hybridization, apomixes, and a long history of cultivation (Azar et al., 2011). Among the citrus species, *Citrus sinensis* L. Osbeck, commonly known as sweet orange, is the most horticulturally important and widely known worldwide (Grosser et al., 2007).

Citrus sinensis L. Osbeck, has been cultivated for thousands of years, and is probably a hybrid among other citrus species in Southern and East Asia, although its evolutionary history is hard to pinpoint. Today, it can be found literally all over the world (Simpson and Ogorzaly, 2001). The largest producer of *C. sinensis* L. Osbeck is Brazil with an annual average production of 18 million tons resulting in 37% of the global production; meanwhile, the USA is the second largest producer with 9.1 million tons per year, comprising 15% of the global market (Pérez-Acero, 2000).

Sweet orange is one of the healthiest and most popular fruits in the world; it possesses high amounts of vitamin C and, owing to its balance of acidity and sweetness, is a widely recognized flavor. Its juice content ranges between 40% and 50%, the peel reaches up to 40%, while the pulp and seeds content varies between 20% and 30%. About 90% of its content is composed of water, with 5% of dissolved sugars.

BOTANICAL ASPECTS

Sweet orange (*C. sinensis* L. Osbeck) belongs to the Rutaceae family that contains about 1700 species growing in warm weather countries; most of these species can be found in Africa (Yañez et al., 2007). It is a hybrid tree originally from India, Vietnam, and south-east Asia (Pérez-Acero, 2000), it is of medium size (7–10 m), erect if containing seeds and with broad crown if grafted, owns shiny leaves and white and bisexual flowers, the fruit is round of about 6–10 cm of diameter with a slightly rough peel, the fruit arises through the growth and development of an ovary and consists of 8–16 carpels clustered around and joined to the floral axis, which forms the core of the fruit. The carpels form locules, or segments, in which seeds and juice sacs (vesicle) grow. The pericarp (rind or peel) is divided into exocarp, or *flavedo*, and mesocarp, or *albedo*. The *flavedo* consists of the outermost tissue layers, which have a cuticle-covered epidermis and parenchyma cells. The *flavedo* is the outer, colored part and the *albedo* is the inner, colorless (white) or sometimes tinted part (blood oranges) (Ladaniya, 2008).

According to its botany, there are four different groups within the *C. sinensis* L. Osbeck species, namely:

- Navel oranges, that can be distinguished by the presence of a navel at the stylar end of the fruit, they are generally seedless, easy to peel with a firm and tasty pulp, tough they are demanding in terms of weather and soil, they include several cultivars such as "Washington", "Thompson", "Navelina", "Navelare", and "Newhall".
- Common oranges, also called white oranges, which represent 65% of the production. According to their maturation time in cold winter countries, they are classified as early, intermediate, and late. In warm countries, this distinction is negligible, they include most of the older varieties grown in different countries, having local names and being difficult to distinguish, the most known cultivars are "Cadenera" from Spain, "Jaffa" from Israel, "Biondo commune" from Italy, "Pera" from Brazil, and "Pineapple" and "Valencia" from the USA.

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- Blood oranges, characterized by the presence of anthocyanin in the fruit, giving it a more or less intense red color to the juice, pulp or peel, they are highly appreciated in European countries, in warm countries they do not develop this color and they are rare in the tropics. The most common cultivars of this group are "Moro", "Taroco", "Sanguinella", "Ruby", and "Doblefina".
- Acidless oranges, also called sugar or sugary oranges because of the low acidity of the juice, two cultivars are "Sucreña" or "Imperial" and "Succari" (Ortiz, 2002).

USAGE AND APPLICATIONS

Citrus is an important crop with world production estimated at 115 million tons per year. Oranges, lemons, grapefruits, and mandarins represent approximately 98% of industrial cultivars (Lagha-Benamrouche and Madani, 2013). The main uses of citrus in food industries include fresh juice or citrus-based drinks (Rouseff et al., 1987). Further, citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, and phenolic compounds that are important to human nutrition (Kamran et al., 2009). Even though citrus fruits are mainly used for juices and nectars, it is of significant economic value for its essential oil (EO) due to its aromatic compounds. Because the juice yield of citrus is less than half of the fruit weight, a very large amount of by-product wastes are produced every year. It has been proposed that citrus by-products, mainly the EO, could be sources of natural antioxidants and antimicrobials (Manthey and Grohmann, 2001). Orange citrus is the largest crop in the world, and as well as the large amount of its edible products, this fruit creates considerable amounts of waste or by-products such as peels, seeds, and pulps which represent 50% of the raw processed fruit. These by-products are considered as a valuable source of functional ingredients namely flavonoids, dietary fibers, and essential oils (Hosni et al., 2010).

Several studies have demonstrated the antimicrobial and antioxidants properties of citrus EO and they reported that there is a relationship between the chemical compounds of the EO with these properties. Although the main use of *C. sinensis* L. Osbeck is to flavor drinks (Chutia et al., 2009), some researchers indicate the possibility of exploiting *C. sinensis* EO as an effective inhibitor of the biodegrading and storage-contaminating fungi in some crops (Sharma and Tripathi, 2008).

CHEMICAL COMPOSITION OF SWEET ORANGE ESSENTIAL OIL

Analytical research regarding aroma compounds of orange fruit has been carried out for years. Many researchers have studied the volatile compounds of this fruit using different analytical methods. As a result of these studies, more than 200 compounds have been described as components of the flavor of sweet orange; the major aroma compounds found in orange juice are hydrocarbons, alcohols, aldehydes, esters, and ketones (Quiao et al., 2008). Citrus essential oils, which previously had been discarded as waste, are used by the food industry as flavor agents, antimicrobials, and antioxidants. Due to their great nutraceutical and economic importance, numerous investigations have been performed aimed at identifying the chemical composition of the essential oils from different citrus species. In the case of sweet orange (*C. sinensis* L. Osbeck), most of the studies have been performed with the EO obtained from the peel of different cultivars.

Orange peel contains an abundance of fragrant substances that are extensively applied for processing into essential oils that are used commercially for flavoring foods, beverages, perfumes, and cosmetics, among others. The presences of terpenes with traces of oxygenated components impart the characteristic aroma of the orange essential oil and peel essential oils. The volatile components present in *C. sinensis* peel are largely terpenes. Mono- and sesquiterpene hydrocarbons, together with several oxygenated derivatives comprising alcohols, aldehydes, ketones, esters, and epoxides, are also found as main compounds (Quiao et al., 2008). In general, the components present in sweet orange EO can be grouped into five classes: monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and other oxygenated compounds (Espina et al., 2011). Limonene is the main compound present (on average 71–95.1%) in sweet orange EO (Sahraoui et al., 2011).

The composition of sweet orange peel essential oil varies depending from the analyzed cultivar. The compounds present in different cultivars are shown in Table 1 (Hosni et al., 2010; Lin et al., 2010; Sahraoui et al., 2011). Volatile components were extracted by steam distillation (SD), hydrodistillation (HD), or supercritical fluid extraction (SFE) and characterized by gas chromatography–mass spectrometry. Fifty compounds have been identified in *C. sinensis* (L.) Osbeck peel EOs, among them limonene, α-pinene, and linalool were common to all cultivars, within studied cultivars. Thomson Navel exhibited the most complex composition with higher number of volatile components (29) while the Meski cultivar contained the lowest number (six). As expected, in studied cultivars the monoterpene hydrocarbon limonene was the main component (Hosni et al., 2010; Lin et al., 2010; Sahraoui et al., 2011).

TABLE 1 Chemical Composition of Essential Oils from Selected Cultivars of Sweet Orange (*Citrus sinensis*)

Compound Name	Cultivar						Reported by
	V	L	ME	VL	T	MA	
Monoterpenes							
(E)-β-Ocymene				*	*	*	III
Myrcene		*					II
α-Pinene	*	*	*	*	*	*	I, II,III
Sabinene	*		*	*	*	*	I, II, III
β-Pinene		*					I, II, III
β-Myrcene	*			*	*	*	I,III
α-Phellandrene			*	*	*	*	III
3-Carnene	*						I
δ-3-Carene	*			*	*		I, III
Limonene	*	*	*	*	*	*	I, II, III
γ-Terpinene		*		*	*	*	II, III
α-Terpineole		*					II
trans-Carveol					*	*	III
trans-Pinocarveol				*	*		III
α-Terpinolene	*			*	*		I, III
Oxygenated Monoterpenes							
Linalool	*	*	*	*	*	*	I, II, III
trans-Limonene oxide	*						I
Citronellal	*						I
β-Cyclocitral				*	*	*	III
Verbenone			*	*	*	*	III
Borneol				*	*	*	III
Terpin-4-ol					*	*	III
β-Citronellol	*						I
Sesquiterpenes							
Cadinol						*	III
Aromadendrene						*	III
α-Copaene	*			*	*	*	I, III
β-Cubebene	*						I
β-elemene	*			*	*	*	I, III
Caryophyllene (E)	*						I
α-Humulene	*			*	*	*	I, III
Germacrene-D	*			*	*	*	I, III

Continued

TABLE 1 Chemical Composition of Essential Oils from Selected Cultivars of Sweet Orange (*Citrus sinensis*)—cont'd

Compound Name	Cultivar						Reported by
	V	L	ME	VL	T	MA	
Valencene	*						I
Germacrene-A	*						I
δ-Cadinene	*						I
Oxygenated Sesquiterpenes							
Caryophyllene oxide	*						I
cis, trans-Farnesol	*						I
β-Sinensal				*	*	*	III
α-Sinensal	*			*	*	*	III
Other Oxygenated Compounds							
Neryl acetate		*		*	*	*	II, III
Decanal	*						I
n-Dodecanal	*						II
Geranyl acetate		*					II
α-Terpinyl acetate				*	*		III
Linalyl acetate				*	*	*	III
Decanol				*	*		III
(E)-(E)-2,4-Decadienal				*	*		
Octyl acetate						*	III
Frenchyl acetate						*	III
trans-Pinocarvyl acetate					*		III
Myrnetyl acetate					*	*	III

I, Sahraoui et al. (2011); II, Lin et al. (2010); III, Hosni et al. (2010), V, Valencia; L, Liucheng; ME, Meski; VL, Valencia late; T, Thomson Navel; MA, Maltaise blanc; *, Compound present in the essential oil.

However, cultivar is not the only parameter that affects the chemical composition of the EO; it depends of several other factors such as environment, fruit stage of maturation, harvesting period, and method of extraction among others. The common commercial technique to produce essential oils from citrus peels is performed via cold-pressing, a process that generates oils that contain furocoumarins, photomutagenic, and photocarcinogenic compounds. As a consequence, different methods of extraction such as HD, SD, microwave steam distillation (MSD), SFE, and controlled pressure drop (DIC) have been studied with the aim to improve EO extraction and composition. Composition of *C. sinensis* L. Osbeck var Valencia EO extracted by different techniques was studied by Sahraoui et al. (2011), who found the same amounts of volatile secondary metabolites obtained from *C. sinensis* var Valencia by MSD and SD.

According to Bourgou et al. (2012) harvesting time is a critical parameter significantly influencing the chemical composition of citrus peel essential oil; these authors studied the *C. sinensis* (L.) Osbeck peel EO obtained at three harvesting periods: stage 1 (green color, immature), stage 2 (yellow color, semimature), and stage 3 (orange color, mature). In their research they

found that peel EO was mainly dominated by limonene which presented a level of 86.40, 81.52, and 85.35% at immature, semimature, and mature stages, respectively. The rest of the compounds were weakly represented with levels lower than 1% except for the monoterpenes β-pinene, camphor, and bornyl acetate; these compounds reached their maximum at semimature stage with levels of 1.80, 4.81, and 4.21%, respectively.

Although compounds present in sweet orange peel essential oil have been extensively studied, some authors reported compounds present in others parts of sweet orange fruit. Quiao et al. (2008) determined the aromatic composition and aroma active compounds of the juice of Jinchen sweet oranges; a total of 49 compounds were identified, with limonene the major component, followed by linalool, terpinen-4-ol, β-myrcene, α-terpineol, octanal, and γ-terpinene. Jinchen sweet orange peel essential oil contained terpenes at higher levels than those found in of the fruit juice; ethyl butanoate, β-myrcene, octanal, linalool, and decanal are responsible for juice aroma, while linalool, α-pinene, and decanal are responsible for the aroma of the peel essential oil.

USAGE AND APPLICATIONS IN FOOD SCIENCE

Antimicrobial Activity

Among the great variety of available EOs, citrus fruit EOs and their major components have gained acceptance in the food industry since they are generally recognized as safe and many foods tolerate their presence (Fisher and Phillips, 2006). Nowadays, citrus EOs are industrially applied to many products, including foods and beverages, as well as cosmetics and drugs, due to their wide spectrum of biological activities such as being effective antimicrobial and antifungal agents (Hosni et al., 2010).

The antimicrobial activity of sweet orange EO is mainly attributed to terpenes which constitute the major part of citrus oils. Even though limonene is the major component of this EO, it has been reported that the antimicrobial activity of *C. sinensis* (L.) Osbeck EO is a result from the synergism among the components present (Settani et al., 2012; Velázquez-Núñez et al., 2013), and consequently the antibacterial effects of this EO are not uniform among bacteria because it depends on the compounds present. The antibacterial activity of different cultivars of *C. sinensis* (L.) Osbeck (Table 2) EO has been tested. Settani et al. (2012) evaluated six different sweet orange cultivars against food-borne pathogenic bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella enterica*) finding that the active extracts of all cultivars were most effective against Gram-positive than Gram-negative bacteria. The ability of sweet orange EO to replace commercial detergents on surface of kitchen utensils has been reported, thus being a green sanitizer. Lin et al. (2010) reported the ability of *C. sinensis* (L.) Osbeck var. Liucheng EO to inactivate Gram-negative bacteria such as *Salmonella* Typhimurium, *Escherichia coli*, and *Vibrio parahahemolyticus* inoculated in stainless steel and plastic surfaces using emulsions of sweet orange EO; these bacteria usually survive on the surfaces after washing with detergents. The antimicrobial effect of a combination of EOs of different cultivars was reported by Espina et al. (2011), who tested a commercial mixture of five sweet orange peel EO against *Enterocococcus faecium* (ATCC 19434), *S. aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 10145), *S. enterica* subsp. *enterica* ser. Enteritidis (ATCC 49214), *E. coli* O157:H7, and *L. monocytogenes* EGD-e using the filter paper disc agar diffusion technique, demonstrating that tested mixture exhibited an antagonist effect. No inhibition against the tested microorganisms was observed.

As the presence of certain phytochemical components and its interactions define antimicrobial activity of essential oils, variation of these compounds due to maturation stage of the plant and the antimicrobial capacity of its EO has been evaluated. Antimicrobial activity of sweet orange EO varied among ripening stages. Comparing the behavior of the compounds present in the oil, limonene (the major monoterpene present) did not vary with ripening stage; however, compounds present in small amounts were affected, therefore, reaffirming the idea that the inhibitory activity of an EO is a result of a complex interaction among its different constituents, which may produce additive, synergistic, or antagonistic effects. Bourgou et al. (2012) found that the antimicrobial activity of sweet orange EO varied with the ripening stage of the fruit, they tested the EO from Maltaise cultivar against *Pseudomonas aeruginosa*. The oil was effective against this microorganism only when the essential oil was obtained from mature fruits (Table 2).

The majority of reported studies of antimicrobial activity of EO have been performed in vitro, demonstrating good antimicrobial and antifungal activities in most cases; however, higher concentrations were required to produce a similar effect when tested in food systems. Direct addition of EOs significantly alters sensory attributes of foodstuffs, turning them in many cases undesirable to the consumer. In order to overcome this problem, the use of citrus EO vapors has been proposed and tested in in vivo assays. Fisher and Phillips (2006) compared the effectiveness of *C. sinensis* (L.) Osbeck EO and three components present in this oil, citral, limonene, and linalool in vapor phase against *Bacillus cereus*, *Campylobacter jejuni*, *E. coli* O157:H7, *S. aureus*, and *L. monocytogenenes* inoculated in cabbage or chicken skin.

TABLE 2 Antimicrobial Activities of Essential Oils from Selected Cultivars of Sweet Orange (*Citrus sinensis*)

Cultivar	Microorganism	Inhibitory Activity	References
Frost Washington Navel			Settani et al. (2012)
Tarocco S. Alfio	*Staphylococcus aureus*	(+)	
Valencia	*Listeria monocytogenes*	(+)	
Frost Valencia	*Salmonella enterica*	(–)	
Lane late VCR			
Washington Navel 3033			
Liucheng	*Vibrio parahaemolyticus*	(+)	Lin et al. (2010)
	Salmonella Typhimurium	(+)	
	Escherichia coli	(+)	
Maltaise	*Escherichia coli*	(–)	Bourgou et al. (2012)
	Staphylococcus aureus	(–)	
	Pseudomonas aeruginosa	(+)	
Sweet orange Mixture (Washington Navel, Navelate, Navelina, Salustiana, Valencia Late)	*Enterococcus faecium*	(–)	Espina et al. (2011)
	Staphylococcus aureus	(–)	
	Pseudomonas aeruginosa	(–)	
	Salmonella enterica	(–)	
	Escherichia coli	(–)	
	Listeria monocytogenes	(–)	

+, inhibition; –, no inhibition.

Their results showed that sweet orange EO added on these foods was not capable to inactivate tested bacteria, while its individual components showed a good bactericidal effect.

Reports regarding antifungal activity of sweet orange EO are scarce; citral has been proved to be one of the most effective antifungal components of sweet orange EO (Espina et al., 2011). Viuda-Martos et al. (2008) studied the effect of this EO on the growth of molds commonly associated with food spoilage, such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, and *Penicillium verrucosum*, using the agar dilution method. The results showed that sweet orange EO had the capacity to reduce or inhibit the growth of tested molds. *Citrus sinensis* (L.) Osbeck EO reduced *A. niger* mycelium growth and Lucini et al. (2006) indicated that mycelia growth inhibition is caused by the monoterpenes present in essential oils. These components increase the concentration of lipidic peroxides such as hydroxyl, alkoxyl, and alkoperoxyl radicals and so bring about cell death.

Besides its antimicrobial activity, sweet orange EO also has the ability to inactivate organisms related with deterioration of fruit at harvest as well as insects related to dissemination of pathogens in fresh fruits and vegetables; sweet orange natural terpenes have insecticidal activity against a variety of insects via topical application (Coats et al., 1991). Palacios et al. (2009) evaluated the effectiveness of *C. sinensis* (L.) Osbeck EO against adult housefly (*Musca domestica* L.). After 30 min of exposure the EO demonstrated high insecticidal effects with a LC_{50} value of 3.9 mg/dm^3.

Antioxidant Activity

Free radicals play an important role in affecting human health by causing several diseases including cancer, hypertension, heart attack, and diabetes, among others; these free radicals are generated during body metabolism (Ghafar et al., 2010). It has been suggested that frequent consumption of fruits and vegetables reduces the risk of these diseases. Furthermore, a great number of plants, herbs, and spices have been reported to have strong antioxidant activities; these antioxidant activities can be explored by food industry using plants as a source of antioxidants to prevent rancidity due to oxidation of lipids

(Lagha-Benamrouche and Madani, 2013). In fact, concerns about the safety of commonly used synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene has led to increased interest on medicinal plants to extract natural and low-cost antioxidants that could replace synthetic additives (Anagnostopoulou et al., 2006).

Citrus fruit extracts also have antioxidant activities. Besides sugars, acids, and polysaccharides, oranges are an important source of phytochemicals such as phenolics, vitamin C, and carotenoids. Some authors have found a direct relationship between phenolic compounds and the antioxidant activity of citrus fruit juices; differences observed with regard to antioxidant activity of juices are probably due to various factors, namely climate, soil, fruit variety, and degree of maturation (Escobedo-Avellaneda et al., 2011). Anagnostopoulou et al. (2006) evaluated the antioxidant activity of seven different extracts, fractions, and residues of navel sweet orange (*C. sinensis* (L.) Osbeck) peel for their radical scavenging activity. Total phenolic content (TPC) values of the successive orange peel extracts ranged from 105 to 3 mg gallic acid (GA)/g dry extract being the methanolic fractions the ones with the highest phenolic contents. On the other hand, radical scavenging activity ranged from 0.5 to 8.9 mg/mg dry extract, and the most active fraction was obtained with ethyl acetate. In general, fractions with a high radical scavenging activity showed a high phenolic content as well; so the antioxidant activity was mainly attributed to the presence of phenolic compounds. These authors conclude that the fractions and residues of navel sweet orange peel extracts are useful as antioxidants in food and medicinal preparations.

Ghafar et al. (2010) investigated the antioxidant activity of four species of citrus (*Citrus hystrix* "wild lime", *Citrus aurantifolia* "common lime", *Citrus microcarpa* "musk lime", and *C. sinensis* (L.) Osbeck "orange") by determining total phenolic, flavonoid, and hesperidin content. TPC values ranged from 135.3 to 490.74 mg GA/mL of juice; the amount of phenolics were in the following increasing order: *C. hystrix*>*C. aurantifolia*>*C. sinensis* (L.) Osbeck>*C. microcarpa*. Flavonoids were reported as the most important natural phenolics, these compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Hesperidin is a member of flavanones and can be commonly found in large amounts in citrus species and has been reported to have antioxidant, anticarcinogenic, antihypotensive, and antimicrobial properties. Also, *C. hystrix* had the highest scavenging activity at a concentration of 35 mg/100 mL of fresh juice. *C. hystrix* exhibited the highest antioxidant, flavonoid, and phenolic content and thus can be used as a readily accessible source of natural antioxidants.

Kamran et al. (2009) investigated and compared the radical scavenging activity of methanolic extracts of 13 commercially available citrus spp. peels and tissues and determined TPC and flavonoids content. They found a range from 66.5 to 396.8 mg GA/g of extract for total phenolic content and a range from 0.3 to 31.1 mg quercetin equivalent/g of extract for flavonoids content. *Citrus sinensis* (L.) Osbeck var. Washington Navel peels had the highest amount of flavonoids. They reported that no correlation between TPC and/or flavonoids content with antioxidant activity in tissues and/or peels. Asjad et al. (2013) studied the radical scavenging activity of 13 commercially citrus spp. peels and tissues separately by determining their phenolics and flavonoids content. TPCs were higher in peels in the range of 99.4–215.8 with respect to tissues in the range of 112.3–229.5 mg GA/g of extract powder. *Citrus sinensis* (L.) Osbeck var. Washington Navel, *Citrus paradise* and *Citrus reticulate* var. Page tissues showed the highest values (229.5 and 215.7 mg GA/g of extract powder, respectively). Further, total flavonoid contents were usually higher in peels in the range of 0.2–25.7 with respect to tissues in the range of 0.9–7.8 mg quercetin equivalent/g of extract powder. *Citrus sinensis* (L.) Osbeck var. Washington Navel and *C. reticulate* var. Page peels showed the highest values. Tested extracts exhibited weak antioxidant activities. *Citrus sinensis* (L.) Osbeck var. Sungin peels showed highest activity while *Citrus aurantium* tissues showed the lowest. *Citrus sinensis* (L.) Osbeck varieties had higher contents of phenols and flavonoids therefore exhibiting strong antioxidant activities.

SUMMARY POINTS

- *Citrus sinensis* (L.) Osbeck is the most common and important species among citrus.
- Limonene is the main chemical component present in *C. sinensis* (L.) Osbeck essential oil.
- The essential oil obtained from sweet orange peel has shown antibacterial and antifungal activities, being more effective against Gram-positive bacteria.
- The antioxidant activity of the *C. sinensis* (L.) Osbeck is related to the amount of phenolic compounds.
- Flavonoids are probably the most important natural phenolic in *C. sinensis* (L.) Osbeck; these compounds possess biological activities including radical scavenging properties.

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Chapter 90

Tagetes (*Tagetes minuta*) Oils

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List of Abbreviations

$ Dollar of the United States of America
£ Sterlling Pound of the United Kingdom
BBT 5-(3-Buten-1-ynyl)-2,2′-bithienyl
BBTOAc 5-(4-Acetoxy-1-butynyl)-2,2′-bithienyl
CaRSV Carnation ring spot viruses
CAS Chemical Abstract Service
CaVMV Carnation vein mottle viruses
CBI Center for the Promotion of Imports from developing countries
E/Z *trans*/*cis*
EPAUS Environmental Protection Agency of the United States
GC Gas chromatography
GC-MS Gas chromatography–Mass Spectrometry
GFDL GNU Free Documentation License
ITC International Trade Center
IUPAC International Union of Pure and Applied Chemistry
Kg Kilograms
M^+ Molecular mass
NRCS Natural Resources Conservation Service
PUFA Polyunsaturated fatty acids
R South African Rand
UK United Kingdom
UNCOMTRADE The United Nations Commodity Trade Statistics Database
UNCTAD The United Nations Conference on Trade and Development
USA United States of America
USDA United States Department of Agriculture
w/w Weight per weight

INTRODUCTION

The plant *Tagetes minuta* L. is a worldwide species popularly known as wild marigold, a common English name prehistorically derived from "Mary's gold." It belongs to the sunflower family, Asteraceae, which comprises the largest family of vascular plants with more than 23,000 species. It is naturalized in a wide range of climatic conditions worldwide but is well known to be native to North and South America. It is now a naturalized species in Europe, Africa, Asia, Australia, New Zealand, United States, and Madagascar following its introduction during the Spanish colonization of South America (Babu and Kaul, 2007).

Tagetes minuta has a long history of human use as food, perfumes, medicines, ornamentals, and in ritual and sociocultural ethnopractices, depending on geographical location and ethnic background (Hamayun et al., 2006). For instance, in Chile and Argentina, the plant is popularly known for its traditional culinary use in stews as a highly prized flavoring agent. While in many parts of the world, *T. minuta* is commonly used to make a hot and cold refreshing beverage as well as herbal tea, a characteristic that has given it the potential to become a new crop for many of the drug-growing areas of the world

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(Chalchat et al., 1995). The essential oil of *T. minuta* is used in many ways, predominantly as flavoring and seasoning agent, and/or imparting aroma in a wide range of foodstuffs and beverages, depending on the essential oil's main constituents (Hamayun et al., 2006).

BOTANICAL ASPECTS OF *TAGETES MINUTA* L.

Morphology

Tagetes minuta is an erect, terrestrial, herbaceous annual and perennial plant, growing up to a height of 4.5 m (Figure 1) (Holm et al., 1997). The leaves are slightly glossy green and are pinnately dissected into four to six pairs of pinnae, while the leaf margins are finely serrate. The inflorescence heads on the plant are numerous, usually flat-topped cymes, 8–12 mm high, apex three to five toothed, ray florets usually three per head, rays 1–2 mm long, disk florets usually three to five per head, and corollas approxiamtely 2.5 mm long. The dark brown achenes of the plant are flattened, 6–8 mm long, and pappus scales 2–3 mm long (Wan and Chen, 2006). The under surface of the leaves bears a number of small, punctate, multicellular glands, orangish in color, which exude a licorice-like aroma when ruptured. Glands may also be found on the stems and involucre bracts. Four or five fused involucre bracts surround each head. The seeds have an unpleasant odor and can reduce the value of grain harvests when they are mixed and stored together (Wanzala et al., 2012).

The genus *Tagetes* consist of 56 species, 27 of which are annuals while 29 are perennials but mostly herbaceous plants (Soule, 1996). A considerable proportion of the species are commercially grown in respective agro-ecological regions worldwide as a multipurpose new crop. Of these species, *T. minuta*, *Tagetes erecta*, *Tagetes patula*, and *Tagetes tenuifolia* are most common with *T. minuta* being the most studied and widely grown species in several countries due to yielding high grade oil used in food, nutraceutical, perfumery, ornamental, pharmaceutical, agarbattis, and agricultural industries (Chalchat et al., 1995; Singh et al., 2003).

Ecology

Tagetes minuta is native to the temperate grasslands of southern South America (Holm et al., 1997). In the nonnative regions of this plant, it naturally grows in the wild and in the arable farming systems as a noxious weed (Holm et al., 1997). The high demand of the essential oil of this plant resulted into depletion of the wild resources, thus necessitating its systematic cultivation in subtropical and temperate agroclimatic zones worldwide, predominantly in the Indian subcontinent (Singh et al., 2003).

FIGURE 1 (A) A plant of *Tagetes minuta* L., 1753 in an arable farming system as a weed left in the farm after harvesting corn. (B) Theaerial parts of *T. minuta* that are harvested for extraction of essential oil. *Picture courtesy of Dr Wycliffe Wanzala.*

Tagetes minuta is now a widespread weed in Africa, South Europe, South Asia, Madagascar, and Australia (Chalchat et al., 1995). *Tagetes minuta* is rich at both high and low altitudes and in either high or low rainfall environments (Holm et al., 1997) and is often found growing in disturbed areas during early plant successional stages. The seeds cling to the hair of animals and human clothes once in contact and are therefore dispersed by humans, domesticated by wild animals. Toward the end of its growing season, the aerial parts of *T. minuta* dry up and may easily be destroyed by fire, but new colonies are formed rapidly in the following season from seeds deposited in the soil. *Tagetes minuta* tolerates a soil pH between 4.3 and 7.0. In South Africa, the marigold is a useful pioneer plant in the reclamation of disturbed land, while in many other countries, the plant is found spread on forested roadside, adjacent to forest margin and dominating arable land, particularly after crop harvest as the commonest weed (Figure 1(A)) (Wan and Chen, 2006).

USAGE AND APPLICATIONS OF TAGETES OIL IN FOOD SCIENCE

The usage and applications of the essential oil of *T. minuta* are deeply rooted in the diversity of useful secondary metabolites found in appropriately desired proportions, thus manifesting in a variety of biological properties (Table 1) (Sadia et al., 2013; Wanzala and Ogoma, 2013). Even from a traditional point of view, however, the nature of chemotypes and their composition affects the quantity and quality of oil, thereby impacting on the usage and applications of the oil, and thus the plant as a whole (Wanzala et al., 2012). Traditionally, the plant has a long history of interaction with humanity and henceforth, its applications to the pharmaceutical and nutraceutical industries, more particularly the preparation of teas and popular potato dish called *ocopa* in South America (Soule, 1996). Infusions, tinctures, and juice from aerial parts of *Tagetes* spp. have been used as traditional food additives worldwide. This traditional claim provided leads into research to help explain the underlying science and as a result. For example, an orange–yellow carotenoid lutein substance found in the florets of *T. erecta* and many other marigolds (*T. minuta* included) has been identified, isolated, and approved by the European Union (INS-Number E161b) for use as a food color and flavor in various foodstuffs, at a usage level in condiments and relishes such as pasta, vegetable oil, margarine, mayonnaise, salad dressing, baked goods, confectionery, dairy products, ice cream, yogurt, citrus juice, and mustard (Timberlake and Henry, 1986). Additionally, the orange pigment extracted from the petals of marigold is in great demand for poultry feed in food production systems, while, on the other hand, the plant is grown in arable farming systems to keep the nematode population in soil under control, hence often grown alongside valued food crops such as tomato, eggplant, chili pepper, tobacco, and potato plants to boost their production, thus ensuring food security. Tagetes oil is therefore a potential agent for protecting food crops on the farm and in storage, thereby increasing food security, particularly in undernourished communities of the world. The oil also provides an opportunity for developing an environmentally friendly and a nontoxic acaricide to enhance increased production of milk, beef, and hides/skin in the livestock industry (Wanzala, 2009; Nchu et al., 2012).

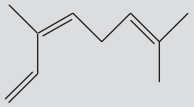
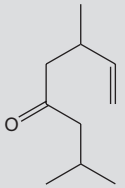
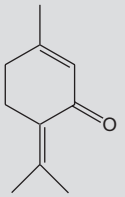
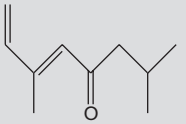
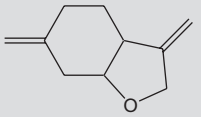
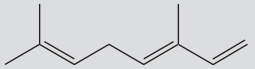
Chemotypes of the Essential Oil of *Tagetes minuta* L.

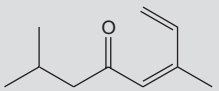
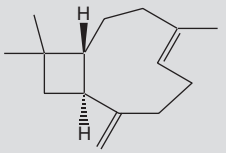
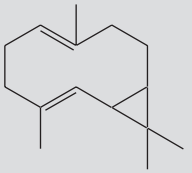
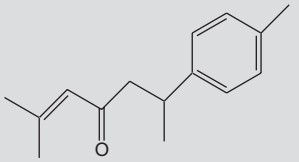
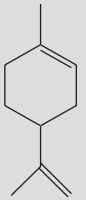
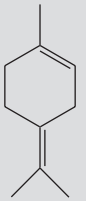
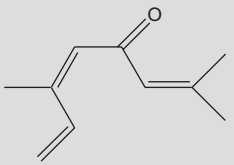
Tagetes minuta essential oil has a strong, sweet, wild, fruity, and slight citrus-like aroma. It has a yellow to reddish–amber coloration with an intermediate viscosity, which may well turn thick and even gel-like upon exposure to air at length (Figure 2) due to easy polymerization. The oil is rich in secondary metabolites, including acyclic, monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, carotenoids, and thiophenes (Wanzala and Ogoma, 2013).

The usage and applications of the essential oils of *Tagetes* spp. are rooted in the composition of secondary metabolites in the oil. Previous reports have shown significant differences in their compositions, thus having different biological properties and impacting on the nature of their usage and applications (Wanzala, 2009). Furthermore, the *Tagetes* species can be unambiguously differentiated taxonomically by the chemical composition of their essential oils (Singh et al., 2003). Gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) analysis of *T. minuta* oils indicated that the geographical location of plants (Chalchat et al., 1995; Singh et al., 2003), stage of growth of harvested plants, plant parts harvested (Chalchat et al., 1995), soil type and its nutrient status (Singh et al., 2003), nature of chemotypes (Gil et al., 2000), plant parts, climate, sunlight, methods of plant harvesting, and oil extraction method (Wanzala, 2009) equally affects the quantity and quality of oil.

In Argentina, dihydrotagetone, α-phellandrene, limonene, o-cymene, as well as the isomers of β-ocimene, tagetone, and tagetenone were the major constituents of *T. minuta* essential oil (Gil et al., 2000). In Saudi Arabia, GC and GC-MS analysis confirmed the presence of tagetone (11.52%), 5-octyn-4-one,2,7-dimethyl (11.52%), propanedinitrile, dicyclohexyl- (10.45%), and 2-pinen-4-one (8.03%) to be the main components with lesser amounts of 1-acetoxy-*p*-menth-3-one (0.17%) and 9-octacenamide(Z) (0.48%) (EL-Deeb et al., 2004). In India, the freshly distilled *T. minuta* oil contained ocimene 54.97%, and dihydrotegetone 32.58% as major constituents (Singh et al., 2003), while in other studies (Z)-tagetone,

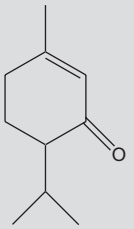
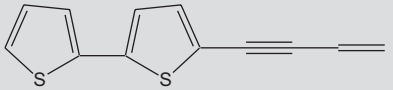
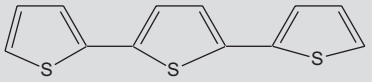
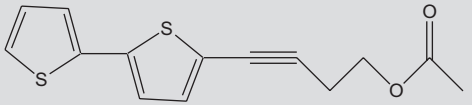
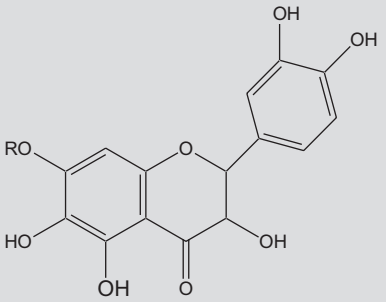
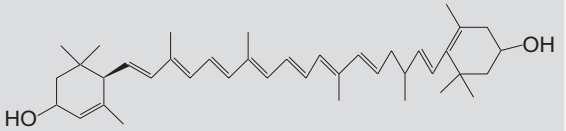
TABLE 1 Some of the Major Chemical Components Evaluated in the Essential Oil of *Tagetes minuta* (Sample Plants were Collected from the Southern Slopes of Mount Elgon in Western Kenya) and Some of Their Corresponding Industrial Applications in Food and Agriculture

Chemical Name	IUPAC Name	Chemical Structure	Molecular Formula	Molecular Mass (g/mol)	Density (g/cm^3)	CAS Number	Uses
Tagetes minuta essential oil	Tagetes oil	Not applicable	Not applicable	Not applicable	Variable (0.850–0.922)	91770-75-1 (Replacing 8016-84-0)	Multipurpose (in food, agriculture and perfumery/ fragrance industries)
cis-Ocimene	3,7-Dimethyl-1,3, 7-octatriene		$C_{10}H_{16}$	136.24	0.800	3338-55-4	Agriculture (insecticides)/ perfumery
Dihydrotagetone	2,6-Dimethyloct-7-en-4-one		$C_{10}H_{18}O$	154.25	0.826	1879-00-1	Flavory in food industry/ perfumery
Piperitenone	2-Methyl-6-propan-2-ylidenecyclohex-2-en-1-one		$C_{10}H_{14}O$	150.22	0.977	491-09-8	Fragrance and flavor concentrates of all types.
trans-Tagetone	(5E)-2,6-Dimethylocta-5, 7-dien-4-one		$C_{10}H_{16}O$	152.23	0.847	6752-80-3	Livestock tick repellant/ perfumery
3,9-Epoxy-*p*-mentha-1,8(10)-diene	3,6-Dimethylidene-4,5,7,7a-tetrahydro-3aH-1-benzofuran		$C_{10}H_{14}O$	150.22	0.967	494-90-6	Peppermint oil formulations
β-Ocimene	(3E)-3,7-Dimethylocta-1,3, 6-triene		$C_{10}H_{16}$	136.23	0.776	3779-61-1	Perfumery

cis-Tagetone	(Z)-2,6-Dimethylocta-5,7-dien-4-one		$C_{10}H_{16}O$	152.23	0.847	3588-18-9	Treatment of wounds, skin infections, bee stings, warts, cancer, mosquito repellant
(*trans*)-β-Caryophyllene	4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene		$C_{15}H_{24}$	204.36	0.905	87-44-5	Antileishmanial
Bicyclogermacrene	(1R, 4E,8E, 10S)-4,8,11,11-Tetramethyl-bicyclo[8.1.0]undeca-4,8-diene		$C_{15}H_{24}$	204.35	0.861	67650-90-2	Terpene with no recorded uses
Ar-Turmerone	(6S)-2-Methyl-6-(4-methylphenyl)hept-2-en-4-one		$C_{15}H_{20}O$	216.32	0.945	532-65-0	Insecticidal activity/Fungicidal activity/ Antidermatophytic property/ antiphenom activity
Lemonene	1-Methyl-4-(1-methylethenyl)-cyclohexene		$C_{10}H_{16}$	136.24	0.841	5889-27-5	Agriculture (insecticides)/ Cosmetic products
α-Terpinolene	Cyclohexene,1-methyl-4-(1-methylethylidene		$C_{10}H_{16}$	136.24	0.861	586-62-9	Flavors and fragrance
(Z)-Ocimenone	2,5,7-Octatrien-4-one, 2,6-dimethyl-		$C_{10}H_{14}O$	150.22	0.847	33746-71-3	Flavors and fragrance

Continued

TABLE 1 Some of the Major Chemical Components Evaluated in the Essential Oil of *Tagetes minuta* (Sample Plants were Collected from the Southern Slopes of Mount Elgon in Western Kenya) and Some of Their Corresponding Industrial Applications in Food and Agriculture—cont'd

Chemical Name	IUPAC Name	Chemical Structure	Molecular Formula	Molecular Mass (g/mol)	Density (g/cm^3)	CAS Number	Uses
Piperitone	6-Isopropyl-3-methyl-1-cyclohex-2-enone		$C_{10}H_{16}O$	152.23	0.933	89-81-6	Production of synthetic menthol and thymol for pharmaceuti cals
BBT	5-(3-Buten-1-ynyl)-2,2′-bithienyl		$C_{12}H_8S_2$	216.	–		Antifungal, nematocidal, antibacterial
α-Terthienyl (α-terthiophene)	2,5-Di(thiophen-2-yl)thiophene		$C_{12}H_8S_3$	248.38	–	1081-34-1	Antifungal, nematocidal, antibacterial
BBTOAc	5-(4-Acetoxy-1-butynyl)-2,2′-bithienyl		$C_{14}H_{13}S_2O_2$	276	–	–	Antifungal, nematocidal, antibacterial
Quercetagetin-7-arabinosyl-galactoside	–	 R=arabinose-galactose	$C_{26}H_{31}O_{17}$	616	–	–	Antimicrobial activity
Lutein	(3R,3′R,6′R)-β,ε-Carotene-3,3′-diol		$C_{40}H_{56}O_2$	568.88	–	127-40-2	Food colorant

IUPAC, International Union of Pure and Applied Chemistry; CAS, Chemical Abstract Service.

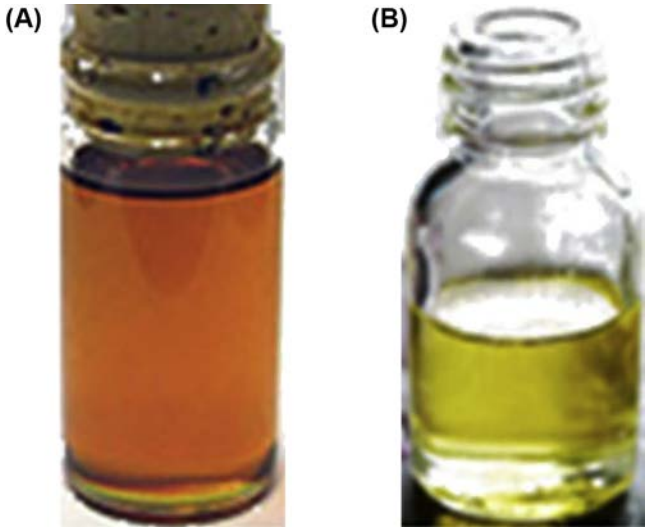


FIGURE 2 Samples of the color of the essential oil of *Tagetes minuta* L., 1753 (reddish–amber–light yellowish fluid liquid (A) and yellowish (B)). The color of the essential oil produced on industrial large-scale form for commercial use (A) and the color of the essential oil produced in the laboratory on small-scale for studies (B). When sample (B) is left exposed to air for some time, its color changes to that of sample (A) due to oil oxidation.

(Z)-β-ocimene, dihydrotagetone, (Z)- and (E)-ocimenone were found as major constituents (Chalchat et al., 1995). In Egypt, the main components of the *T. minuta* essential oil were monoterpenes of which *trans*- and *cis*-tagetone were present in 52.3–64.2% (Mohamed et al., 2002). In Iran, GC and GC-MS analysis revealed the main components to be α-terpineol (20.8%), (Z)-β-ocimene (17.7%), dihydrotagetone (13.7%), (E)-ocimenone (13.3%), (Z)-tagetone (8.4%), and (Z)-ocimenone (6.1%) (Moghaddam et al., 2007). In Madagascar, the main components in the essential oil of *T. minuta* were limonene (3.6–11%), (Z)-β-ocimene (1.0–17.1%), (E)-β-ocimene (0.5–14.6%), *p*-cymene (0.3–20.4%), β-caryophyllene (1.1–12.7%), (Z)-tagetenone (26.7%), (E)-tagetenone (31.3%), α-muurolene (36.5%), and verbenone (1.4–15.4%) (Wanzala and Ogoma, 2013). In Kenya, characterization of the essential oil showed that *T. minuta* oil comprised mainly *cis*-ocimene (43.78%), dihydrotagetone (16.71%), piperitenone (10.15%), *trans*-tagetone (8.67%), 3,9-epoxy-*p*-metha-1,8(10) diene (6.47%), β-ocimene (3.25%), and *cis*-tagetone (1.95%) (Wanzala and Ogoma, 2013). In other studies, out of 104 chemical components, the major constituents of *T. minuta* essential oil were tagetone, E/Z-ocimenone, E/Z-ocimene, germacrene, limonene, *trans*-anethole, and dihydrotegetone. Some of the major chemical components in the essential oil of *T. minuta* and their applications in industry are listed in Table 1.

General Biological Properties of *Tagetes minuta* L. and Its Essential Oil

Validation of some of the folkloric claims about *T. minuta* through evaluation of its essential oil has shown the plant to contain compounds and/or blends that have a wide range of bioactive and therapeutic properties (particularly in aromatherapy) such as antihelminthic, carminative, arthropod repellency, sedative, weedicidal, antiseptic, diaphoretic, spasmolytic, germicides, stomachic, antispasmodic, antiprotozoal, bactericidal, emmenagogue, nematocidal, insecticidal, fungicidal, antiviral, and other microbicidal properties (Sadia et al., 2013). Also, the essential oil of *T. minuta* has been shown to possess bronchodilatory, tranquilizing, hypotensive, and antiinflammatory bioactivities (Singh et al., 2003). There is empirical evidence that the secondary compounds in *Tagetes* species are effective deterrents of numerous organisms, including fungi (Chan et al., 1975), pathogens, bacteria (Grover and Rao, 1978), trematodes, nematodes (Graham et al., 1980), and numerous insect pests through several different mechanisms (Maradufu et al., 1978). It has been reported that Z-β-ocimene and dihydrotagetone, which are constituent compounds of the essential oil of *T. minuta*, were found to be antiviral, active against carnation ring spot and carnation vein mottle viruses. The essential oil of *T. minuta* generally affects a variety of microbial organisms (Senatore et al., 2004). Allelopathic activities of *Tagetes* spp., particularly those against nematodes, have been reviewed (Sadia et al., 2013). Nematocidal activity of *T. minuta* roots is attributed to thienyls, which provide inhibitory effects to parasitic root nematodes and other microbes affecting roots of plants in arable farming systems (Soule, 1996) while the biocidal effects of the essential oil from *T. minuta* flowers and leaves are generally due to a wide range of terpenoids (Singh et al., 2003). Dihydrotagetone and Z-β-ocimene isolated from *T. minuta* oil showed strong nematicidal activity against eggs and juveniles of a root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) with dihydrotagetone showing a higher level of toxicity than Z-β-ocimene (Adekunle et al., 2007).

Ethnobotanic Usage and Applications of *T. minuta* L. in Food Science

Tagetes minuta has a long history of human interest and is used as beverage, condiment, ornamental, medicinal decoction, and in ritual/religious practices (Soule, 1996). In southern South America where the plant is native, its leaves, stems, and flowers are used as a culinary herb in Peru, Ecuador, and parts of Chile and Bolivia, where it is called by the Incan term, *huacatay*. The *huacatay* paste is used to make the popular potato dish called *ocop*a. In Peru, since the ancient Inca civilization, *T. minuta* is made into puree and seasoning that give local dishes a unique herbal flavor which is thought to taste like a mixture of mint, coriander, and basil oils. The plant is also popular in making ethnomedicinal teas in some areas (Soule, 1996). Farmers, who do not practice industrialized agriculture leave plants of *T. minuta* in their arable farming fields. This second crop is beneficial in several ways such as the rapid growth of *T. minuta* quickly shades out other plant species that may be of less use to the farmer and it can be harvested for personal use or for sale in city markets; as well, it has been reported to aid in the retention of humidity in the arable farming fields (Jimenez-Osornio, 1991). In Nepal, marigold garlands are used almost in every household especially during the *Tihar* festival and in Portugal, as well as in India, the plants are used for daily worships and rituals particularly in the Day of the Dead celebrations as the plant was regarded as the flower of the dead in prehispanic Mexico. In Ukraine, related plants of marigold, traditionally known as *Chornobryvtsi* are regarded as one of the national symbols and are often mentioned in songs, poems, and tales. There has been increasing interest in the use of *T. minuta* by indigenous people in India due to increased demand of the essential oil, which is widely used in daily lives while the plant's flowers are used to decorate Hindu temples (Anjaria, 1989). More importantly, an orange pigment extracted from petals of marigold is in great demand for poultry feed in food production systems, while, on the other hand, the plant is grown in arable farming systems to keep the nematode population in soil under control, is often grown alongside valued food crops such as tomato, eggplant, chili pepper, tobacco, and potato plants to boost their production (Wanzala and Ogoma, 2013). Due to the plant's antinematicidal activity, it is used as an intercrop in rotation arable farming systems to protect crops from damage (Singh et al., 2003). In addition, marigold produces antibacterial thiophenes as exudates by the roots in the arable farming soils thereby reducing risks of arable farmers contracting infections while in contact with affected and infected soils (Soule, 1996). This therefore implies that the tagetes (marigold oil) producing plants should not be planted near any nitrogen-fixing leguminous plants whose bacterial activity increases soil fertility, otherwise arable farming productivity and food security for thiophenes may kill the useful bacteria. Dried leaf powder of *T. minuta* was used as a mulch to suppress the growth of two rice paddy weeds, *Echinochloa crus-galli* and *Cyperus rotundus*, thus increasing rice yield and enhancing food security (Singh et al., 2003).

In many regions of the world, the plant is popularly used as anthelmintic, diuretic, antispasmodic, and to treat stomach and intestinal diseases and relief stomach upsets following a meal. The plant's decoction and/or tea preparations may be consumed either warm or cooled and may be sweetened to individual's taste. *Tagetes minuta* is popularly used in rice dishes and as flavoring agent in stews in Chile and Argentina since antiquity. For instance, in northern Chile, *suico* (a tradition preparation of *T. minuta* that enhances flavor of food) is so highly prized that many people have made a traditional habit of collecting wild plants to dry a sufficient supply to last the winter season in their respective homesteads (Soule, 1996). Leaf infusions and extracts from *Tagetes* spp. have been used in folk medicines to treat intestinal and stomach diseases, which have been also suspected of causing food poisoning and some of them have been found to have bioactivity against Gram-positive and Gram-negative bacteria (Tereschuk et al., 1997).

In Kenya, an infusion of *T. minuta* is used for the treatment of snake bites in the Luo and Kamba communities and aerial parts of the plant used for protection against mosquito bites in the tribes of western Kenya and its scent (essential oil) used as antitick agent in the livestock industry (Wanzala, 2009).

Value Addition Usage and Applications of *T. minuta* L. in Food Science

Identification of essential oil of *Tagetes minuta* with a wide range of biological activity such as anthelminthic, carminative, repellent property, weedicidal, diaphoretic, spasmolytic, germicides, stomachic, antispasmodic, antiprotozoal, bactericidal, emmenagogue, nematocidal, insecticidal, fungicidal, antiviral, and other microbicidal activity (Tereschuk et al., 1997), and its application in food science became inevitable (Chatchal et al., 1995; Saha et al., 2012). For a long time in the history of humanity, infusions, tinctures, and juice from aerial parts of *Tagetes* spp. have been used as traditional food additives worldwide. This provided leads into research for underlying science and as a result, for instance, an orange–yellow, carotenoid lutein substance in the florets of *T. erecta* and many other marigolds (*T. minuta* included) has been identified and approved by the European Union (INS-Number E161b) for use as a food color and flavor in various foodstuffs, at a usage level in condiments and relishes such as pasta, vegetable oil, margarine, mayonnaise, salad dressing, baked goods, confectionery, dairy products, ice cream, yogurt, citrus juice and mustard (Timberlake and Henry, 1986).

In such applications, the European Union approved a maximum level of 0.05% of the essential oil of *T. minuta* in cosmetic products and a level in the consumer product not exceeding 0.01%. In the United States, however, the powders and extracts from marigolds are only approved as colorants in poultry feed. In Europe, Asia, Africa, and America, where a variety of insects at various stages of their developments, are used for food and feed security, marigolds have been recorded as a potential food plant for some of these insects such as Lepidoptera caterpillars including the dot moth and as a nectar source for butterflies (Soule, 1996). Conversely, dried leaves of Mexican marigold are used in cooking to give an apple-like aroma to foodstuffs such as soup, meat dishes, and vegetables. In addition to marigold's use in food additives as coloring and flavoring agent, it is innovatively used as fodder in animal food, principally the dried flowers meal and extract used as supplement for poultry feed (Saha et al., 2012). In South America, India and many parts of Asia, essential oil of *T. minuta* is used for flavoring milk, cheese, bakery products, jams, confectionery, and soft drinks (Mohamed et al., 2002) and for spraying in food stores, green houses, and tissue culture laboratories to stop fungal growth and other destructive microbial agents. In parts of Peru, *T. minuta* is used as a vegetable called *huacatay*, which is among the vegetables with the highest levels of vitamin C (Holm et al., 1997). *Huacatay* paste is used to make the popular Peruvian potato dish called *ocopa* (a traditional dish of Arequipa, Peru).

Usage and Applications of Essential Oil of *Tagetes minuta* in Agriculture

Arable Farming System: Plants

In arable farming system, wild marigold is sometimes an alternative host for *Sclerotinia sclerotiorum* ((Lib.) de Bary, 1884), a fungal pathogen that can infect a variety of crops (Soule, 1996), thus showing its agricultural economic importance in arable farming systems used for food production worldwide. The essential oil of *T. minuta* has been found to be effective against three species of common grain pests of fungus, namely, *Tribolium castaneum* (Herbst, 1797), *Rhyzopertha dominica* (Fabricius, 1792), *Callosobruchus analis* (Fabricius, 1781)(Wanzala, 2009) thus can be used to protect grains in storage. While working on a biopesticides registration action document for tagetes oil (PC Code: 176,602), the United States Environmental Protection Agency found this oil to be useful as an insecticide/acaricide for the control of mites, whiteflies, aphids, thrips, mealybugs, scales, and pyslla on a variety of food crops. The essential oil is thus a potential agent for protecting food crops on farm and in storage, thereby increasing food security, particularly in undernourished communities of the world.

Livestock Farming System

Tagetes minuta essential oil has over 95% efficacy against *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1888), *Rhipicephalus sanguineus* (Latreille, 1806), *Amblyomma cajennense*(Fabricius, 1787), and *Argas miniatus* (Koch, 1844) at a concentration of 20%. The oil has the potential to control *Rhipicephalus appendiculatus* (Neumann, 1901), *Amblyomma* spp. etc. (Wanzala, 2009) and other livestock tick species (such as *Hyalomma rufipes*(Koch, 1844)) (Nchu et al., 2012) that cause socioeconomic losses to farmers. The essential oil therefore provides an opportunity for developing an environmentally friendly and a nontoxic acaricide to milk, beef, and hides/skin production in livestock industry. The oil also has repellent effect against blowflies and effectively used for blowfly dressing in livestock industry (Jacobson, 1983).

Production and Market Value of Essential Oil of *Tagetes minuta* L.

The essential oil of *T. minuta* is isolated from the aerial parts of the plant picked when the seeds are just starting to form. Worldwide, production of the essential oil of *T. minuta* was around 1.5 tonnes per annum in 1984 (Lawrence, 1985) and is currently estimated at between 8 and 15 tonnes per annum and priced at $50 per kg on an upward trend. However, for unknown reasons, world records of production and market value of the essential oil of *T. minuta* are not easy to access in the literature (Table 2). Nevertheless, some organizations such as Floracopeia Aromatic Treasures in India price their essential oil at US $1.07 per mL while Mountain Rose Herbs company in the USA that rates South Africa and France as the largest producing countries in the world, sell ½ oz *T. minuta* essential oil at US $11.75. Hermitage soil, a leading supplier of specialist oils in the United Kingdom since 1979, sells *T. minuta* essential oil at £8.20. The International Trade Center (ITC) identifies Egypt and Zimbabwe as the major world producers of *T. minuta* essential oil. Other countries contributing to the international production and marketing of the *T. minuta* essential oil include: Nepal, India, Brazil, Madagascar, Australia, Ukraine, Chile, Bolivia, Peru, Ecuador, Paraguay, Morocco, Kenya, China, United States of America, Argentina, and Madagascar (Craveiro et al., 1988) (Table 2). In 1996, *T. minuta* was on the list of aromatic plants cultivated on a large-scale rating for

TABLE 2 Annual Global Production of *Tagetes minuta* Essential Oil in Some Countries and/or Regions

Country and/or Region	Quantity Produced (tonnes)	Quality Rating	Level of Production	Source Government Agencies, Institutions, and/or Trading Companies Transacting the Essential Oil Business
[b]South Africa	6.5	High quality	Large-scale farming	CBI market information database. www.cbi.eu
India	4.0	Low quality	Large-scale farming	CBI market information database. www.cbi.eu
[b]Zimbabwe	2.0	High quality	Small-scale wild collections	CBI market information database. www.cbi.eu International Trade Center (ITC)
Europe	[a]Negligible	High quality	Small-scale farming	CBI market information database. www.cbi.eu
[b]Madagascar	– (Price: US $155/kg)	High quality with 42–47% *cis*-β-ocimene	Both large-/small-scale farming and wild collections	International Trade Center (ITC) Biolandes http://www.biolandes.com/ouverturepdf.php?file=F2959.pdf&lg=en Natural Extracts Limited http://www.naturalextracts.com/ne/price_sheet.php
China	–	Low quality	Large-scale farming	CBI market information database. www.cbi.eu
[b]Egypt	100–250 kg	High quality	Both large-/small-scale farming	Cairo Aromatic Limited www.cairoaromatic.com
[a]France	183.3–458.2 kg (Approximated from related existing figures)	High quality	–	International Trade Center (ITC) UNCTAD COMTRADE database, United Nations Statistics Division, 2002
Morocco	63.9–159.7 kg (Approximated from related existing figures)	High quality	Both large- and small-scale farming	UNCTAD COMTRADE database, United Nations Statistics Division, 2002

Note: Exact information was not available and/or difficult to access in literature; CBI, Center for the Promotion of Imports from developing countries; UNCTAD, The United Nations Conference on Trade and Development; COMTRADE, The United Nations Commodity Trade Statistics Database.
[a]*European countries are mainly importers of the* Tagetes minuta *essential oil.*
[b]*Leading countries in the production of* Tagetes minuta *essential oil, worldwide.*

industrial processing in Asia, with Nepal taking the lead, just a head of South Africa, which was producing 6000–7000 kg per annum as per 2003 international records of evaluation of market value of essential oil industry at a price ranging from R400 to R1000 per kg. However, this market value is determined by sustainable supply of considerably sufficient volumes and a high quality of the oil. In turn, the quality is determined by the composition and, for instance, oils containing 40–55% *cis*-β-ocimene are highly rated and valued at the international market by the consumers while the biocidal activity is determined by the presence of tagetenone in the composition.

The rate of isolation of the essential oil from *T. minuta* by steam distillation is normally 0.1–0.4 w/w (Wanzala and Ogoma, 2013). However, at a farm level, the production of essential oil from *T. minuta* ranges from 12.5 to 75 kg per hectare (Panda, 2004). Nevertheless, in European countries the market value of *T. minuta* essential oil is potentially increasing as food manufacturers are increasingly preferring to use this oil in a wide range of foodstuffs (such as frozen deserts) and beverages (such as alcoholic beverages and soft drinks) because of its complex and unique flavors such as that of an apple, banana and mixture of sweet basil, tarragon, mint, and citrus. Further, the oil also has preservative properties.

SUMMARY POINTS

- *Tagetes minuta* has a wide range of bioactivity, thus presenting itself as an excellent preventive measure agent and as a food preservative for a wide range of foodstuffs and beverages.
- The presence of antifungal acetylinic thiophenes from *T. minuta*, presents the plant as a potential on-farm biopesticide agent in arable farming systems.
- The wide range of microbicidal activity of the essential oil of *T. minuta* against virus, bacteria, fungi, arboviruses, protozoa, and helminths presents the oil as a potentially agent to relief food poisoning.
- The essential oil of *T. minuta* has the potential to be developed as a weedicide and anthelminthic to help increasing productivity in arable farming systems thereby increasing food productivity as the plant is grown to suppress a wide range of perennial weeds and to protect crops against nematodes and slugs.
- *Tagetes minuta* is a potential food storage agent and an environmentally friendly and nontoxic acaricide.
- There is a need for validation of traditionally claimed usages and applications of the essential oil of *T. minuta* in the food industry.

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Chapter 91

Tangerine (*Citrus reticulata* L. var.) Oils

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List of Abbreviation

ABTS 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulpohonic acid)
BHA Butylated hydroxy anisole
BHT Butylated hydroxy toluene
CREO *Citrus reticulata* essential oil
DPPH 2.2′-Diphenyl-1-picrylhydrazil
FRAP Ferric reducing antioxidant power
FZ Fluconazole
GRAS Generally recognized as safe
LD 50% Lethal dose
MIC Minimum inhibitory concentration
OCT Ochratoxine
Tet Tetracycline
ZDI Zone diameter of inhibition

INTRODUCTION

There are various types of citrus plants, and their fruits are available all over the world. These plants are used widely in both medicine and food industries as well as in household cooking. The most important horticultural citrus crops commercially cultivated in India include *Citrus reticulate*, followed by *Citrus sinesis* and *Citrus auroantifolia*.

Citrus reticulata (order: Sapindales, family: Rutaceae) is a shrub or small tree that grows up to 20 feet in height. The plants have aromatic flowers and glossy leaves, as well as globose fruit with sweet aromatic pulp and light yellow-orange to flame-orange peel, which is loose and easily removed (Nogata et al., 2003). The different bioactive molecules of different chemical structures have been analyzed, and the presence of such components confirms the application of *C. reticulata* for various ailments by traditional practitioners (Kumar and Bhaskar, 2012). *Citrus reticulata* fruit is one of the most popular *Citrus* fruits for fresh consumption; it is referred to as a *tangerine* (with the name usually confined to the types with red-orange skin), a *mandarin*, or *Kamala lebu* in Bengali. There are many reports on the antimicrobial, antifungal, and anti-oxidant effects of *C. reticulate* essential oils (CREOs), as well as the direct food-related application of various plants parts including fruits, fruit peels, flowers, and leaves.

BOTANICAL ASPECTS

Citrus reticulata (family: Rutaceae) is a small, spiny tree that may reach a height of 25 feet, with a dense top of slender branches (Figure 1). It is evergreen and grown widely in India (NISCAIR, 2000). The plants begin to bear fruit when they are around 3 years old. The fruit consists of three layers: (a) an outer peel with oil glands that exude the essential oil (EO), producing the typical orange odor; (b) a whitish, thread-like mesocarp; and (c) an endocarp with 8–10 segments containing juice sacs. The leaves are ovovate and oblong; petioles are narrowly winged, and sweet-scented flowers are borne singly or a few in groups in the leaf axils. The fruit is globose or oblate; the peel is bright-orange or red-orange when ripe and is loose, separating easily from the segments. The seeds are small and pointed at one end; the cotyledons are green in most cultivars (Das et al., 2014; Tiwari, 2009). Limonene, the major constituent of the CREO, contributes to the aromatic odor of the oil; hence, the plant belongs to the limonene chemotype. The plant is important for its fruit juice and the EOs of the plant's fruit peels, flowers, and leaves.

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FIGURE 1 *Citrus reticulata* twigs with green, semi-mature fruits. *Photographed by Dr Shyamapada Mandal.*

USAGE AND APPLICATIONS

The citrus peel has long been used in traditional herbal medicines. CREO is soothing to the nervous system and produces a tonic effect on the digestive system during flatulence, diarrhea, and constipation. The dried external part of the peel possesses stomachic and tonic properties, and it is also used to relieve pain in tooth and ear. The inner white peel, when consumed along with the juice and pulp, exerts a laxative effect (Das et al., 2014). The CREO (fruits and leaves) is used to relieve vomiting (Chopra et al., 1956), and the fruit peel regulates the skin's moisture, softening hard and rough skin, and it has a cleansing effect on oily skin (Khan et al., 2010). The citrus peel is rich in flavonoid glycosides that have a wide range of biological effects, including antioxidant, anti-inflammatory, and anticarcinogenic properties (Bravo, 1998); *Citrus* flavonoids include hesperidin, neo-hesperidin, nobiletin, and tangeritin (Manthey and Grohmann, 2001). *Citrus reticulata* fruit is rich in electrolytes (potassium, magnesium), minerals, and vitamins, especially vitamin C, as well as folate, β-carotene, and vitamins B1, B2, and B3 (Agu et al., 2013).

Evidence suggests an association between citrus peel consumption and protection against coronary heart disease and stroke (Hertog et al., 1997; Keli et al., 1996). It has been reported that compared with the conventional colonic preparation, the use of *C. reticulata* as an adjunct can improve a patient's tolerance, decrease the incidence of adverse events, and maintain the quality of colonic cleansing (Lan et al., 2012).

Sultana et al. (2012) reported that the potential antimicrobial activity of the fruit peel's EO can be useful in the treatment of skin disorders and/or in aromatherapy; also, it can be incorporated into cosmetic formulations. The gas chromatography/mass spectrometry analysis of ethanolic peel extract of *C. reticulata* revealed the presence of maltol; 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one; glycerol; 5-hydrxoy methylfurfural; nitroisobutylglycerol, and heptamethoxyflavone, thus justifying the use of this *Citrus* plant to treat various aliments in folk and herbal medicine (Kumar and Bhaskar, 2012).

USAGE AND APPLICATIONS IN FOOD SCIENCE

The nonmedicinal uses of *C. reticulata* are of great importance in the food and aroma industries. The *Citrus* peel, including that of *C. reticulata*, is commonly used for flavoring food and beverages worldwide. *Citrus reticulata* fruits are rich in ascorbic acid (23–54 mg/100 g of edible portion) and calcium (25–46 mg/100 g of edible portion), and the water content of the fruit is 80–90% of the edible portion (Tiwari, 2009). *Citrus reticulata* ripe-fruit peel crush is mixed with the famous Bengali sweets called *rosogolla* at the time of its preparation to get an excellent citrus aroma and taste; the peel extract is also used to prepare chutney and the milk-based food *payes*.

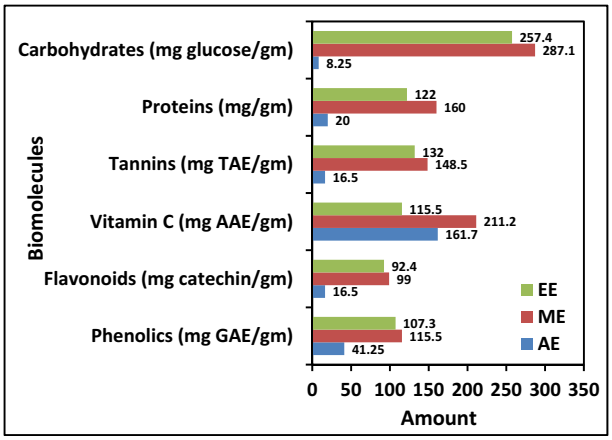

FIGURE 2 Bioactive compounds of *Citrus reticulata* fruit peel. *Adapted from Justin et al. (2014).*

Justin et al. (2014) reported on the various biomolecules such as phenols, flavonoids, vitamin C, tannins, proteins, and carbohydrates present in *C. reticulate* fruit peel (Figure 2). Flavonoids, phenolics, and vitamin C act as antioxidants; tannin is responsible for the prevention of chronic diseases and for health care (Vasundara et al., 2013); protein is vital to the development and correct functioning of the body; and carbohydrates are used as an ideal source of energy. Thus, *C. reticulata* fruit peels can be used as agents in the food industry and as clinically biotherapeutic agents (Justin et al., 2014). CREOs are used in foods, beverages, liquors, and confectionaries as flavoring agents. The current knowledge on *C. reticulata* usage and applications in food science are due to investigations on its biological activities carried out on the essential oils or pure compounds isolated from various parts of the plant.

Antibacterial Activity

CREO from immature fruit exhibited the highest antibacterial activity against *Escherichia coli* (zone diameter of inhibition |(ZDI) 26 mm), which was comparable of positive control activity with the test antibiotic (ZDI 26 mm). In the case of *Staphylococcus aureus* (ZDI 20 mm), the oil was mostly active at the mature stage of fruits (Bourgou et al., 2012). Javed et al. (2011) determined the antibacterial activity of CREO (var. Tangerine and var. Mandarin) against food spoilage bacterial: *E. coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas fluorescence*, *Proteus myxofaciens*, and *Staphylococcus epidermidis*. As reported by Sultana et al. (2012), the antibacterial activity of peel CREO, in terms of ZDI, tested against *S. aureus* and *E. coli*, is represented in Figure 3. Ayoola et al. (2008) reported the broad-spectrum antibacterial activity of fruit peel CREO by determining ZDI against Gram-positive (*S. aureus* and *E. faecalis*) and Gram-negative (*S. typhi*, *K. pneumonia*, *E. coli*, and *Pseudomonas aeruginosa*) bacteria as 11 mm and 10–19 mm, respectively, as well as the minimum inhibitory concentrations (MICs) of CREO against the isolates (Figure 4).

The fresh fruit peel CREO (0.1% v/v, 0.5% v/v, 1.0% v/v) displayed concentration-dependant inhibitory activity against *S. aureus* and *E. coli* (Figure 5) (Tailor and Singh, 2011). Martinez et al. (2003) showed that the tangerine oil (*C. reticulata* Blanco) of variety Dancy was bioactive against *Bacillus subtilis*, *S. aureus*, and *Listeria monocytogenes* at a concentration >1%. The antibacterial activity of CREO was evaluated against Gram-positive bacteria (*S. aureus*: ZDI 14 mm, *Bacillus cereus*: ZDI 12 mm, *L. monocytogenes*: ZDI 13 mm, *Micrococcus luteus*: ZDI 13 mm) and Gram-negative bacteria (*E. coli*: ZDI 12 mm, *K. pneumoniae*: ZDI 13 mm, *P. aeruginosa*: ZDI 12 mm, *Proteus vulgaris*: ZDI 14 mm) using a disk diffusion technique (Kirbaslar et al., 2009).

The antibacterial activity in terms of MIC of CREO alone and in combination with garlic (*Allium sativum*) EO is represented in Figure 6. More potent antimicrobial activity was observed from the EO blend of *A. sativum* and *C. reticulata* working synergistically against the micro-organisms tested (Johnson et al., 2013).

The antibacterial activity of CREOs has been reported to be associated with their components such as monoterpenes, which diffuse into and damage cell membrane integrity. In addition, the bacterial growth inhibition may be caused by either additive, synergistic, or antagonistic interactions between the phytochemical components of the essential oils, even for those present in low concentrations (Bourgou et al., 2012).

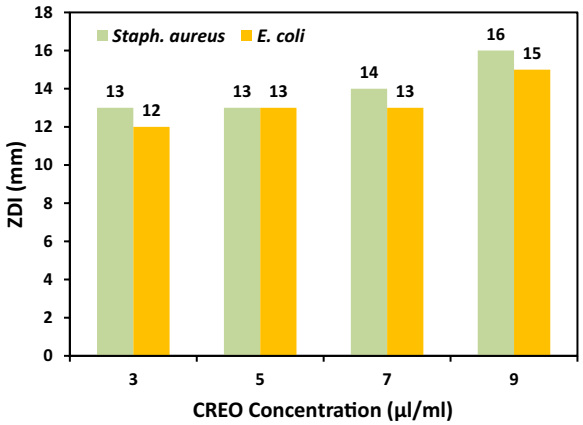

FIGURE 3 Antibacterial activity of CREO against pathogenic bacteria. CREO, *C. reticulata* essential oil; ZDI, zone diameter of inhibition. *Adapted from Sultana et al. (2012), who determined the tetracycline (50 µg/mL) ZDIs of* S. aureus *and* E. coli *to be 11 mm and 17 mm, respectively.*

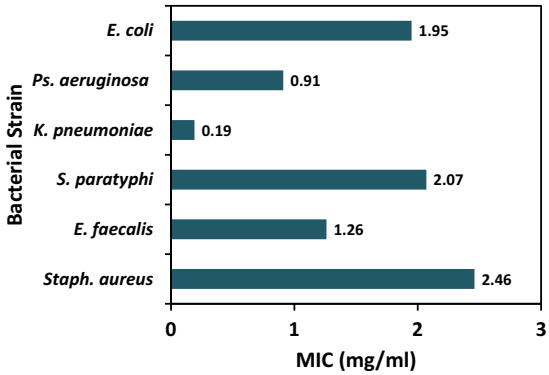

FIGURE 4 MICs of fruit peel CREO against gram-positive and gram-negative pathogenic bacteria. CREO, *C. reticulata* essential oil; MIC, minimum inhibitory concentration. *Adapted from Ayoola et al. (2008).*

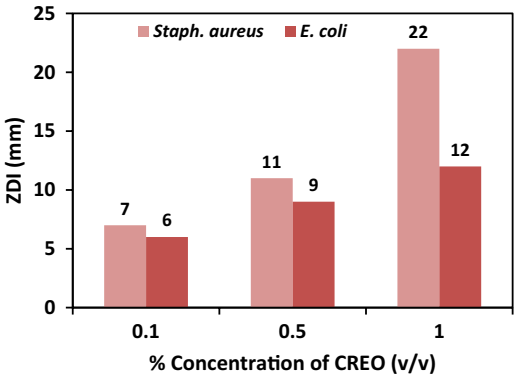

FIGURE 5 Antibacterial activity of fresh peels oil of *Citrus reticulata* Blanco fruits. CREO, *C. reticulata* essential oil; ZDI, zone diameter of inhibition. *Adapted from Tailor and Singh (2011), who used an agar-well diffusion method for the determination of zone diameter of inhibition.*

Antifungal Activity

The fungal species of the genus *Aspergillus* are responsible for spoilage and poisoning of food. *Aspegillus niger* and *Aspergillus ochraceus* are known to produce ochratoxine. *Aspergillus flavus* is known to produce aflatoxins, which are a group of common, extremely hazardous, carcinogenic metabolites (Passone et al., 2012); these are the chemicals at risk in food products. The contamination of spoilage micro-organisms during storage is thus a major problem for the food industry and consumers.

CREO components such as pinene, limonene, and nerol exhibited 100% inhibition of the growth of *A. ochraceus*, *A. niger*, and *A. flavus* when used at concentrations of 1–2 µL. Thus, CREO can inhibit ochratoxigenic and aflatoxigenic molds (Mihai and

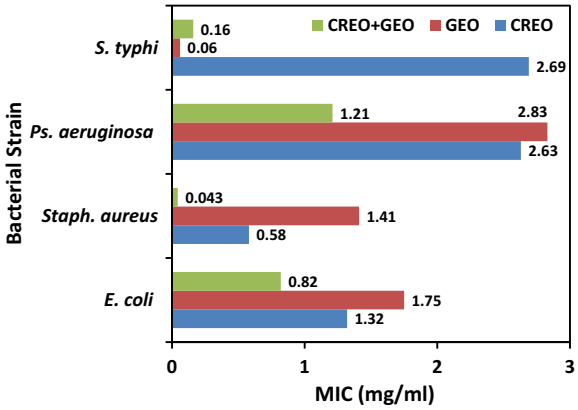

FIGURE 6 Antibacterial activity of CREO alone and in combination with GEO against bacterial pathogens. CREO, *C. reticulata* essential oil; GEO, garlic essential oil. *Adapted from Johnson et al. (2013).*

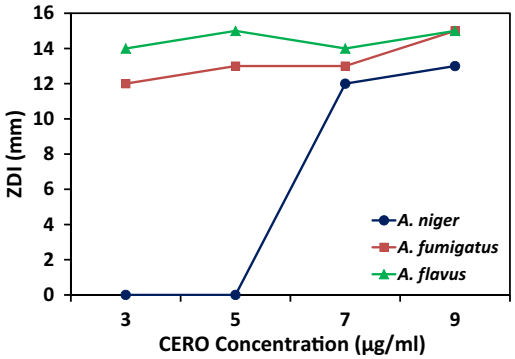

FIGURE 7 Antifungal activity of CREO against food spoilage fungal isolates. CREO: *C. reticulata* essential oil; ZDI: zone diameter of inhibition. *Adapted from Sultana et al. (2012), who determined fluconazole (50 µg/mL) ZDIs against* A. niger, A. fumigatus, *and* A. flavus *to be 22, 12, and 14 mm, respectively.*

Popa, 2014). CREO was found to be active against fungus cultures (*Candida albicans*: ZDI 13 mm, *Kluyveromyces fragilis*: ZDI 15 mm, *Rhodotorula rubra*: ZDI 14 mm, *Debaryomyces hansenii*: ZDI 14 mm, *Hanseniaspora guilliermondii*: ZDI 12 mm), as reported by Kirbaslar et al. (2009). Tao et al. (2014) demonstrated the dose-dependent inhibition of *Penicillium italicum* and *Penicillium digitatum* with CREOs; they reported that the antifungal activity of the oils against *P. italicum* was attributed to citronellol, octanal, citral, decanal, nonanal, β-pinene, linalool, and γ-terpinene, whereas the activity against *P. digitatum* was attributed to octanal, decanal, nonanal, limonene, citral, γ-terpinene, linalool, and α-terpineol. These results suggest that CREOs generate cytotoxicity in *P. italicum* and *P. digitatum* by disrupting cell membrane integrity and causing leakage of cell components.

The antifungal activity of fruit peel CREO against some common foodborne and phytopathogenic fungi—*Alternaria alternata*, *Rhizoctonia solani*, *Curvularia lunata*, *Fusarium oxysporum*, and *Helminthosporium oryzae*—has been reported by Chutia et al. (2009); complete inhibition of fungal growth was observed at 0.2 mL/100 mL for *A. alternata*, *R. solani*, and *C. lunata*. The MIC of *F. oxysporum* and *H. oryzae* was recorded as >0.2 mL/100 mL, while CREO (0.15 mL/100 mL) completely inhibited the growth of *C. lunata*. Fungal spore production was fully inhibited at 0.2 mL/100 mL for *A. alternata*, *R. solani*, and *C. lunata*, but 0.5% for *F. oxysporum* and 0.25% for *H. oryzae* were needed for the inhibition of fungal spore formation (Chutia et al., 2009). The fresh fruit peel CREO displayed concentration-dependant inhibitory activity against *Aspergillus nidulan*, *A. niger*, and *F. oxysporum*, with ZDI of 7, 11, and 12 mm, respectively, at concentrations of 0.1% (v/v), 0.5% (v/v), and 1% (v/v) (Tailor and Singh, 2011). Based on Sultana et al. (2012), the antifungal activity of peel CREO, in terms of ZDI, tested against *A. niger*, *A. fumigatus*, and *A. flavus* is represented in Figure 7.

Antioxidant Activity

Antioxidants are used in the food industry to increase the shelf life of foods. Synthetic antioxidants, such as butylated hydroxy anisole and butylated hydroxy toluene, have been used in foods (Reddy et al., 2005); however, due to their toxicity, liver-damaging activity, and carcinogenicity, their safety profile has been in doubt (Nanditha et al., 2008). Mehmood et al. (2013)

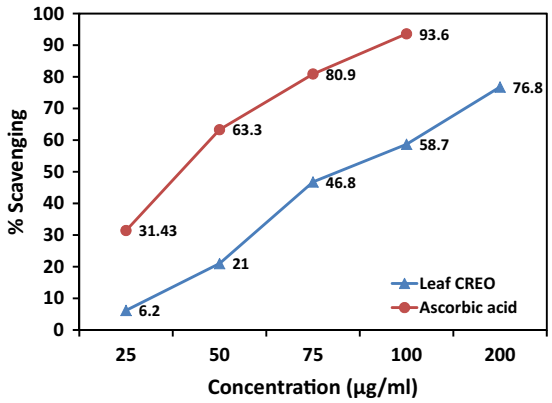

FIGURE 8 DPPH radical scavenging activity of leaf CREO. CREO, *C. reticulata* essential oil; DPPH, 2.2′-Diphenyl-1-picrylhydrazil. *Adapted from Fayed (2009).*

determined the antioxidant activity of leaf and fruit peel CREOs in different systems, such as 2,2′-azinobis(3-ethylbenzothiazoline-6-sulpohonic) acid (ABTS), ferric reducing antioxidant power (FRAP), 2.2′-diphenyl-1-picrylhydrazil (DPPH), lipid peroxide, superoxide anion radical, and iron chelation. There was a linear correlation between the inhibition of ABTS radical cations and the amount of leaf CREO ($R^2=0.9978$) and fruit peel CREO ($R^2=0.985$). The superoxide anion radical scavenging was 15.25% and 48.75%; FRAP values were 1.2 and 0.173 mM $FeSO_4$; and metal (iron) chelating activities were 56.1% and 61.82%, respectively, for the leaf and fruit peel CREOs. CREOs had considerable resistance to lipid peroxidation and time-dependant quenching of DPPH radicals. Fayed (2009) reported that leaf CREO, in a DPPH system, showed antioxidant activity with IC_{50} of 79.84 µg/mL, compared to that of the ascorbic acid (IC_{50} 38.49 µg/mL); the DPPH free radical scavenging activity of the leaf CREO is represented in Figure 8. The essential peel oil of tangerine was able to reduce the stable radical DPPH to yellow-colored DPPH-H reaching 76.75% of DPPH scavenging effect at its 100% concentration (100 µL), whereas the reference standard, ascorbic acid, gave a 99.67% DPPH scavenging effect at its 100% concentration (Shahzad et al., 2009).

CREO may thus be used to minimize the rancidity of foods and restrict the formation of toxic oxidation products, hence maintaining the nutritional quality and shelf life of foods as well as food safety. However, further studies are required to determine the required dosage of CREOs or their components for particular food types.

Tangerine Chemistry and Biological Activity

Citrus reticulata fruit peels are harvested at different stage of maturity. Stage 1 (green color, immature), stage 2 (yellow color; semi-mature), and stage 3 (orange color:mature) yielded EOs of 0.22%, 2.7%, and 1.13%, respectively (Bourgou et al., 2012). The amount of CREO chemicals belonging to the four different classes is shown in Figure 9.

Kirbaslar et al. (2009) identified 45 components in *C. reticulata* peel EO. The major monoterpenes of the oil were limonene (90.7%), γ-terpinene (3.9%), myrcene (2.1%), α-pinene (0.5%), and sabinene (0.3%). The major sesquiterpene was (E)-β-farnesene (0.1%). The major oxygenated components were aldehydes: octanal (0.2%) and decanal (0.1%); alcohols: linalool (0.4%), α-terpineol (0.1%); and esters: geranyl acetate (0.2%) and neryl acetate (0.1%).

CREO isolated by hydrodistillation from mature fruit peels contained 37 different components, of which limonene (46.7%), geranial (19%), neral (14.5%), geranyl acetate (3.9%), geraniol (3.5%), β-caryophyllene (2.6%), nerol (2.3%), and neryl acetate (1.1%) are regarded as the major components. Some other compounds include linalool (0.7%), 6-methyl-5 hepton-2-one (0.7%), decanol (0.6%), and b-bisobolene (0.6%) (Chutia et al., 2009). Kamal et al. (2011) reported limonene (64.1–71.1%) and β-myrcene (3.27–4.5%) as the major monoterpene hydrocarbons, linalool (1.1–2.56%) and decanal (2.33–7.71%) as the main oxygenated monoterpenes, germacarene D (0.3–1.07%) as the major sesquiterpene hydrocarbon, and α-sinensal (0.93–5%) as the main oxygenated sesquiterpne. Thus, the fresh peel, ambient-dried peel, and oven-dried peel CREOs mainly consisted of four chemical classes: monoterpene hydrocarbons, oxygenated monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes (Table 1). The fruit peel CREO (3.5%, w/w, light yellowish-green yield) is composed of nine monoterpenes including five terpenic alcohols (45.5%), two terpenic esters (18.2%), and two terpenic hydrocarbons (18.2%); the predominant components were α-pinene (30.5%), terpinen-4-ol (17.4%), benzyl acetate hydrocarbon (12.8%), *p*-cymene (8.8%), *p*-cymene-8-ol (6.5%), α-terpineol (6.3%), nerol (5.7%), and α-terpenyl acetate (1.5%) (Tailor and Singh, 2011).

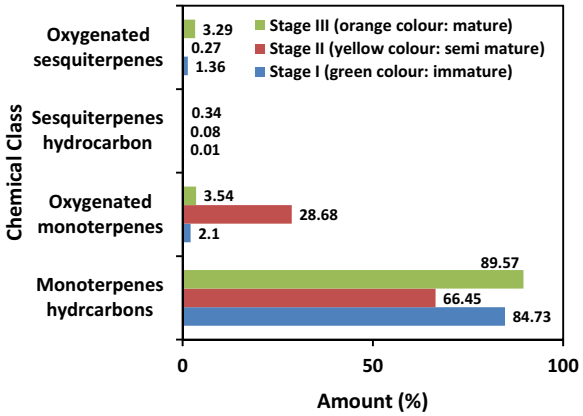

FIGURE 9 Variations of levels (%) of chemical classes of CREOs obtained at different ripening stages of the fruits. *Adapted from Bourgou et al. (2012).*

TABLE 1 Classes of Compounds in CREO from Variously Treated Fruit Peels

	Proportion (%) of CREO Compounds		
Compound Class in CREO	Fresh Peels	Air-Dried Peels	Oven-Dried Peels
Monoterpene hydrocarbons	74.8	70.78	76.79
Oxygenated monoterpenes	11.53	18.22	15.93
Sesquiterpene hydrocarbons	6.19	2.9	*
Oxygenated sesquiterpenes	7.46	7.61	4.3

CREO, *C. reticulata* essential oil; *, Data not provided or present in trace amount.
Text data converted in to table from Kamal et al. (2011).

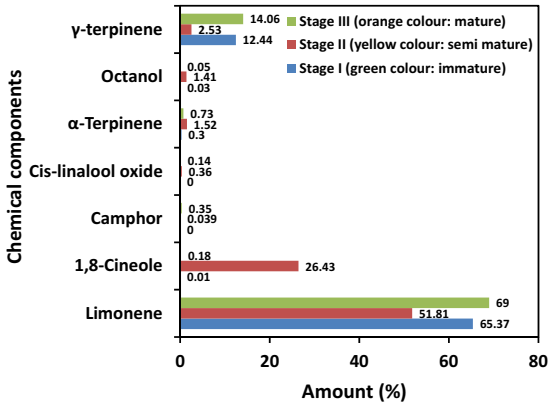

FIGURE 10 Chemical composition (%) of fruit peel CREOs obtained at different ripening stages of the fruits. *Adapted from Bourgou et al. (2012).*

As has been demonstrated by Bourgou et al. (2012), the immature-stage fruit peel CREO contained predominantly monoterpene hydrocarbons (mainly limonene: 65.37%, followed by γ-terpinene: 12.44%). Borneol (1.22%) and caryophyllene oxide (1.18%) were the most represented oxygenated monoterpenes and sesquiterpenes, receptively. The decrease of limonene (51.81%) and γ-terpinene (2.53%) levels at the semimature stage was accompanied by a concomitant increase of 1,8-cineole (26.43%) and E-β-ocimene (7.93%) levels. CREO was dominated by limonene (69%) and γ-terpinene (14.06%) levels at maturity (Figure 10).

The aldehydes are reported to be the important contributors to the characteristic flavor of *C. reticulata*. Among the aliphatic aldehydes, octanal (0.5%) and decanal (0.2%) were the most prominent. Nonanal, dodecanal, (E)-2-undecenal, and tridecanal occurred at <0.05%. Perillaldehyde and α-sinensal were the main terpene aldehydes (0.1%), whereas citronellal, geranial, and β-sinensal occurred at <0.05% (Njoroge et al., 2006).

The oil of fresh *C. reticulata* fruit peels was characterized mainly by monoterpenes (99.1%): six monoterpene hydrocarbons (97.7%) and three monoterpene alcohols (1.0%). The predominant monoterpene was limonene (92.4%), followed by gamma-terpinene (2.6%) and phellandrene (1.8%) (Sultana et al., 2012). Javed et al. (2014) reported that the tangerine fruit peel oil had carvone (16.97%), carveol (8.77%), 3-cyclohexene-1-methanol (8.91%), and limonene oxide (6.85%) in addition to limonene (58.5%) when the CREO yield was 0.28%.

Safety and Toxicity

Citrus reticulata seed extract is given as a treatment for malaria in Ecuador, but it is known to cause respiratory distress and a strong contraction of the spleen (Tiwari, 2009). *Citrus reticulata* trees may have adverse respiratory reactions in close proximity. Saw dust of the wood, formerly used for polishing jewelry, may cause asthma. Excessive contact with fruit peel CREOs can produce dermatitis; sucking of the fruits often causes skin irritation around the mouth; and excess peeling of fruits may cause the appearance of rash and blisters between the fingers. Sensitive people may have respiratory reactions when in contact with fruit peel CREO (Tiwari, 2009).

However, the main advantage of EOs is that they can be used in any food and are generally recognized as safe. The dermal 50% lethal dose (LD_{50}) of undiluted *C. reticulata* (tangerine) leaf oil (described as "petitgrain bigarade oil") was reported as >2 g/kg in rabbits, while the dermal LD_{50} of undiluted mandarin peel oil (*C. reticulata*) was >5 g/kg in rabbits (Ford et al., 1992a,b). Thus, dose determination of the CREOs or its components is mandatory before using the agent in foods, as a food ingredient, as a preservative, or as medicine.

SUMMARY POINTS

- CREOs have broad-spectrum antibacterial activity against food-spoilage bacteria. Thus, *C. reticulata* oil and its components may be used as an alternative to synthetic fungicides or preservatives.
- Due to the antifungal, antibacterial, and antioxidant activities of CREO and its components, it can be exploited as a suitable alternative to chemical additives for use in the food industry, attending to the needs for safety and satisfying the demand of consumers for natural components.
- CREOs can be used as food preservatives in some food products in which *A. flavus*, *A. niger*, and *A. ochraceus* growth and the potential production of mycotoxins are considered to be health hazards.
- CREOs can prevent the spoilage of food products and can increase the shelf life of foods, thus ensuring the microbiological safety of food products.
- Four major classes of chemicals—monoterpene hydrocarbons, oxygenated monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated sesquiterpnes—are present in CREOs; the prime component is limonene.
- The presence of different bioactive molecules of various chemical structures confirms the application of *C. reticulata* for various ailments by traditional practitioners.
- *Citrus reticulata* has the ability to protect against coronary heart disease and stroke, and it can be used as an adjunct in colonic preparations.

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Chapter 92

Tarragon (*Artemisia dracunculus* L.) Oils

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INTRODUCTION

Essential oils of aromatic plants have the ability to prevent food deterioration and food contamination. Therefore, they can be used as natural and safe preservatives of botanical origin for foodstuff. *Artemisia dracunculus* L. (tarragon) has been used for flavoring foods in different countries for a long time. Tarragon and its essential oil have antibacterial and antifungal activity. They may be suitable candidates for use as natural botanical and safe preservatives for foodstuff.

BOTANICAL ASPECTS

Artemisia dracunculus L. (syn. *Artemisia simplicifolia* Pamp.), an aromatic plant belonging to the Asteraceae (Compositae) family, has many common names, including biting dragon, wild tarragon, dragon plant, estragon, little dragon, mugwort, French dragon, British dragon, pinon wormwood, wild tarragon, true tarragon, herbaceous sagewort, German tarragon, and tarragon (Mozaffarian, 2008; Small and Deutsch, 2001). The common English name *tarragon* is a corruption of the French *estragon* (meaning "little dragon") and is derived from the Arabic word *tarkhun* (Small and Deutsch, 2001).

Tarragaon is a wild species native to southeastern Russia, Afghanistan, Pakistan, Mongolia, Turkey, and western North America and is cultivated in other countries (Mozaffarian, 2008). Figure 1 shows most of the areas where the plant is cultivated.

Artemisia dracunculus is a large, shrubby, perennial rhizomatus (60–120 cm tall) that is strongly aromatic to inodorous. It has relatively numerous erect or ascending stems, which are sparsely hairy to glabrous and somewhat woody, and a long, brownish root that is gnarled and fibrous. The leaves are alternate, dark, shiny, narrow gray–green, entirely linear to lanceolate or oblong, and they are usually glabrous. They have smooth edges and grow to about 8 cm (Figure 2).

The small globose rayless flowerheads are arranged in long, loose, terminal panicles; the florets are bisexual and sterile. They are whitish, then later reddish. The sepals of the epicalyx are oblong-elliptic and mostly green (Figure 3). The inner sepals are ovate with a broad embranous edge. The ray florets are female. The disc florets are androgynous and infertile. The corolla is yellow with a glabrous receptacle. The flowering time is mid-summer to late fall. The achene is up to 0.6 mm (Mozaffarian, 2008).

Although there are many different reports about the components of the volatile oil extracted from *A. dracunculus*, the main components of this plant oil are methyl ethers, ocimene, myrcene, α-pinen, β-pinene, camphene, limonene, and linalool (Anonymous, 2007; Volak and Stodola, 1998). However, the prevalent components (>10%) of the volatile oil of *A. dracunculus* in different countries are summarized in Table 1 (Obolskiy et al., 2011). The extracts of the plant contain flavonoids such as quercetin and patulin glycosides, hydroxycoumarins such as herniarin and scopoletin, and isocoumarins including artemidin and polyenes (Anonymous, 2007).

USAGE AND APPLICATIONS

Artemisia dracunculus L., like most of the *Artemisia* species, is a medicinal plant that has been widely used in folk medicine. Table 2 shows the pharmacological activity of *A. dracunculus* and its preparations (Obolskiy et al., 2011). Research shows that this herb has pharmacological activity including carminative, digestive, anti-inflammatory, antipyretic, antiseptic, antispasmodic, antiparasitic, antimicrobial, anthelmintic, and fungicidal effects (Anonymous, 2007; Duke, 2001; Volak and Stodola, 1998). Tarragon essence is used also in aromatherapy (in the forms of massages, face masks, suspensions,

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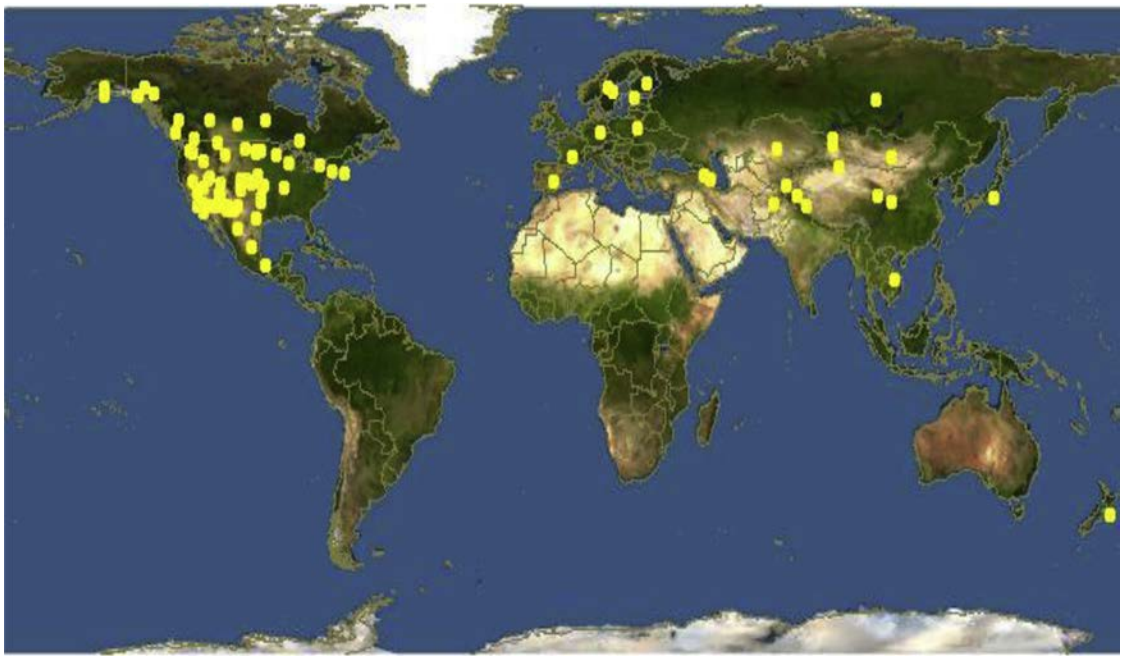

FIGURE 1 Cultivation areas of *Artemisia dracunculus* L., shown by yellow (white in print version) dots. *Note: This figure was published in the internet.*

FIGURE 2 The branched stems of *Artemisia dracunculus* L. *Note: This figure was published in the internet.*

FIGURE 3 The flowers of *Artemisia dracunculus* L. *Note: This figure was published in the internet.*

TABLE 1 Major Components (>10%) of the Volatile Oil of *Artemisia dracunculus* in Different Countries (Obolskiy et al., 2011)

Origin of *A. dracunculus*	Major Essential Oil Constituents
Canada	Methyleugenol (up to 35%), terpinolene (up to 19.1%), estragole (up to 16.2%)
Cuba	Methyleugenol (up to 17%)
Denmark	Methyleugenol (up to 39%), sabinene (up to 24.75%), elemicin (up to 10.37%)
France	Estragole (up to 74%), 7-methoxy coumarin (up to 13%), β-ocimene (up to 10%), methyleugenol (up to 5%)
Georgia	Estragole (up to 82%)
Iran	α-*trans*-Ocimene (up to 20%), limonene (up to 12%)
Italy	*trans*-Anethole (up to 53%)
Russia	Methyleugenol (up to 14%), terpinen-4-ol (up to 41.34%), sabinene (up to 39%), elemicin (up to 57%), β-ocimene (up to 12%), estragole (up to 3.39%)
United States	Terpinolene (up to 25%), *cis*-ocimene (up to 22.2%)

TABLE 2 Pharmacological Activity of *Artemisia dracunculus* and Its Preparations (Obolskiy et al., 2011)

Preparation/ Compound	Tarragon Origin	Effect	Model	Dose Tested
Antibacterial Activity				
Essential oil	Turkey	In vitro Moderate inhibitory activity against *Pseudomonas syringae glycinea* (RK-470), *Xanthomonas* axanopodas pv *vesicatoria*, *Brevibacterium casei*, *Proteus vulgaris*, and others	Disc diffusion method	600–1200 µg/ disc
Antifungal Activity				
Essential oil 5-phenyl-1,3-pentadiyne, capillarin, methyleugenol	NS[a]	Fungistatic against *Colletrotichum fragariae*, *Colletrotichum gloeosporioides*, and *Colletrotichum acutatum*	Bioautography on silica gel thin-layer chromatography plates; microbioassay	NS
Essential oil	Turkey	Moderate inhibitory activity against *Phythium ultimum*, *Sclerotinia sclerotiorum*, *Botyrtis* sp., *Fusarium seminectum*, etc.	Contact assay; growth inhibition	20 µL of essential oil per plate

[a]*NS: not stated.*

baths, and compresses) in the treatment of gastrointestinal spasms, muscle function disorders, dyspepsia, hiccups, aerophagia, anxiety, intestinal worms (ascaris and pinworm) (Duke, 2001; Bown, 2002; Valnet, 1990), menstrual problems, delayed menstruation, and uterus stimulation (Valnet, 1990).

Artemisia dracunculus also has been used for flavoring foods in many countries. The pleasant, spicy aroma of the plant and its essential oil, along with its antibacterial and antifungal activity, are the main reasons for the wide use of this herb in the food industry.

The oil extracted from tarragon and its major components are suitable and safe natural compounds. They have the ability to prevent food deterioration and control various contamination in food. Following standardization of the safety limit of tarragon oil, it will be possible to use it as natural preservative for foods.

USAGE AND APPLICATIONS IN FOOD SCIENCE

During the storage of foodstuff, various contaminations caused by microorganisms lead to food spoilage. Also, in humid environments and at high temperatures, fresh fruits and vegetables are destroyed by these microorganisms. This is a major problem in the food industry, as well as an environmental problem.

One of the major causes of the degradation of materials and foods is oxidation. The reduction of molecular oxygen results in reactive oxygen species (ROS). ROS are very reactive and can damage transient chemical species formed in cells (Halliwel and Gutteridge, 1990; Mantle et al., 2000). ROS can be implicated in the pathogenesis of more than 50 diseases (Mojarab et al., 2009). In addition, these free radical species can deteriorate the lipid components in foodstuff. The effect of ROS on food components, as well as the contamination of food during storage with microorganisms, leads to food deterioration. Therefore, the food industry has to use preservatives to prevent food contamination and spoilage.

The use of synthetic preservatives over a long period can lead to some health and environmental problems, such as carcinogenicity and other toxicities. Therefore, the replacement of synthetic compounds with natural compounds from botanical sources has generated considerable interest. This can delay the destruction of food, vegetables, and fresh fruits in storage while avoiding environmental and health problems. Essential oils extracted from different plants and their major components are suitable and safe natural agents to be used as natural preservatives for food. They have the ability to prevent food deterioration and control contamination in food.

Tarragon has a long history of use as a spice and remedy, and it is safe to use in functional foods and/or as a dietary supplement. This plant has been used as a flavoring agent in food, salad, and vinegar (Ribnicky et al., 2004). The essential oil extracted from tarragon and its major components can be used as safe natural compounds in the food industry as a replacement for synthetic preservatives and additives.

Tarrago as an Antimicrobial for Food Preservation

Raeisi et al. demonstrated the antibacterial activity of tarragon essential oil in Iranian white cheese and in culture media on *Staphylococcus aureus* and *Escherichia coli*. This research study showed the possibility of using this essential oil as a natural preservative to protect foods like cheese against the two tested bacteria (Raeisi et al., 2012).

The antimicrobial activity of *A. dracunculus* essential oil against 10 different microorganisms was evaluated by Lopes-Lutz et al. using drop agar diffusion. The antimicrobial activity of the volatile oil against the tested microorganisms showed varying degrees of growth inhibition. Although more investigation is needed, the antifungal activity of the volatile oil extracted from tarragon may be suitable for use as a natural agent in pharmaceutical products and the food industry (Lopes-Lutz et al., 2008).

Alinezhad et al. studied the antifungal activities of extracts and volatile oils of eight different bioactive plants against *Aspergillus parasiticus* (a aflatoxin-producing microorganism) using a microbioassay method. *Aspergillus parasiticus* growth was inhibited remarkably by *A. dracunculus* essential oil using different concentrations. The antifungal effect of this essential oil extracted from tarragon can be a valuable in protecting food and agricultural products from contamination and deterioration (Alinezhad et al., 2012).

The antifungal activities and antibacterial effects of the essential oil of *A. dracunculus* grown in Turkey (Turkish tarragon) were examined by Kordali et al. The volatile oil was obtained from tarragon by hydrodistillation. The tested oil showed high antifungal effects on the growth of most of fungi, which were agriculturally pathogenic. Although the oil exhibited antifungal activity on the tested pathogenic fungi, the inhibition zone of the essential oil was lower when compared with the penicillin inhibition zones (Kordali et al., 2005).

Tarragon as an Antioxidant

Cejudo-Bastante et al. tested several aromatized vinegars available commercially in the market for their antioxidant activity. Various raw fruits, vegetables, spices, and herbs were the source of these vinegars' aromatization. The antioxidant effect of vinegar aromatized by tarragon was determined using ultra-high performance liquid chromatography with an ultraviolet detector and gas chromatography-mass spectrometry. The vinegar aromatized by tarragon showed high antioxidant activity, correlated to the presence of different compounds in the oil sample (Cejudo-Bastante et al., 2013).

The antibacterial and antioxidant properties of the volatile oil of tarragon, as well as some other oils, on hake protein edible films have been studied. When different volatile oils were added, there was an increase in the solubility of films in water but a reduction in the water vapor permeability. The antioxidant effect on hake protein films increased with the incorporation of the volatile oil of tarragon (Pires et al., 2013).

Ayoughi et al. examined the antioxidative effects of essential oil obtained from the aerial parts of *A. dracunculus*. In this study, they used β-carotene/linoleic acid assay and a 2,2′-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH) method to measure the antioxidant activity of the oil. The results of the study showed antioxidative activity for the essential oil of this plant. The oil extracted from *A. dracunculus* had the ability to reduce the soybean oil rate of oxidation when tested under 60 °C as a condition to accelerate the rate of oxidation. These data support other studies that evaluated the antioxidant activity of this oil (Ayoughi et al., 2011).

The reduction of molecular oxygen results in ROS, which are very reactive, can damage the transient chemical species formed in cells, and may lead to several diseases. The free radical species can deteriorate food components during the storage. Antioxidant agents can be added to foodstuff to reduce the rate of their oxidation. The antioxidant activity of *A. dracunculus* essential oil was examined by Lopes-Lutz et al. using beta-carotene/linoleate assay and a DPPH radical scavenging method (Lopes-Lutz et al., 2008). Weak antioxidant activity was reported for the oil when the above methods were used. However, the authors advise that further research is needed to evaluate this oil's antioxidant activity (Lopes-Lutz et al., 2008).

SUMMARY POINTS

- Tarragon (*A. dracunculus* L.) is an ornamental plant that is used in the food industry, as well as a medicinal plant since ancient times.
- Tarragon and its products are used extensively as flavoring agents.
- Tarragon essential oil has various pharmacological activities, antimicrobial activity, and antioxidant activity.

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Chapter 93

Tasmanian Pepper Leaf (*Tasmannia lanceolata*) Oils

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INTRODUCTION

Tasmannia lanceolata is the main commercial species of native pepper in Australia. The leaves and berries are known for their pepper flavor and are used in savory dishes (Ahmed and Johnson, 2000). The leaves are used dried and milled or as an essential oil extract. In culinary applications, the leaves are used as dried or fresh herbs or as purees. The Tasmanian pepper leaf contains a pungent sesquiterpene, polygodial, which has demonstrated a wide range of biological properties, including both antibacterial and antifungal activity and anti-insect feeding properties (Clarke, 2012). Other plants known to have this terpene, which is the pungent factor causing the hot effect in the mouth, are *Persicaria hyropiper* (water pepper, smart weed, or march pepper), *Persicaria odorata* (Vietnamese mint or coriander or Laksa plant in Malaysia) (Starkenmann et al., 2006), *Pseudowintera colorata* (Horopito) from New Zealand (McCallion et al., 1982), *Drimys winteri* from South America (Winters Bark) (Williams and Harvey, 1982), and two East African *Warburgia* trees, *Warburgia ugandensis* and *Warburgia stuhlmannii*, which are used as local food spice (Kubo et al., 1976).

The fruit of *T. lanceolata* was used by early European settlers in Australia as a substitute for black pepper. There is renewed interest in using the leaf and berry as a spice; it is currently used in novelty food products such as marinades, pickles, oils, and health care products (Clarke, 2012; Pengelly, 2002). This chapter reviews the application of Tasmanian pepper leaf extracts in food, its botanical aspects, and its chemical and functional properties.

Botanical Aspects

Tasmannia lanceolata (Poir.) A.C. Smith is a member of the family Winteraceae, previously known as *Drimys aromatica*, *Drimys lanceolata*, *Tasmannia aromatica*, and *Wintera lanceolata*. Some of the common names are Tasmanian pepper leaf, mountain pepper, and Tasmanian pepper bush (Hegarty et al., 2001; Lim, 2013). It grows as a branched shrub from 2 to 5 m, with dark shiny green leaves and a distinct crimson young stem. The plant has separate male and female white to creamy white flowers and produces a dark purple (almost black) fleshy fruit containing many small black seeds. The leaves and berries are aromatic, hot, and spicy. It grows at high altitudes in Tasmania, Victoria, and New South Wales and is endemic to southeastern Australia and Tasmania. *Tasmannia lanceolata* grows well in cool environments, free from water stress, in neutral, well-drained, and fertile soil (Lim, 2013; Pengelly, 2002). The leaves and berries are mainly wild harvested between March and June (Clarke, 2012; Figure 1).

USAGE AND APPLICATIONS

Traditionally, the leaves have been used by indigenous Australians as a therapeutic agent to treat stomach and skin disorders and colic; more recently, it has been used as a quinine substitute (Cock, 2013). The main interest in the therapeutic field is the treatment of fungal infections, particularly for topical applications. In New Zealand, creams for skin-based antifungal infections have been developed and commercialized using the extracts of *Pseudowintera colorata*, also belonging to the Winteraceae (Pengelly, 2002). It is used in health care products such as toothpaste, where the pungent molecules present in *T. lanceolata* have enhanced the freshness.

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FIGURE 1 Tasmanian pepper leaves, berries, and flower. *Courtesy of Essential Oils of Tasmania Pty Ltd.*

Pungent molecules, like polygodial extracted from *T. lanceolata*, have been successfully used as a potent detachment substance for blue mussels on ship hulls. The detachment activity increased significantly when used in combination with sorbic acid, anethole, and indole. These mussels can cause marine fouling and are a serious problem on ship hulls, cooling, and aquaculture systems (Ban et al., 2000). Tasmanian pepper leaf extracts, which are rich in chlorogenic acid, have been used as a co-pigment to increase the color intensity in a model soft drink solution, where Davidson's plum was used as an anthocyanin-based food colorant (Jensen et al., 2011).

Usage and Applications in Food Science

Tasmanian pepper leaf is harvested from March to June. The essential oil from the leaves is extracted by a solvent extraction process in commercial operations. After the plant material has been extracted with a solvent such as hexane, it produces a waxy aromatic compound referred to as concrete. This concrete is soluble in ethanol. A concrete yield of 6% or more should be maintained to ensure commercial viability (Menary et al., 1999). Although it is possible to prepare an essential oil of Tasmanian pepper leaf by steam distillation, the yields are low, the oil contains very little pungent molecules such as polygodial, and it is dominated by volatile monoterpenes (Read, 1995). A more concerning issue with steam distillation is that it can concentrate less desirable volatile compounds in the small yield of oil. The volatiles in the leaf extract and essential oil from steam distillation were predominantly monoterpenic, with α-pinene, β-pinene, limonene, linalool, bicyclogermacrene, and 1,8-cineole as the major components (Southwell and Brophy, 1992). Polygodial is a sesquiterpene that was reported as a compound contributing to the pungent taste in *T. lanceolata* leaves (Loder, 1962; Read and Menary, 2000). The Tasmanian pepper leaf is currently wild harvested, but there are regrowth and small plantation operations with selected clones. These clones have been selected on the basis of high oil; some yield in excess of 50% polygodial in the volatiles (Clarke, 2012; Menary, 2003).

The compounds listed in Table 1 are the predominant ones found in *T. lanceolata*, from petroleum ether extract of the dried leaves (Menary, 2003).

The food and beverage industries use Tasmanian pepper leaf extracts in a number of products, including as a seasoning in savory dishes and snack foods and in beverages and confectionery. They are also used in cooked or preserved meats, cheese, pates, and sauces (Clarke, 2012). These extracts have also been used as a flavor enhancer in chewing gum to enhance the flavor of mint, peppermint, and spearmint (Menary, 2003). The sensory description developed for the aroma and flavor of Tasmanian pepper leaf in the lexicon for Australian native foods include "aroma of Australian bushland," "dry paperbark," and "herbal"; the flavor is an intense heat that slowly develops on the palate (Smyth et al., 2012).

ANTIMICROBIAL PROPERTIES

Tasmannia lanceolata extracts have shown broad-spectrum antibacterial and antifungal activity, as shown in Table 2, indicating its potential as a natural preservative for the food industry.

Polygodial, the principal pungent component in *T. lanceolata*, showed fungicidal activity at 50 μg/mL against *Zygosaccharomyces bailii*, a food spoilage yeast. These yeasts are known to cause spoilage in high-sugar products such as honey, syrups, and molasses. The fungicidal activity of polygodial increased significantly in combination with anethole and sorbic acid, indicating the synergistic effects of combining two compounds in comparison to a single compound (Fujita and Kubo, 2005b). In another study, the combination of polygodial with anethole displayed fungicidal activity against *Candida albicans* and *Saccharomyces cerevisiae*. The lethal concentration of polygodial against *C. albicans* was lowered from 3.13 to 0.098 μg/mL and for *S. cerevisiae* from 1.56 to 0.049 when polygodial was combined with 100 μg/mL of anethole. However, when 400 μg/mL of indole was combined with 1.56 or 0.039 μg/mL of polygodial, the number of viable cells of

TABLE 1 Major Components in *Tasmannia lanceolata* Extract

Component	Percentage
Linalool	1.81
Bicyclogermacrene	1.51
Myristicin	1.00
Calamenene	3.42
Cadina-1,4-diene	1.58
Spathulenol	1.94
Guaiol	4.46
Drimenol	1.91
5-Hydroxycalamenene	1.47
Polygodial	36.74
n-Pentacosane	1.54
hexacosanal	2.71

TABLE 2 In Vitro Testing of Tasmanian Pepper Leaf Extracts and Key Components against Food-Related Organisms

Tasmanian Pepper	Inhibited Microorganism	References
Water, ethanol, hexane extracts	*Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus*	Weerakkody et al. (2010)
Water, ethanol, hexane extracts	**Pathogenic bacteria** *Aeromonas hydrophila, Bacillus cereus, Clostridium perfringens, E.coli 0157:H7, Listeria monocytogenes, Shigella sonnei, S. aureus, Salmonella enteritidis, Vibrio cholera, Yersinia enterolica* **Spoilage bacteria** *Acinetobacter baumannii, Bacillus subtilis, Lactobacillus plantarum, Pediococcus cerevisiae, Psuedomonas aeruginosa, Psychrobacter phenylpyruvica* **Spoilage yeasts** *Candida albicans, Candida colliculosa, Candida lipolytica, Candida stellata, Hanseniaspora uvarum, Pichia anómala,* Pichia membranifaciens, *Rhodotorula mucilaginosa, Schizosaccharomyces octosporus*	Zhao and Agboola (2007)
Polygodial	*Sclerotinia libertiana, Mucor mucedo, Rhizopus chinensis, Aspergillus niger, Penicillium crustosum*	Kubo and Taniguchi (1988)
Polygodial	*Zygosaccharomyces bailii, Saccharomyces cerevisiae*	Fujita and Kubo (2005a,b)
Polygodial	*Salmonella choleraesuis*	Kubo and Fujita (2001)

C. albicans reduced but remained static, and *C. albicans* was still viable after 48 h of incubation. The combination of polygodial and indole has a fungistatic effect on *C. albicans*, indicating that this combination is good as a preventive measure (Himejima and Kubo, 1993).

The antifungal mechanism of polygodial is suggested to be its ability to function as a nonionic surfactant, disrupting the lipid–protein interface and denaturing integral protein conformation in the cell membrane of the organism (Kubo et al., 2001). This surfactant concept could also be applied in part to *Salmonella choleraesuis* as the bactericidal effect occurred rapidly after the addition of the polygodial. This rapid lethality supports its ability to function as a nonionic surfactant. In addition to the surfactant concept, polygodial can also enter the cells of the microorganisms through the pores

TABLE 3 Average Usual and Maximum Use Levels (ppm) for *Tasmannia lanceolata* Extract FEMA GRAS™ Flavoring Substances

Food Category	Usual Use/Maximum Use Levels (ppm)
Nonalcoholic beverages	1/2
Alcoholic beverages	1/4
Chewing gum	30/40
Hard candy	4/7
Seasonings/flavors	100/200
Snack foods	10/20
Soft candies	4/7

caused by membrane damage (Kubo et al., 2005). Polygodial indicated potent antifungal activity against yeast such as *C. albicans*, *S. cerevisiae*, and also filamentous fungi including *Trichophyton* and *Penicillium marneffei*. It showed moderate-to-low susceptibility to fungi such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, and *Candida lipolytica* (Lim, 2013). *Tasmannia lanceolata* extracts have shown promising broad-spectrum antimicrobial activity, mainly attributed to polygodial. However, there are other terpenes with known antimicrobial activity in these extracts. Thus, it is a synergistic combination of these chemical components that give Tasmanian pepper leaf extracts its potent antimicrobial activity.

FLAVORING AGENT

Tasmannia lanceolata extract has been approved as a generally regarded as safe (GRAS) flavoring ingredient by the Flavor and Extract Manufacturers Association (FEMA No. 4755), under the conditions of the intended use in food flavorings in accordance with the 1958 Food Additives Amendment to the Federal Food, Drug and Cosmetic Act. The accepted levels as a flavoring substance in food are given in Table 3 (Marnett et al., 2013).

Due to its pungency, Tasmanian pepper leaf has been used in wasabi paste to enhance the pungent effect of allyl isothiocynate, as well as to improve the sensory properties of artificial sweeteners. These effects are attributed to the pungency and trigeminal stimulatory effect of polygodial (Starkenmann et al., 2011).

SAFETY OF TASMANIAN PEPPER LEAF ESSENTIAL OILS

Tasmannia lanceolata extract has been listed under the GRAS flavoring substances 26. It was evaluated and approved by the Expert Panel of the Flavor and Extract Manufacturers Association of the United States (FEMA). It is classified as FEMA No. 4755; its primary name is *Tasmannia lanceolata* extract with the synonyms *Drimys lanceolata* extract and *Drimys aromatica* extract (Marnett et al., 2013). The issue of the presence of safrole in the extract has been addressed to fulfill the requirements of the Organisation of the Flavor Industry, which has a limit of 1 mg/kg in foods and beverages. There are clonal materials that have very low or no safrole and thus have been selected for cultivation (Menary, 2003).

SUMMARY POINTS

- The leaves of Tasmanian pepper leaf (*Tasmannia lanceolata*) solvent are extracted for the production of the essential oil.
- Polygodial is the major chemical component contributing to the bioactivity and flavor of the essential oil.
- Tasmanian pepper leaf essential oil has broad-spectrum antimicrobial activity and is a potent antifungal agent.
- Tasmanian pepper leaf essential oil is used in the food and beverage industry as a flavoring agent.
- Opportunities for the trigeminal effect of pungent molecules, such as polygodial, should be explored for novel food applications.
- Combining two or more phytochemicals, such as polygadiol and anethole, enhanced the antimicrobial activity; this is a promising strategy for further investigation.

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Chapter 94

Thyme (*Thymus vulgaris* L.) Oils

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List of Abbreviations

BHA Butylated hydroxy anisole
BHT Butylated hydroxy toluene
FIC Fractional inhibitory concentration
GRAS Generally recognized as safe
MAP Modified atmosphere packaging
MBC Minimum bactericidal concentration
MFC Minimum fungicidal concentration
MIC Minimum inhibitory concentration
TBHQ tert-butyl hydro-quinone
TEO *Thymus vulgaris* essential oil
ZDI Zone diameter of inhibition.

INTRODUCTION

The thyme plant, *Thymus vulgaris* L., exhibits polymorphic variation in monoterpene production. Intraspecific chemotype variations are seen in *Thymus* and are named geraniol, α-terpineol, thuyanol-4, linalool, carvacrol, and thymol after its dominant monoterpene (Thompson et al., 2003). Thymol and carvacrol are the two most active components of thyme essential oil, with a wide range of antimicrobial and antioxidant properties that improve the shelf life of food. The leafy parts of thyme and its essential oil have been used in foods for flavor, aroma, and preservation and are added to meat, fish, and food products. The strong toxic properties of thyme oil and its active compounds, such as thymol and carvacrol, against a large number of microorganisms have been described by Soliman and Badea (2002). The use of thyme in the cosmetic industry has increased the economic importance of this medicinal crop worldwide; the plant thus has changed from a traditional herb to a serious drug in rational phytotherapy.

Thymus vulgaris oil and extract components, acting alone or in combination, may result in a broad spectrum of antimicrobial activity. This activity may be attributed to thymol or the result of synergism between other main components in the oil. Although *T. vulgaris* has generally recognized as safe (GRAS) status, its oil should be reserved for topical use; internally, it may lead to dizziness, vomiting, and breathing difficulties (Newall et al., 1996).

BOTANICAL ASPECTS

Thyme (*Thymus vulgaris* L.), belonging to the Lamiaceae family, is a perennial subshrub with a lifespan of approximately 10–15 years. Its stem becomes woody with the age, and it has both horizontal and upright habits. The plant is gynodioecious; both hermaphrodite and female individuals are found in populations (Ozguven and Tansi, 1998).The full-grown plant may be 25 cm in height, with a quadrangular stem and branches; the stalkless leaves are 6–12 mm in length and are oval to oblong in shape.

Thymus vulgaris blooms in June to July. The flowers are arranged in dense terminal heads with uneven calyx; they are yellow, white, or purple in color. The seeds are round, very small, and retain their germinating power for 3 years.

Thyme is cultivated all over the world. Reproduction of the plant is possible by seeds and vegetative parts, such as by root cuttings; the root cutting reproduction of the plant has been well described by Ozguven and Tansi (1998).

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USAGE AND APPLICATIONS

Thymus vulgaris has been used since ancient times to achieve healing, cure chest congestion, and induce saliva; the fresh leaves are taken to relieve sore throats. The plant is also used as an effective remedy for chest infections (bronchitis, pharyngitis, whooping cough) as well as to treat worms in children.

The plant has been used for its flavor in cooking. *Thymus vulgaris* essential oil (TEO) is used as an antiseptic, antiviral, and antimicrobial agent in folk medicine. Thyme also possesses carminative and antioxidative effects. Fachini-Queiroz et al. (2012) showed that the constituents, thymol and carvacrol, of TEO present effects on the inflammatory response; the anti-inflammatory properties of TEO are partially involved in the hepatoprotective effect of the essential oil (Grespan et al., 2014).

Baydar et al. (2004) reported the use of TEO in medicating bandages before the discovery of modern antibiotics. *Thymus vulgaris* is used for skin problems such as oily skin, acne, dermatitis, eczema, and insect bites. Thyme oil has been used as a fumigant in places of worship by many ancient civilizations. Sokovic and van Griensven (2006) reported that TEO showed very high activity against major fungal (*Verticillium fungicola* and *Trichoderma harzianum*) and bacterial (*Pseudomonas tolaasii*) pathogens of button mushroom, *Agaricus bisporus.*

USAGE AND APPLICATIONS IN FOOD SCIENCE

The nonmedicinal use of *T. vulgaris* is of great importance, being used in food and aroma industries. TEO is used as a flavor enhancer in a wide variety of foods, beverages, and confectionery products; it also possesses antimicrobial and antioxidant properties, and thus is used as a food preservative. Thyme is used to season and suppress offensive odors, such as tri-methylamine odor, in foods. The synthetic antioxidants (butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and tert-butyl hydro-quinone (TBHQ)) are effective, but BHA and BHT are reported to be carcinogenic. TEO and *T. vulgaris* extracts possess excellent antioxidative activity—better than those of the synthetic antioxidants. The current knowledge on *T. vulgaris* use and applications in food science are due to investigations of its biological activities carried out on extracts, essential oils, or pure compounds isolated from the herb *T. vulgaris*.

Food Preservation and Antispoilage Activity

Spoilage and poisoning of food by microorganisms is a major problem, especially in developing countries. Foodborne illnesses are associated with bacterial pathogens, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella enteritidis*. These bacteria present a major public health concern, and there is also an immense economic cost associated with the deterioration of food products by food pathogens. A variety of microorganisms lead to food spoilage and foodborne diseases; Javed et al. (2012) reported on the foodborne pathogenic fungi, such as *Alternaria alternate*, *Fusarium oxysporum*, and *Aspergillus flavus*, and food spoilage bacteria, such as *Pseudomonas syringae*, *Bacillus subtilis*, *E. coli*, *Staphylococcus* sp., and *Aeromicrobium erythreum.*

TEO is proven to be highly effective against food pathogenic microorganisms, including *Campylobacter jejuni*, *S. enteritidis*, *E. coli*, *S. aureus*, and *L. monocytogenes* (Smith-Palmer et al., 1998). Szczepaniak et al. (2011) reported that a mixture of thyme components (thymol, carvacrol, and cymene) inhibited the growth rate of the bacterium *Brochothrix thermosphacta* (a prevalent microflora of meat refrigerated in modified atmospheres with high oxygen content) by 25.7%, and thus extended the shelf-life of minced pork during an in situ study.

TEO maintains normal pH range (5.7–6.6) in fish flesh (which on spoilage due to improper storage shows increased pH) because the oil is excellent at preventing the growth of spoilage microorganisms of Enterobacteriaceae family and *Pseudomonas*. Angis and Oguzhan (2013) found TEO (0.8%, v/w) to be very effective in extending the shelf life of fresh rainbow trout fillets in modified atmosphere packing (50% CO_2 + 50% N_2).

Rabiei et al. (2011) demonstrated the treatment of apples (*Malus domestica*) with TEO (100 ppb) for improving the postharvest quality after 5 months of cold storage at 0–2 °C and 85–90% relative humidity. Shabnum and Wagay (2011) reported the potential of thyme oil volatiles as an antifungal preservative for strawberry fruits that are quite susceptible to decay (gray mold and soft rot incidence) caused by *Botrytis cinerea* and *Rhizopus stolonifer*; the gray mold and soft rot incidences were reduced by 75.8% and 74.8%, respectively, at 14 days of storage by exposure to higher concentration of thyme oil volatiles. It is interesting to note that thyme extracts possess the strongest antioxidant capacity because of its higher polyphenol content, and they can be used as preservative ingredients in the food.

Karami-Osboo et al. (2010) reported on the antigrowth activity of TEO and its major components (carvacrol; 4.78% and thymol; 49.5%) against *Erwinia amylovora*, suggesting the suitability of the agents in controlling fire blight disease of fruits such as apples and pears. Because of its broad-spectrum antimicrobial activity, increased efficacy over common synthetic

preservatives, and non-mammalian toxicity, TEO may be formulated as a safe and economical phytopreservative against postharvest fungal infestation and aflatoxin contamination of food produce (Kumar et al., 2008).

TEO is an effective antifungal agent that can prevent conidial germination and growth of food spoilage and mycotoxin-producing fungi like *Fusarium moniliforme*, *A. flavus*, and *Aspergillus fumigates* at concentrations of 1000 ppm, indicating the suitability of the oil as a food preservative (Nguefack et al., 2004). The food preservative potential of TEO against mycotoxigenic strains of *Aspergillus ochraceus*, *Penicillium expansum*, and *Penicillium verrucosum* was reported by Nguefack et al. (2009). Burt and Reinders (2003) reported bacteriostatic and bactericidal activities of TEO (at 625 µL/L and 1250 µL/L, respectively) against *E. coli* O157:H7 from foodstuffs such as meat and meat products, milk, yogurt, water, salad vegetables, fruits, and fruit juices, and recorded a zone diameter of inhibition (ZDI) of 25 mm against the pathogen. Kassem et al. (2011) reported the reduction of microbial load (Enterobacteriaceae, *Staphylococcus*, proteolytic and lipolytic bacteria, and total mold counts), in stored beef burger treated with TEO (0.04% and 0.06%). Smith-Palmer et al. (2001) reported TEO as a potent inhibitor of *L. monocytogenes* and *S. enteritidis* in soft cheese, and thus it is an excellent food preservative.

Antibacterial Activity

Thymus vulgaris oil and extracts possess broad-spectrum antibacterial property against food spoilage and food poisoning bacteria, and that related to foodborne diseases of humans. People in industrialized countries suffer from foodborne disease (World Health Organization, 2002) resulting from infection with foodborne pathogens such as *E. coli*, *Salmonella enterica* serovar Enteritidis, *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Typhi, *Shigella flexneri*, *B. cereus*, and *S. aureus*.

TEO exhibited antibacterial activity against *S. aureus*, *S. enteritidis*, *E. coli*, and *Bacillus cereus* with ZDIs of 38, 46, 39, and 35 mm, respectively (Dobre et al., 2011a). Sheeladevi and Ramanathan (2012) reported that the TEO had antibacterial activity against foodborne bacteria: *Campylobacter* sp. (ZDI 20 mm), *Listeria* sp. (ZDI 24 mm), *Yersinia* sp. (22 mm), *Salmonella* sp. (21 mm), and *Pseudomonas* sp. (22 mm), with minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs) of 80–100 µL/mL and 320–400 µL/mL, respectively.

The concentration-dependent growth inhibition of *S. aureus* and *E. coli* with *T. vulgaris* hot alcoholic (25, 20, 15, 10, and 5 mg/mL) and aqueous (25, 20, 15, 10, and 5 mg/mL) extracts has been reported by Fayad et al. (2013) as having ZDIs of 21, 15, 14, 11, and 9 mm (*S. aureus*) and 19, 14, 13, 10, and 8 mm (*E. coli*), respectively. The concentration-dependent inhibitory activity of TEO against *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (both clinical and standard strains) has been reported by Yazdi et al. (2013): the oil (10–40%, v/v) had similar activity against *E. coli* (ZDIs 9–18 mm) and *S. aureus* (ZDIs 12–22 mm).

TEO (with 0.5%; v/v, supplementation) in sterilized milk displayed a bactericidal activity, effectively limiting the cell numbers of test pathogenic microorganisms such as *E. coli*, *S. aureus,* and *L. monocytogenes*. The inhibitory effect of the essential oil increased with its increasing concentration (Celikel and Kavas, 2008).

The MICs for *E. coli*, *S. typhimurium*, *S. aureus*, and *L. monocytogenes* of TEO have been recorded as 0.45–1.25, 0.450->20, 0.2–2.5, and 0.156–0.45 µL/mL (v/v), respectively (Burt, 2007). The oil component carvacrol had MICs of 0.225–5, 0.225–0.25, 0.175–0.45, 0.375–5, and 0.1875–0.9 µL/mL (v/v). Thymol had MICs of 0.225–0.45, 0.056, 0.140–0.225, 0.45, and 0.45 µL/mL (v/v) for *E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes,* and *B. cereus*, respectively (Burt, 2007). The bacterial strains tested were found to be sensitive to TEO and showed very effective bactericidal (*Bacillus subtilis*, *S. aureus*, *Staphylococcus epidermidis*, *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *Mycobacterium smegmatis*) activity with MICs of 75–1100 µg/mL and ZDIs of 22–29 mm (AlMaqtari et al., 2011).

TEO has been found to be active against food intoxication causing bacterial isolates, including *S. enteritidis* with ZDIs 16–45 mm and MICs with 1.56 mg/mL (Miladi et al., 2013). The oil was found effective against *Micrococcus luteus* NCIMB 8166 and *L. monocytogenes* ATCC 19,115 strains, while the Gram-negative bacteria showed less sensitivity to the oil (Figures 1 and 2). The Irani and Omani thyme oils inhibited completely the growth of *S. aureus*, *Streptococcus pyogenes*, *E. coli*, and *P. aeruginosa* at concentrations 0.39, 0.78, 1.56, and 1.56% (v/v), respectively (Nzeako et al., 2000).

Synergistic antibacterial activity was seen against *S. aureus*, in combination with TEO and *Cinnamomum zeylonicum* essential oil, with a fractional inhibitory concentration (FIC) index of 0.26. TEO in combination with *Aniba rosaeodora* and *Melissa officinalis* essential oils demonstrated a synergistic effect against *E. coli* (FIC indices 0.23 and 0.34, respectively) (Kon and Rai, 2012). Nezhad et al. (2012) reported excellent antibacterial activity of thyme against *E. coil*, and two other bacterial strains (*Pectobacterium carotovorum* and *Ralstonia solanacearum*) infecting tomatoes and potatoes, showing ZDIs of 30, 34.8, and 16.5 mm, respectively; the MICs and MBCs are given in Figure 3. Thus, potatoes and tomatoes can be protected from infection with *R. solanacearum* and *P. carotovorum*, which damage the crops, through the application of TEO without using chemical agents.

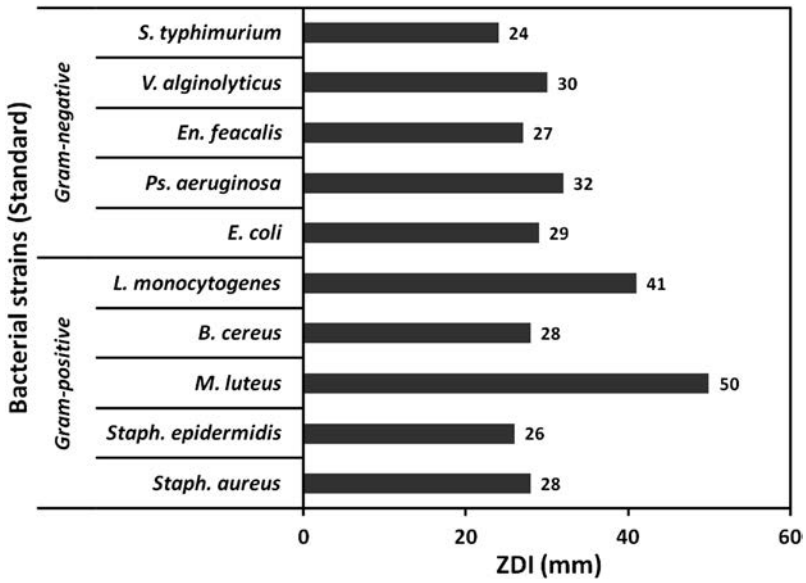

FIGURE 1 The antibacterial activity of *Thymus vulgaris* essential oil against standard strains of potential pathogenic bacteria. ZDI, zone diameter of inhibition; *V. alginolyticus*, *Vibrio alginolyticus*; *En. faecalis*, *Enterococcus faecalis*. *Adapted from Miladi et al. (2013).*

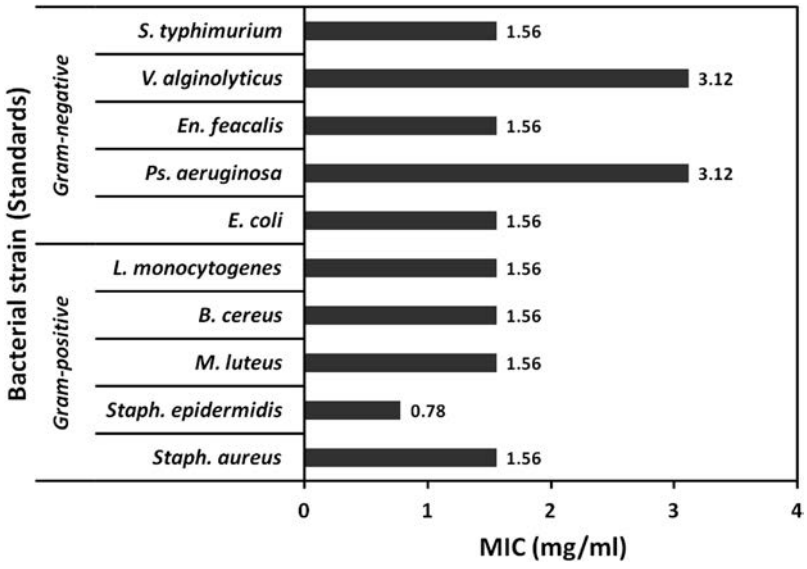

FIGURE 2 The MIC values of *Thymus vulgaris* essential oil against standard strains of potential pathogenic bacteria. MIC, minimum inhibitory concentration; *V. alginolyticus*, *Vibrio alginolyticus*, *En. faecalis*, *Enterococcus faecalis. Adapted from Miladi et al. (2013).*

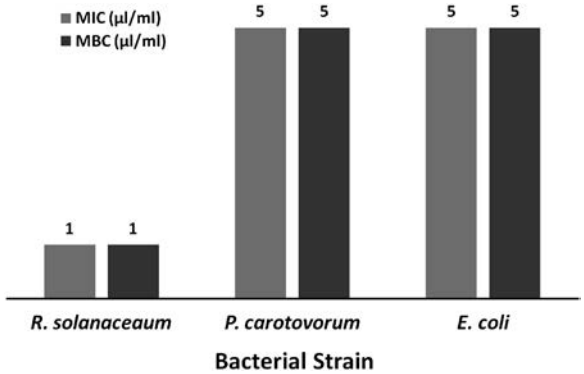

FIGURE 3 MICs and MBCs of *Thymus vulgaris* essential oil against *Ralstonia solanacearum*, *Pectobacterium carotovorum*, and *Escherichia coli*. *Adapted from Nezhad et al. (2012).*

Antifungal Activity

The fungal colonization of food reduces its shelf life and thus its market value. Fungi may make food unfit for human consumption, in some cases due to mycotoxin production (Kumar et al., 2008). The volatile vapor of white thyme oil exhibited antifungal activity against food spoilage fungi *Aspergillus* spp., with a ZDI 55 of mm (*A. oryzae*) to 70 mm (*A. brasiliensis*), and 62 mm against *A. flavus* (Dobre et al., 2011b). Soliman and Badeae (2002) and Kalemba and Kunicka (2003) reported TEO (500 ppm) to possess strong antimicrobial properties against foodborne fungi, including *A. flavus*, *A. parasiticus*, *A. ochracus*, and *F. moniliforme*.

According to Klaric et al. (2007), thymol and TEO, the main components of which are *p*-cymene (36.5%) and thymol (33%), showed strong fungicidal and/or fungistatic activities against *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Mucor*, and *Rhizopus*. TEO and thymol displayed strong antifungal activity against *Rhizopus oryzae* (related to gastrointestinal disease in humans) with MICs of 128–512 µg/mL for both, while *p*-cymene did not show significant inhibition of growth against strains of *R. oryzae* (MIC >1024 µg/mL for 100% of strains; de Lira Mota et al., 2012).

TEO inhibited completely the mycelial growth of *A. flavus* at 0.7 µL/mL and exhibited a broad fungi-toxic spectrum against many food-contaminating fungi, such as *F. oxysporum*, *Cladosporium herbarum*, *Curvularia lunata*, *A. terreus*, *A. niger*, *A. fumigatus*, *A. alternata,* and *Botryodiploidia theobromae* (Kumar et al., 2008).

Bouddine et al. (2012) reported TEO (10%; v/v) as an antifungal agent showing a ZDI of 70 mm against the toxinogenic as well as pathogenic *A. niger*, as found in grains, fruits, forage, vegetables, and dairy products. The phenolic compounds, thymol and carvacrol, at 10%, had ZDI of ≥85 mm and 56 mm against *A. niger*. Thymol had better fungicidal activity (MFC = 0.025%) than carvacrol (MFC = 0.05%). TEO (MICs 1.6–19.6 µg/mL), which is rich in thymol (MICs 1.6–13.74 µg/mL) and other antifungal components, could be used for the disinfection of moldy walls in dwellings at low concentration; thus, the chances of food/vegetable contamination with fungus is reduced (Klaric et al., 2007).

Riccioni and Orzali (2011) reported very low MIC (<0.05%) of *T. vulgaris* oil against seed-borne fungal pathogens such as *Ascochyta rabiei*, *Colletotrichum lindemuthianum*, *Fusarium graminearum*, *Fusarium culmorum*, *Drechslera avenae*, *Alternaria radicina*, and *Alternaria dauci*. The authors concluded that the oil can be used in agriculture for safe and eco-friendly seed treatments, and to prevent and control seed-borne diseases.

Antioxidant Activity

The antioxidant activity of TEO and thyme extracts is of great importance because these agents extend the shelf life and preserve foods from toxic effects of oxidants. The major phenolic components of thyme extracts or thyme essential oil include thymol and carvacrol; these components have excellent antioxidant activity that may be higher than the well-known BHT and α-tocopherol antioxidants (Lee and Shibamoto, 2002). TEO exhibited effective radical scavenging capacity with an IC_{50} value of 437 µg/mL; thus, it acts as a natural antioxidant agent (Miladi et al., 2013). Selmi and Sadok (2008) reported the role of thyme oil as a scavenger of radicals involved in lipid oxidation, protecting lipids from oxidation during frozen and refrigerated storage; the authors reported radical scavenging activities (IC_{50} = 18.6 µg/mL) that was superior to that of the standard BHT.

At concentrations of 5–5000 µg/mL, TEO significantly inhibited lipid peroxidation (IC_{50} = 8461 ± 7.778 µg/mL), demonstrating the antioxidant property of TEO. The free radical-scavenging capacity (RSC) of TEO at concentrations of 60–2500 µg/mL showed significant antioxidant activity in vitro (IC_{50} = 1377 ± 1.6970 µg/mL). Figure 4 shows the antioxidant activity of TEO (Grespan et al., 2014).

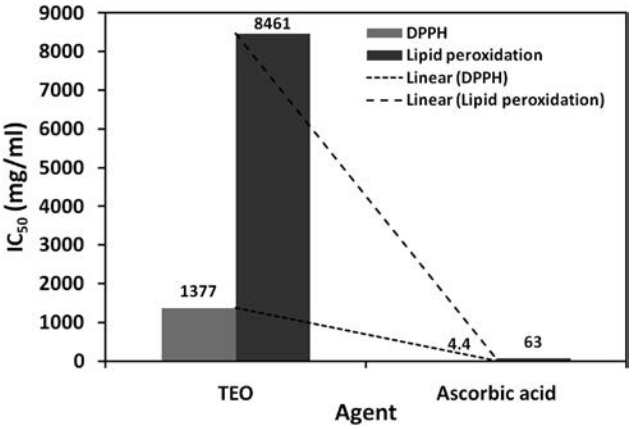

FIGURE 4 Antioxidant activity in terms of IC_{50} values of two agents: thyme essential oil (TEO: test) and ascorbic acid (positive control) by lipid peroxidation and 2,2-diphenyl-1-picrylhydrazil (DPPH)-scavenging activity. *Adapted from Grespan et al. (2014).*

Thymus vulgaris extends the shelf life of refrigerated fish and fish products. As reported by Selmi and Sadok (2008), tuna fish (*Thunnus thynnus*) flesh—the lipid content of which undergoes auto-oxidation during chilled storage—could be stored up to 18 days at 0 °C when treated with powdered thyme. As reported by Albarracin et al. (2012), the reduction of oxidative processes in *Oreochoromis niloticus* fillets by using TEO at days 9 and 12 is represented in Figure 5.

Thyme Chemistry and Biological Activity

The isolated active substances of *T. vulgaris* are volatile oils (21.1%), saponins (59.2%), and tannins (9.7%) (Fayad et al., 2013). The hexane, ethyl, and acetate *T. vulgaris* leaf extracts contain steroids, the methanol and butanol extracts contain flavonoids, and the ethyl acetate and butanol extracts contain saponins (Hossain et al., 2013); the total flavonoid contents of the five crude extracts of *T. vulgaris* is given in Figure 6.

It has been reported that TEO is rich in phenolic compounds (thymol, carvacrol, and γ-terpipene) that have strong antibacterial activity (Baranauskiene et al., 2003a; Dorman et al., 2004), which can be bactericidal or bacteriostatic depending upon their effective concentrations (Nessrien et al., 2007). Thymol, found in TEO, is an antioxidant containing OH; it transmits the hydrogen to peroxide after lipid oxidation and lessens the formation of free radical H_2O_2 (Lee et al., 2004). Carvacrol kills pathogenic microorganisms by disintegrating the cytoplasm, and it prevents an increase in plasma triglyceride and cholesterol (Lee et al., 2003). Imelouane et al. (2009) reported that the major components of TEO with antibacterial activity against *S. aureus*, *S. epidermidis*, *Streptococcus* sp., and *E. coli*, include camphor (38.54%), camphene (17.19%), α-pinene (9.35%), 1,8-cineole (5.44%), borneol (4.91%), and β-pinene (3.9%). According to de Lira Mota et al. (2012), the major components of *T. vulgaris* include *p*-cymene and thymol; the antifungal activity (fungicidal and/or fungistatic) of TEO against *Rhizopus oryzae* can be attributed to thymol, mainly the hydroxyl group of this compound, because *p*-cymene (benzene) does not possess substantial antifungal activity.

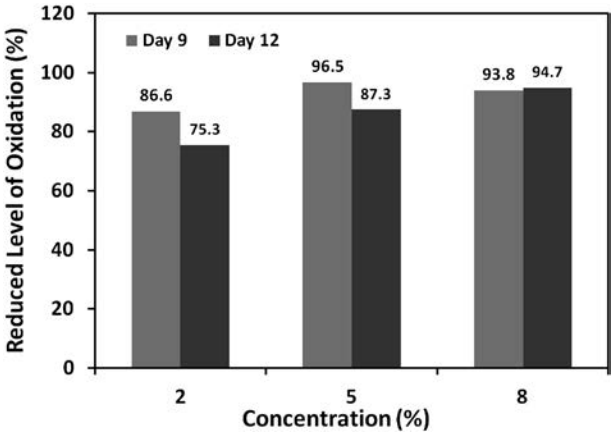

FIGURE 5 Reduction of oxidative processes in *Oreochoromis niloticus* fillets by using *Thymus vulgaris* essential oil. *Adapted from Albarracin et al. (2012).*

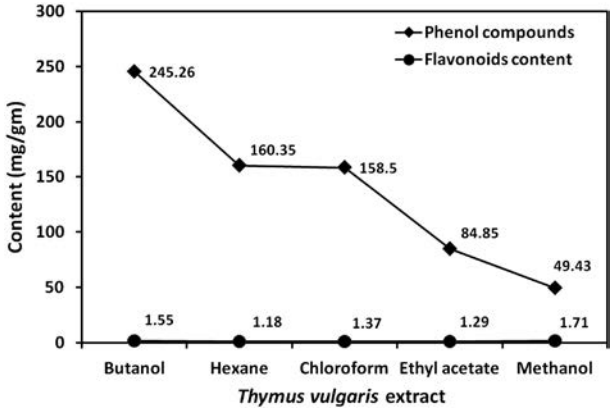

FIGURE 6 Total flavonoid content of the five crude extracts of *Thymus vulgaris*. *Adapted from Hossain et al. (2013).*

TABLE 1 Major Components of *Thymus vulgaris* Essential Oil (TEO)

TEO Components (%)					
Monoterpene Hydrocarbon γ-Terpinene	Monoterpene Hydrocarbon *p-Cymene*	Terpenoidic Phenol *Carvacrol*	Terpenoidic Phenol *Thymol*	Monoterpene Alcohol *Linalool*	References
16.5	18.6	2.4	44.7	–	Porte and Godoy (2008)
3.2	11.7	70.3	0.6	2.2	Pina-Vaz et al. (2004)
2–31	10–56	2–11	10–64	–	Burt (2007)
0.18–2.12		2.46–4.55	7.44–14.32	1.59–3.20	Ozguven and Tansi (1998)
–	24.36–51.26	2.46–4.55	7.44–16.58	–	Mericli-Ilisulu and Tanker (1986) and Piccaglia and Marotti (1991)
0.3	21.6	4.2	29.4	0.7	Nowak et al. (2013)
15.8	6	62.3	2.5	–	Kon and Rai (2012)
0.3	38.9	–	46.6	3.8	de Lira Mota et al. (2012)
27.31	10.9	1.49	30.61	–	Roldan et al. (2010)
14.1	9.9	–	46.2	4	Ozcan and Chalchat (2004)
6.9–18.9	9.1–18.5	2.4–4.2	44.4–58.1	–	Baranauskiene et al. (2003b)
–	43.19	0.45	44.33	4.67	Ramadan et al. (2013)
–	30.53	3.37	30.86	2.73	Grigore et al. (2010)

The *p*-cymene (1-methyl-4-(1-methylethyl)-benzene) and γ-terpinene (1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene) are the precursors of carvacrol (2-methyl-5-(1-methylethyl)phenol) and thymol (5-methyl-2-(1-methylethyl)phenol) (Burt, 2007). The essential oil obtained by hydrodistillation from the aerial parts of *T. vulgaris* is a pale yellow liquid with 1.57% (v/w) yield (Ozcan and Chalchat, 2004). The composition of TEOs (Table 1) from a particular species of plant can depending on the harvesting seasons, ecological and climatic conditions, and/or geographical source, and it may have variation from year to year because of perennial plants (Merino et al., 1990).

Safety and Toxicity

Thymus vulgaris has been given GRAS status by the US Food and Drug Administration, meaning that the plants are generally recognized as safe for human consumption without limitations on intake and commonly accepted by consumers. TEO showed a predominance of carvacrol (45.54%), α-terpineol (22.96%), and endo-borneol (14.29%) as the major components. When tested orally, the oil showed a high LD_{50} value (4 mg/kg), suggesting the safety and nontoxicity of TEO for animals (Grespan et al., 2014).

Thyme oil should be reserved for topical use. When taken internally, it may lead to dizziness, vomiting, and breathing difficulties (Newall et al., 1996). There should be restrictions on the use thyme in cases of high blood pressure. Certain chemotypes containing high levels of thymol and carvacrol are toxic and should be avoided in aromatherapy (DAAFFRSA, 2012).

SUMMARY POINTS

- The thyme plant, *T. vulgaris*, exhibits polymorphic variations in monoterpene production. Intraspecific chemotype variations are seen in the plant; these are named geraniol, α-terpineol, thuyanol-4, linalool, carvacrol, and thymol after its dominant monoterpene.
- *Thymus vulgaris* essential oil and extracts are active growth inhibitors of bacteria and fungi that cause food spoilage and/or foodborne diseases.

- The essential oil of *T. vulgaris* is highly active as a fungitoxicant and could safely be used as a natural preservative.
- The use of *T. vulgaris* as a spice and natural preservative in food products is valuable for increasing the shelf life of foods, and plant oil and extracts could possibly replace chemical additives and synthetic antimicrobial agents in the future.
- The strong antibacterial action of *T. vulgaris* essential oil and extracts has been attributed to the phenolic monoterpenes (carvacrol and thymol); a possible synergistic effect with other minor components, such as monoterpene hydrocarbons (γ-terpinene and *p*-cymene), is indicated.
- The essential oil of *T. vulgaris* can be recommended for use as part of an antimicrobial packaging system for postharvest preservation of fresh vegetables and fruits, because the oil shows inhibition of phytopathogenic microorganisms.
- Thyme is generally regarded as safe; however, it may be dangerous in some cases. Further research is needed to evaluate the effectiveness of *T. vulgaris* essential oil in food ecosystems to establish its utility as a natural antimicrobial agent in food preservation and safety.

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Chapter 95

Turmeric (*Curcuma longa*) Oils

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List of Abbreviations

EO Essential oil
TO Turmeric oil
CL *Curcuma longa*

INTRODUCTION

The uses of medicinal and aromatic plants started since time immemorial in Indian History because of a vast number of natural resources. India is a land of agriculture that supported by climatic features in different zones which results store house of huge flora and fauna and hence the commercial importance of Indian medicinal and aromatic plants is widely demanding and competitive in world market. Turmeric is an important medicinal and aromatic plant which is considered as one of the golden resource with immense export potential as medicine, beauty aid, cooking spice, and as a dye. It has more than 100 compounds; curcumin is one of most important curcuminoids and rhizomes contains 3–8% yield of turmeric oil (TO). It has strong socio-cultural ties to the people of India and has at least 6000 years of documented use as the spice (Ravindran et al., 2007), hence it is known as the golden spice of India and also as "Kitchen Queen". The ancient Vedic people of India regarded it as "the herb of the sun" because of its yellow–orange rhizome. Turmeric is an herbaceous perennial and underground stem ginger-like plant. The plant is native to tropical south-east Asian countries, but is widely cultivated throughout the world. Currently, India is the major producer (over 500,000 metric tons annually) and consumer of turmeric followed by China, the Caribbean, and Latin America. India accounts for about 80% of world turmeric production and 60% of world exports (Bengmark et al., 2009).

BOTANICAL ASPECTS

Plant Profile

Kingdom: *Plantae*, plants
Subkingdom: *Tracheobionta*, vascular plants
Superdivision: *Spermatophyta*, seed plants
Division: *Magnoliophyta*, flowering plants
Subclass: *Zingiberidae*
Order: *Zingiberales*
Family: *Zingiberaceae*, ginger family
Genus: *Curcuma*
Species: *longa*
Botanical name: *Curcuma longa*

Turmeric is a rhizome and herbaceous perennial aromatic plant belongs to the family Zingiberaceae. The plant is about 60–90 cm in height with a short stem tufted oblong leaf. The flowers are white to light yellow and they are between 10 and 15 cm in length and they group together in dense spikes, which appear from the end of spring until the middle session. The whole turmeric rhizome has a rough, segmented skin. The rhizome is yellowish-brown with a dull orange interior that looks bright yellow when powdered. Dried rhizomes are slightly acrid in taste. Rhizomes measure about 2.5–7.0 cm in length and 2.5 cm in diameter with small tubers branching off (Kirtikar and Basu, 1993) (Figures 1 and 2).

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FIGURE 1 Turmeric plant from the medicinal garden of Krupanidhi College of Pharmacy, Bangalore. *Source: Kuntal Das.*

FIGURE 2 Turmeric rhizome procured from the local market of Yesvanthpur, Bangalore, India. *Source: Kuntal Das.*

The plant requires temperatures between 20 and 30 °C and an annual rainfall of about 1500 mm is optimum. The plant thrives in well-drained, fertile, sandy and black soil, rich in humus, and uniform in texture. Natural drainage and irrigation facilities are the best for the cultivation of turmeric. The harvest is carried out during January to March. The rhizome matures in about 9 months (Khanna, 1999).

The plant is grown extensively in tropical climate of Indonesia, Malaysia, Singapore, China, Brazil, and India. In India, the plant is cultivated in the coastal regions of Tamil Nadu, Karnataka, Maharashtra, Gujarat, Goa, Kerala, Andhra Pradesh, Assam and West Bengal (Lal, 2012) (Figure 3).

There are some of the important turmeric varieties, namely Madras variety, Duggirala, Tekurpeta, Sugandham, Amalapuram, Erode local, Alleppey, Moovattupuzha, and Lakadong that are exported from India (Lal, 2012). Furthermore, various Indian cultivars were released by the Indian Institute of Spice Research which are listed in Table 1.

Among the varieties Allepy turmeric contains about 3.5–5.5% volatile oils, and 4–7% curcumin, whereas the Madras type contains only 2% volatile oils and 2% curcumin (Lal, 2012). TO is extracted generally by steam or hydrodistillation method and by supercritical fluid extraction of the powdered rhizome (Gopalan et al., 2000). Several reports are revealed on isolation of TO by hydrodistillation using Clevenger's apparatus (Reddi, 2013) but the essential oil (EO) content varied with the parts of plants, varieties, and the cultural practice.

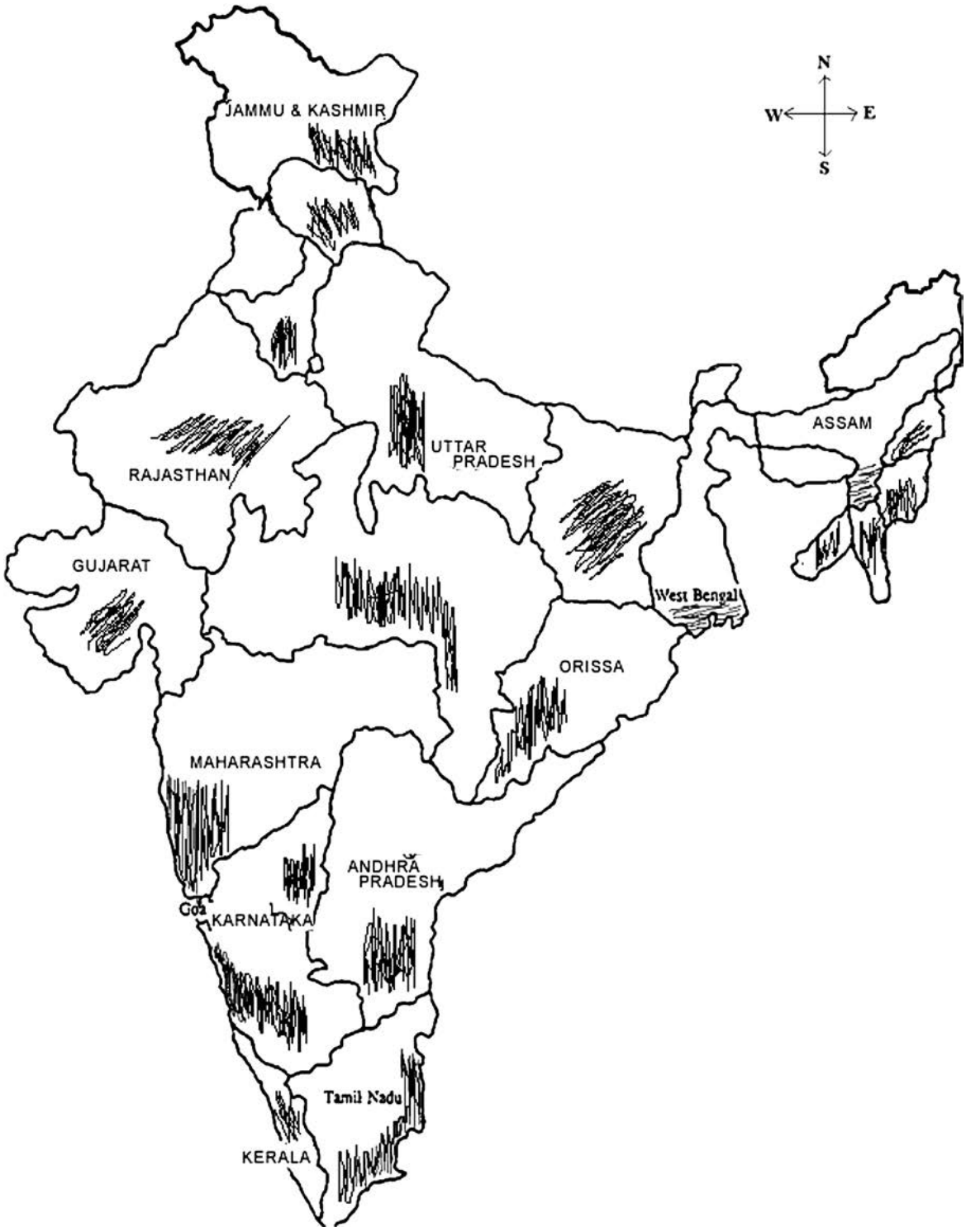

FIGURE 3 Cultivation of turmeric in different states of India. *Source: Kuntal Das.*

The plant contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), and moisture (23.1%). The EO (5–8%) obtained by steam distillation of rhizomes has α-phellanderene (1%), sabiene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%), and sesquiterpines (53%), etc. (Rathaur et al., 2012). Furthermore ar-tumerone (49.1%) followed by curlone (16.8%), α-phellandrene (5.3%), ar-curcumene (3.5%), eucalyptol (2.6%), β-sesquiphellandrene (1.8%), β-caryophyllene (0.8%), β-bisabolene (0.6%), and δ-3-carene (0.3%) are reported using gas chromatography

TABLE 1 Some Important Indian Cultivars of *Curcuma longa*

Cultivars	Rhizome Color	Curcumin (%)	Essential Oil (%)	Oleoresin (%)
Suvarna	Deep orange	4	7	13.5
Suguna	Orange	4.9	6	13.5
Sudarshana	Orange	7.9	7	15
Prabha	Red yellow	6.5	6.5	15
Prathibha	Red yellow	6.5	6.2	16.2

Taken from Anandaraj et al. (2001).

and mass spectrum analysis, isolated from the steam distilled extracted EO from the rhizome of turmeric plant (Singh et al., 2011).

USAGE AND APPLICATIONS

Turmeric production in India began in ancient times. Erode (a city of Tamil Nadu, south India) is the world's largest producer and most important trading center of turmeric throughout Asia. Hence, in history Erode was known as "Yellow City" or "Turmeric City" followed by second largest state of India is Maharashtra (Sangli, a town of western state of Maharashtra). India produces more than 600,000 tons of turmeric annually which is 80% of world production of around 800,000 tons (Kumar and Sakhya, 2013). The Bengal type *C. longa* (CL) is preferred for used in industries in India as dyes. CL and its products are mostly exported to countries such as United Arab Emirates, the United States, Bangladesh, Japan, Sri Lanka, Malaysia, and the United Kingdom for the consumers' interest and about 65% of Indian turmeric exports to the world market (The American Spice Trade Association, Inc, 2002).

Turmeric has been used in traditional and Ayurvedic medicine since ancient times, with various biological applications such as in the treatment of many inflammatory conditions. In India, turmeric has been traditionally used primarily for arthritic, muscular disorders, biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, and sinusitis (Shishodia et al., 2007). The active constituents of CL are yellowish orange volatile oils called curcuminoids known as curcumin, which has demonstrated antioxidant, antineoplastic, antiviral, antiinflammatory, antibacterial, antifungal, antidiabetic, anticoagulant, antifertility, cardiovascular protective, hepatoprotective, and immunostimulant activity in animals (Bengmark et al., 2009; Martin et al., 2011; Singh et al., 2011; Behal et al., 2012). Curcuminoids inhibit leukotriene biosynthesis via the lipoxygenase pathway and decrease prostaglandin formation (Bengmark et al., 2009). It is also used in the preparation of oils, ointments, and poultice, in cosmetic product to prepare various natural herbal creams, lotion, and hair dye, etc. (Negi et al., 1999). TO of curcuma exerts triglyceride-lowering activity on serum as well as liver triglycerides (Yasni et al., 1994). A study revealed the concentration-dependent antiangiogenic effect of CL tea powder by th decreasing the vascularization of the chorioallantoic membrane of 10-day-old duck embryos (Maniago et al., 2014).

Since time immemorial in India, turmeric was used commercially to dye cloth yellow. Turmeric is still used as a common food additive to reach the desired yellow tinge in many fine dishes. In various countries, the food industry uses curcumin as a coloring agent in cheese, spices, mustard, cereals, pickles, potato flakes, soups, ice cream, and yogurt (Shishodia et al., 2007).

USAGE AND APPLICATIONS IN FOOD SCIENCE

EOs play an important roles in industries such as the food and beverage industries due to their uniform quality and appearance as pleasant color. In the United States, turmeric is considered as a spice by the food industry, whereas the Food and Drug Administration classified it as a food colorant. The flavor and fragrance industries have grown due to increased demand for ethnic foods, innovation in beverage products, growth in processed food consumption, and increasing demand for EO extracted from natural sources like TO and therefore the demand of TO is gaining momentum in various industrial sectors apart from crude turmeric powder. TO is yellow to light brown viscous liquid which is extensively used as a flavor ingredient in major food products including alcoholic and nonalcoholic beverages like in preparations of soda, pop, coke, fizzy drink, tonic, seltzers, mineral, or carbonated beverage in Western countries because the major compounds found in TO are sesquiterpene, ketones, β-, and ar-turmerone which are highly beneficial for human consumption. In India

and other South Asian cuisines, as well as medicinal uses and use in the textile industries as a dye, CL is widely used as a gold-colored spice that imparts flavor and color to the food and other food beverages due to presence of nonhazardous curcumin which was first isolated almost two centuries ago and its structure was determined in 1910 (Aggarwal et al., 2007). CL is a free radical scavenger as well as hydrogen donor and it binds with metals particularly iron and copper and acts as strong antioxidant agent (Hatchera et al., 2012). Several studies in the literature have revealed that the turmeric plant has antioxidant properties by both direct oxygen radical quenching and by induction of antioxidant responses via nuclear factor (erythroid-derived 2)-like 2 activation. The antioxidative activity in various models of antioxidant such as 2,2-diphenyl-1-picrylhydrazyl scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, ferric ions reducing power, and ferrous ions chelating was estimated (Ak and Gulcin, 2008). The ability of CL and TO to cross the blood–brain barrier may afford protection against neurodegenerative diseases (Safdar, 2011) and also improve overall function of β-cells, with minimum adverse effects (Chuengsamarn et al., 2012). With the several beneficial activities of turmeric plant, the demand in food preparations has increased. CL and its various preparations are used in nutraceuticals compound to develop immunomodulatory and neuroprotective therapies for the treatment of various neurodegenerative disorders as it acts as a potent modulator of the microglial transcriptome and triggers a phenotype with antiinflammatory and neuroprotective properties (Karlstetter et al., 2011). Hence, TO is the most important EO for the development of pharmaceuticals, nutraceuticals, or food ingredients with functional properties.

TO in Food Flavoring and Food Preservations

The interest in EOs and their application in food preservation has been amplified by an increasingly averse consumer perception of synthetic preservatives. Furthermore, maintaining sustained nutritional value, texture, and flavor is an important aspect of food preservation because food-borne diseases are a growing public health problem worldwide which can be minimized by effective preservation strategies. CL EO itself contains high percentage of ketonic sesquiterpenes like α- and β-turmerone, ar-turmerone which are responsible for aromatic taste and smell (Golding et al., 1982) and hence TO is extensively used for food flavoring in many continental food preparations. TO is also used for food preservations alone or in combination with other EOs. The use of the chemical pickling method for preserving food in an edible antimicrobial liquid takes several days and this method is carried out by placed the foods in an edible liquid that inhibits or kills bacteria and other microorganisms but due to the health hazards of the chemicals, the EOs with different preservation techniques are better alternatives for food protection. TO inhibits the growth of a variety of bacteria, parasites, and pathogenic fungi. It is often use alone or blended with other oils to make delicious foods. TO acts as a food preservative with its strong antimicrobial and germicidal activities (Apisariyakui et al., 1995; Negi et al., 1999). TO is helpful in preserving the raw foods like cucumbers, peppers, corned beef, etc. Furthermore, the oil of CL acts as a good masking agent for unpleasant tastes and smells in the food products like meat and other meat products. TO also helps in the inhibition of oxidative deterioration in the meat products due to its antioxidant activity because the oil contains turmerones that exhibit a potent antioxidant activity in various model systems. Fresh CL powder is used as seasoning to improve the flavor of foods like dressings, soups, sauces, etc.

TO in Bakery Products

The fragrance in baked products is made richer and smoother with the addition of freshly extracted TO in liquid form. Flavor imparts a uniqueness to the bake products when it is added alone or in combination with other oils. There are a number of EOs available to add flavor to baked products in which TO plays a vital role. The EO of TO is used as a fundamental ingredient in baking and also is used to add moisture and texture to backed products like cakes, cookies, puddings, etc. Antimicrobial and antifungal compounds for food preservation are receiving increased attention due to consumer awareness about natural food products and a growing concern of microbial resistance toward conventional preservatives. Therefore, TO is used in some baked products like cakes and creamy products because TO inhibits oxidation of such preparations which become rancid after prolonged storage owing to the oxidation of polyunsaturated fatty acids because of its presence of natural antioxidants, antifungal, and antimicrobial activities. TO itself increases shelf life of bakery products that are kept for long storage. Baked products like biscuits are the most consumed food product, contributing over 60–70% of the total production in the baking industry and EOs play a major role. Nevertheless, TO is used as one of the important ingredients used as an EO to enhance flavor and stability on the storage of creams that are used in biscuit preparations and on sheets to form sandwiches. The manufacture of prepacked sandwiches is now a large industry, consuming large quantities of bread that can be stored with the help of TO. The antifungal activity of TO (Cho et al., 2006) helps to minimize fungal growth of baked products over longer storage like cream rolls, fruit cakes, vegetable bread rolls, etc. Baked products including cookies, *namkeen*, dry cake, etc. are stored with the help of vapor of TO alone or in combination with other EOs.

TO in Seltzers, Mineral, or Carbonated Beverages

TO also plays a vital role in preserving seltzers products like soda water and carbonated water. These sparkling waters with natural flavor contain EOs for calorie-free refreshment. TO is added to mineral water in dropwise to enhance the flavor and increase storage of the drinks with its self-fungicidal, germicidal activities. TO is preferred because of its sweet pleasant taste and it also acts as an effective free radical scavenging agent. One of the benefits of adding therapeutic grade TO to water is to eliminate toxins, petroleum residues, metals, and other harmful substances without any side effects. TO also helps to energize, uplift the mind and spirits, and boost health. TO is also added to soda water to increase metabolism and soothe digestion. Hence, TO is used in many nutraceutical preparations.

TO in Other Food Products

Other products like chewing gums, all kinds of mouth refreshing confectioneries, and all kinds of mints and some other fruity oils are greatly important to consumers of EOs especially all kinds of mints and some other fruity oils. These confectioneries generally contain a sweetening component, food grade acid composition, cooling agents, and flavoring agents, especially EO of herbal plants. Chewing gum is a functional food, having a significant impact on oral hygiene over the short term. TO is used in chewing gum and bubble gum to enhance gum health and as a tooth whitener with its germicidal and antibacterial activity. Not only does it impart aromatic flavors due to the presence of sesquiterpenes, it also reduces gum disease. Products containing TO mask foul mouth odors and and help kill bacteria and other germs, as well as providing a sensation of mouth moistening and freshness upon consumption without side effects.

TO in Alcoholic and Nonalcoholic Beverages

The demand for alcoholic beverages products like beer, wines, brandy, whiskies, rum, gin, etc. is increased in the global market. India has one of the largest markets for alcoholic beverages in the world. During the composition of a new alcoholic spirit EOs must be added to alcohol dropwise, dissolved, and only then can juices, syrup, and water be introduced, as TO is added to gin, wines as a primary or supplemental flavor. This EO is used for enhancing flavor in alcoholic preparations for its aromatic odor. TO is also used in nonalcoholic beverages like fruit and vegetable juices, syrup, and tonic preparations as good hygienic chemical substituent. The development of the soft drink industry in India is of great importance because it is a major consumer of EOs like TO. The EO of CL is used to prepare soft drinks and help in preservation without spoiling the appearance of the beverage or causing a peculiar taste to products like soda, pop, fizzy drink, etc. and to make right the metabolic functions such as decomposition of food and absorption of nutrients by toning up the liver, stomach, and intestines, helping stronger and healthier growth. In some of the liquid nutraceuticals, TO is used for proper excretion, to regulate endocrine secretion of hormones and enzymes, and also to tone up the nervous system by boosting immunity to protect from infections and other critical diseases.

India, the most important manufacturers of TO, ensures the best quality of turmeric and TO are exported to different countries in the world and fulfils the demand, especially for preparation of foods and beverages.

SUMMARY POINTS

- The turmeric plant is native to south India and is widely cultivated in different parts of world.
- Turmeric is known as “Kitchen Queen” and an alternate to saffron.
- Steam distillation is the preferred process for the extraction of TO from the dried rhizomes of CL from the consumer point of view.
- Turmeric is made use of in aromatherapy, perfumery, dyes, and cosmetics production.
- TO is extensively used as a flavor ingredient in major food products including alcoholic and nonalcoholic beverages.
- TO is used in flavors, food supplements, beverages, candy, baked products, puddings, and other soft drinks to enhance flavor and as well therapeutic benefits.

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Chapter 96

Vetiver Grass (*Vetiveria zizanioides*) Oils

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List of Abbreviations

EO Essential oil
VZ EO *Vetiveria zizanioides* essential oil

INTRODUCTION

Vetiveria zizanioides (vetiver grass) is also known as "*Khus–khus*" in India, "Miracle grass" in Thailand, and "*Xiang-Gen-Cao*" or "*Yan-Lan-Cao*" in China and Taiwan (Wang, 2000). *Vetiveria zizanioides* is an eco-friendly plant, and it is the fastest-growing perennial tussock grass of the Poaceae (Gramineae) family (Figure 1). It can prevent soil erosion and is also helpful in the rehabilitation of metal-polluted soils. This plant is also useful because of its tolerance to high levels of heavy metals (Pripdeevech et al., 2006). In addition, *V. zizanioides* has also been cultivated for many industrial applications, including the production of a commercially and medically valued essential oil called vetiver oil, which can be distilled from the roots of *V. zizanioides*. *Vetiveria zizanioides* essential oil (VZ EO) has potent antioxidant, anti-inflammatory, antimicrobial, and antifungal activities (Chou et al., 2012; Kim et al., 2005; Luqman et al., 2005). For that reason, VZ EO has commonly been used as a functional ingredient and fragrance in foods, aromatic products, and cosmetics.

BOTANICAL ASPECTS

Plant Profile

Kingdom: Plantae, plants
Subkingdom: Tracheobionta, vascular plants
Superdivision: Spermatophyta, seed plants
Division: Magnoliophyta, flowering plants
Class: Liliopsida, monocotyledons
Subclass: Commelinidae
Order: Cyperales
Family: Poaceae (Gramineae), grass
Genus: *Vetiveria* Bory, vetiver grass
Species: *Vetiveria zizanioides* (L.) Nash, vetiver grass

Botany

Vetiveria zizanioides is an evergreen, gramineous, and perennial herb, and its appearance is similar to that of lemongrass (Figure 1). Under normal circumstances, the height of vetiver can reach up to 1 m or more. During its flowering stage, it can grow to approximately 1.5–2 m, and it can be increased to 3 m under some conditions. In addition, *V. zizanioides* stems in large clumps from a frequently branching root stock and grows in a cluster type; the leaves form thin strip shapes, and the leaf sheaths are entwined with one another (Bhushan et al., 2013; Wu and Lian, 2002).

* Ying Shih and Chih-Chien Lin are the lead authors of this chapter.

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FIGURE 1 Image of *Vetiveria zizanioides*. The image was provided by International Association of Aromatherapy (Taipei, Taiwan).

Habitats and Range

Most botanists believe that *V. zizanioides* is native to the tropical regions of northern India. According to its ecological type, it can be divided into wild type and cultivated types. The wild type vetiver grass is also known as north India type, which can produce seeds and has high potential to become a weed. Therefore, it is not suitable for cultivation. However, since domestication and breeding, the current cultivated vetiver grass type will bloom, but it does not produce a functional seed. This type is mainly distributed in the regions of southern India, and it is also known as the south India type. South India vetiver grass only propagates through ramets; therefore, this type of vetiver grass may not engage in uncontrollable propagation and can be used as a harmless and functional cultivated plant (Wang, 2000; Wu and Lian, 2002).

Propagation

In most areas, vetiver (south India type) will not bloom, and even the flowers produce sterile seeds. Moreover, because vetiver grass does not send its lateral stems underground and its stolons into the ground, it must use the ramet method to propagate. This also explains why vetiver grass does not spread and becomes a weed. *Vetiveria zizanioides* has strong growing potential, and the ramet roots can grow 60 cm deep within 10 weeks after planting. Moreover, this plant grows fast, and it can grow into a grass hedge within 3–4 months (Wu and Lian, 2002).

USAGE AND APPLICATIONS

Vetiver grass has broad applications because of its special physiological and ecological features. It has a wide range of habitats with a variety of environmental and soil conditions. Vetiver grass is currently used in more than 120 countries. In India, people traditionally use vetiver grass as a boundary fence. In Thailand, people use vetiver grass to build traditional houses. In Australia and some other countries, people use vetiver grass for land protection, including the prevention of soil erosion and the treatment of polluted soil. In mainland China, people extract aromatic oils (essential oils) from vetiver roots. In addition, the cultivation histories of vetiver are very long in many countries, such as Taiwan, Indonesia, Fiji, Sri Lanka, Burma (Myanmar), and other countries in West Africa (Wang, 2000; Wu and Lian, 2002).

Several of the most important vetiver grass applications consist of soil and water conservation systems in agricultural environments; mountain slope stabilization; the rehabilitation of mines, contaminated soil, and saline land; and wastewater treatment (Mickovski et al., 2005). *Vetiveria zizanioides* has been shown to tolerate high concentrations of a range of heavy metals with no effects on growth and development. Previous studies have also demonstrated *V. zizanioides* has the ability to accumulate up to 19,800 ± 2400 and 3350 ± 66 mg Pb/kg dry weight in root and shoot tissues, respectively. These results also report no phytotoxic indications, such as growth retardation and chlorosis under hydroponic conditions (Andra et al., 2009). Moreover, another study demonstrated that *V. zizanioides* can be considered an excellent accumulator for only Pb and Zn. The phytoextraction experiments showed a poor efficiency for vetiver in Cr and Cu uptake, as less than 0.1% of these metals was taken up in the shoots and roots after 30 days. In contrast, vetiver has a relatively high ability to take up Pb and Zn, with 0.4% in shoots and 1% in roots for Pb and 1% in both the shoots and roots for Zn after 30 days (Antiochia et al., 2007). Therefore, *V. zizanioides* is recognized as a suitable plant for solving many environmental problems.

Vetiver has added commercial value because its roots yield aromatic compounds that are applied to food and cosmetic uses. VZ EO has traditionally been used in the treatment of many diseases, such as mouth ulcers, headaches, inflammation, fever, and gastritis (Luqman et al., 2009). Therefore, the use of VZ EO is one of the most important subjects related to vetiver grass. Moreover, the applications of vetiver grass to food science frequently involves the use of VZ EO.

USAGE AND APPLICATIONS IN FOOD SCIENCE

In the European Union, the most extensive use of EOs is in food (as preservatives and flavorings), cosmetics (for fragrances and aftershaves), and pharmaceuticals (functional activities). Moreover, individual compounds in EOs are also used as food additives, whether extracted from the plant resource or synthesized (Burt, 2004). Hence, EOs are one of the most vital functional food additives with natural properties in food science.

Although VZ EO is not a commonly used essential oil in foods, the application potential of VZ EO has been recognized in many studies on food flavors, food preservation, and food additives. This attention is related to the fresh and elegant scent of VZ EO and its potent antioxidant, anti-inflammatory, antimicrobial, and antifungal activities. VZ EO has been documented as a nationally permissible natural food additive in China (China Number System for food additives, N102). In addition, VZ EO is an edible oil that fetches a high price in the traditional markets of China (Wang et al., 2009). Therefore, VZ EO has already been recognized by the Chinese government as a safe and valuable food additive. Moreover, VZ EO has also been used in India in many ways as a food additive, including as a flavoring for syrups, ice cream, and beverages and for food preservation (Lavania, 2003). Therefore, the prospective uses and applications of VZ EO in food science are fairly extensive.

Safety

Food safety is the most important concern in relation to any other issue. Thus, the safety of food additives is always one of the most important factors to evaluate during their development. VZ EO has been used as an edible oil in the daily lives of people in many countries, including China and India. When people use diluted VZ EO, it rarely causes problems. This low risk might be related to the fact that VZ EO is a safe essential oil for foods and cosmetics. In addition, Chou et al. tested the cytotoxicity of VZ EO on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. The results indicated that all test concentrations (5–12.5 μg/mL) of VZ EO did not induced cytotoxicity in LPS-stimulated RAW 264.7 macrophages. Moreover, all tested VZ EO concentrations were able to slightly enhance the cell growth of RAW 264.7 macrophages, likely as a result of its antioxidant activity (Chou et al., 2012). The results suggest that a proper concentration of VZ EO causes no cytotoxicity and exerts protective effects in the cells. Taking these results together, we can suppose that VZ EO is a safe and functional edible oil for use in foods.

Chemical Composition

The chemical composition of VZ EO was previously evaluated in many studies. Chou et al. found 25 components in VZ EO using gas chromatography–mass spectrometry, and the major compounds were cedr-8-en-13-ol (12.4%), α-amorphene (7.80%), β-vatirenene (β-vetivenene, 5.94%), and α-gurjunene (5.91%) (Chou et al., 2012). These results are shown in Table 1 and the chemical structures are shown in Figure 2. In addition, other groups reported that khusimol, α-vetivone, and β-vetivone are also major constituents of VZ EO; the chemical structures of these compounds are shown in Figure 3 (Kim et al., 2005; Pripdeevech et al., 2006). Pripdeevech's study also revealed that different cultivation systems (normal soil or soil with added microbes or semihydroponic conditions) may produce different volatile constituents in *V. zizanioides*. Consequently, the different constituents of VZ EO might be related to the diversity of planting sites or different essential oil extraction procedures. Moreover, different cultivation conditions also influence the resulting VZ EO.

Among these major components, β-vatirenene, α-vetivone, and β-vetivone exhibit antioxidant activities by scavenging 2,2-diphenyl-1-picrylhydrazyl free radicals and chelating ferrous ions (Kim et al., 2005). In addition, Chou et al. demonstrated that VZ EO can attenuate LPS-induced inflammation responses in RAW 264.7 macrophages, including NO production and cell apoptosis, which is controlled through the regulated expression of the inflammation-related enzymes heme oxygenase-1, inducible nitric oxide synthase, cyclooxygenase-2 (the inducible cyclooxygenase), the inflammatory cytokine tumor necrosis factor-α, interleukin-1β, and interferon-β. Chou's group also supposed that the anti-inflammatory activity of VZ EO was correlated with its antioxidant activity (Chou et al., 2012). Therefore, these results indicate that VZ EO is a potential alternative natural food additive with potent antioxidant and anti-inflammatory activities.

TABLE 1 Components of *Vetiveria zizanioides* Essential Oil

Compounds	KI	%
2,3,5,5,8,8-Hexamethyl-cycloocta-1,3,6-triene	1314	2.28
1,5,9,9-Tetramethyl-2-methylene-spiro[3.5]non-5-ene	1328	3.96
(+)-Sativen	1339	2.83
4,8,8-Trimethyl-2-methylene-4-vinylbicyclo[5.2.0]nonane	1407	4.53
α-Amorphene	1440	7.80
2-Isopropenyl-1,3,5-trimethylbenzene	1465	2.43
α-Gurjunene	1479	5.91
β-Vatirenene	1489	5.94
δ-Cadinene	1499	2.57
β-Guaiene	1523	4.28
Dehydro-aromadendrene	1545	5.45
Cubenol	1580	2.09
(+)-Ledene	1605	4.77
Epiglobulol	1632	2.21
Widdrol	1651	2.13
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro- naphthalen-2-ol	1690	1.97
3-(2-Isopropyl-5-methylphenyl)-2-methylpropionic acid	1745	3.17
Cedr-8-en-13-ol	1769	12.36
Ethyl 4-(4-methylphenyl)-4-pentenoate	1804	2.12
Isovellerdiol	1842	2.38
α-Curcumene	1867	2.44
3,3,8,8-Tetramethyl-tricyclo[5.1.0.0(2,4)]oct-5-ene-5- propanoic acid	1890	4.82
Solavetivone	1906	4.20
3,8-Dimethyl-4-(1-methylethylidene)-2,4,6,7,8,8a-hexahydro- 5(1H)-azulenone	1925	4.89
(−)-Spathulenol	1938	2.47

KI: Kovats Index to a series of n-alkanes (C5–C26).
Note: This table is taken from Chou et al. (2012).

Usage and Applications in Food Flavor

Vetiver grass is a scented herb, and its flavor is similar to that of lemongrass and citronella because of its botanical relationships. The odor of VZ EO is a fine and elegant scent, with a slight smokiness but still a fresh and soothing smell. The scent of VZ EO is mainly attributable to its volatile aromatic compounds. Therefore, the special scent of VZ EO is an important property for its uses in foods and cosmetics. Moreover, VZ EO merges very well with many frequently used EOs, including the EOs of cedarwood, chamomile, ginger, jasmine, juniper, lavender, lemongrass, patchouli, rose, sandalwood, spikenard, and vanilla. Therefore, VZ EO is easy to apply in many fields, including food flavoring.

In India, people use diluted VZ EO as a food flavoring for ice cream and in the manufacture of fresh and grass-scented ice products. In addition, this taste is also suitable for beverage products. Drinks and flavored syrups containing diluted VZ EO are made and sold in the Indian markets (Lavania, 2003). Although these applications to food flavorings are not the major applications of VZ EO, the potential for using VZ EO in food flavoring is still recognized. Because VZ EO can combine very well with many other EOs, it can be used to produce a large variety of ingredients for food additives.

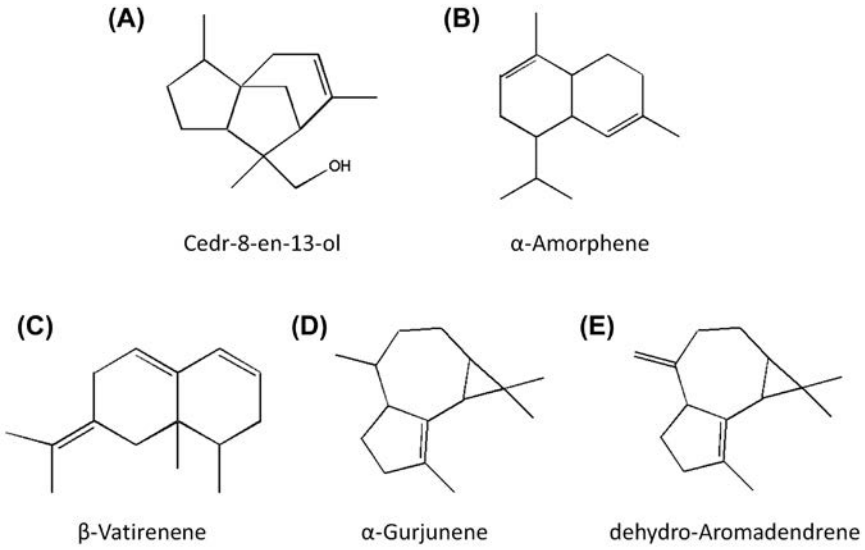

FIGURE 2 Chemical structures of the major compounds in *Vetiveria zizanioides* essential oil identified in Chou's study. (A) Cedr-8-en-13-ol, (B) α-Amorphene, (C) β-Vatirenene (β-Vetivenene), (D) α-Gurjunene, and (E) Dehydro-aromadendrene.

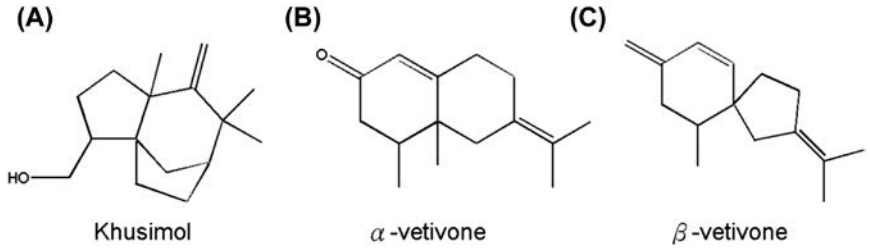

FIGURE 3 Chemical structures of the major compounds of *Vetiveria zizanioides* essential oil identified in other studies. (A) Khusimol, (B) α-Vetivone and (C) β-Vetivone.

Moreover, VZ EO can also be used in other foods that require a fresh scent, such as chewing gum, cool candy, and throat lozenges. Therefore, VZ EO is a good food additive for flavors.

Usage and Applications in Food Preservation

EO applications have a wide variety of purposes. Specifically, the antimicrobial and antifungal activities of EOs constitute an important basis for various applications, including raw and processed food preservation.

The antimicrobial and antifungal activities of VZ EO have been investigated by several groups. Hammer's group evaluated the antimicrobial activities of 52 EOs, including VZ EO extracted from leaves, against *Acinetobacter baumannii*, *Aeromonas veronii* biogroup *sobria*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica* serotype *typhimurium*, *Serratia marcescens*, and *Staphylococcus aureus* using an agar dilution method. The results indicated that VZ EO has the ability to inhibit the growth of *C. albicans*, *E. coli*, and *S. aureus*. In addition, when compared with 52 other EOs, VZ EO had the lowest minimum inhibitory concentration (MIC), 0.008% (v/v) for *S. aureus* (Hammer et al., 1999). In a study by Luqman's group, two genotypes of VZ EO (*gulabi* and KS-1) were used to evaluate antibacterial activity against wild-type and drug-resistant strains of *Mycobacterium smegmatis* and *E. coli* using disk diffusion and microbroth dilution methods. The results showed that two types of VZ EO have antimicrobial activities against *M. smegmatis* and *E. coli*. The MIC of the intact and spent root extracts of both varieties against *M. smegmatis* ranged from 62.5 to 250 μg/mL, while that against *E. coli* ranged from 0.5 μg/mL to 60 μg/mL. Moreover, VZ EO from the genotype *gulabi* has better inhibitory activity against drug-resistant strains of *M. smegmatis* and *E. coli* than KS-1 (Luqman et al., 2005). Another study, from Nantachit's group, demonstrated that alkaloid compounds from VZ EO showed antifungal activity against *Trichophyton mentagrophytes*. The most active compound, vetiverin, was an alkaloid with an MIC of 1628 μg/mL against *T. mentagrophytes* (Nantachit et al., 2010). Taking these

results together, we can suppose that VZ EO has a potent ability to inhibit the growth of various microorganisms. Therefore, the effective antimicrobial and antifungal activities of VZ EO indicate that it has great potential for food preservation applications.

Conclusions

In conclusion, VZ EO is a commercially and medically valuable essential oil. Although VZ EO is not a commonly used essential oil in foods, it has potential applications in food flavoring, food preservation, and food additives. VZ EO has a fresh and elegant scent as well as potent antioxidant, anti-inflammatory, antimicrobial, and antifungal activities. In addition, VZ EO is a safe and valuable food additive for flavoring syrups, ice cream, and beverages and for food preservation. Therefore, the prospective uses and applications of VZ EO in food science are fairly extensive.

SUMMARY POINTS

- VZ EO is a commercially and medically valuable essential oil.
- VZ EO has frequently been used as an active ingredient and fragrance in foods, aromatic products, and cosmetics.
- VZ EO has potent antioxidant activity.
- VZ EO has potent anti-inflammatory activity.
- VZ EO has potent antimicrobial and antifungal activities.
- VZ EO is a potential food flavor and preservative.

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Chapter 97

Wormwood (*Artemisia absinthium* L.) Oils

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INTRODUCTION

Wormwood, a perennial herb widely distributed in the Northern Hemisphere, is a well-known aromatic and medical plant. A long list of various biological properties, such as hepatoprotective, digestive, diuretic, tonic, antiseptic, antiparasitic, antipyretic, antimicrobial, antioxidant activities, etc., has been reported for wormwood extracts and essential oils (EOs). Numerous investigations are focused on the composition of wormwood EOs. The most important component of the oils is thujone, however, (Z)-epoxyocimene, sabinyl, bornyl or chrysanthenyl acetate, 1,8-cineole, chrysanthenol, caryophyllene, chamazulene, and some monoterpenes have been found to be among other principal constituents. Compositional variability of the EOs influences organoleptic properties, physiological properties, and the range of oil applications. Due to characteristic pesticidal and herbicidal nature, wormwood could be used against many pests. In addition, wormwood EOs display potential antifungal and antimicrobial activity, inhibiting the growth of many food-borne pathogens. Furthermore, the antioxidant and radical scavenging activities of EOs of *Artemisia absinthium* are well documented. The oils are very active against many parasites and worms, including those causing damage for domestic animals.

Wormwood is not only well known as a medical plant worldwide; mostly it finds its niche as a spicy and flavoring herb. Aerial parts of wormwood have been used as a culinary herb due to its specific odor and bitter taste. EOs of *A. absinthium* are largely used in the alcoholic drinks industry. The oil is applied in various liqueur and aperitif formulations as it is an important ingredient for flavoring some beverages and wines, but the plant is mostly known as a raw material used in the manufacture of the bitter spirit, absinthe.

BOTANICAL ASPECTS

Artemisia absinthium L. (wormwood) is a species of the genus *Artemisia*, one of the largest and most widely distributed (containing up to 500 species) from the family Asteraceae (synonym: Compositae), tribe Anthemideae.

The origin of the word Artemisia is most probably from the Greek goddess Artemis, while absinthium is from the ancient Greek (or borrowed from ancient Persian language) word ἀψίνθιον (absinthion).

Wormwood is also known by common names such as in Engl.: green ginger, grand wormwood, American or Western wormwood, Madder wort, wormwood sage; in French: *Absinth (e)*, *Armoise Amère*, *Herbe aux Vers*, *Herbe d'Absinthe*, *Herbe Sainte*, in German: *Wermut*, *Wermutkraut*, *Bitterer Beifuβ.*

The plant is herbaceous and perennial with a strong sage odor. The stems are straight and firm, woody at the base, growing up to 0.5–1.1 m (sometimes to 1.5 m in height or even larger), branched and leafy. The leaves are silvery-green, long (the basal leaves up to 20 cm in length and 3–7 cm in broad), spirally arranged, divided two or three times into deeply lobed leaflets, bearing EO-producing glands. Leaves and stems of the plant are covered with fine silky hairs that give a grayish tint. Flowering tops are branched with numerous light yellow flowers. The small flowers are of spherical form. The wormwood rhizomes are fibrous, taproot is short, twiggy, with many shoots and covered by silky hairs as overground parts of the plant.

The flowering period is from July to September or even October. The above-ground parts die at the end of the growing season. The ripe fruit of wormwood, being a small achene, is not crowned by a tuft of hairs or pappus, so is dispersed by gravity. The seeds are long (up to 1 mm), cylindrical, flat, and gray-brown in color. One plant can produce up to 100,000 of seeds.

The aerial parts of wormwood have a strong characteristic odor, while the root has a warm and aromatic taste.

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The plant is easily propagated by division or cutting of roots, or by ripped seeds in the autumn. Wormwood is native to temperate regions, its habitats ranging from Europe, Scotland, Scandinavia, to the Mediterranean sea, to far south up to north Africa, in the East up to Siberia and northern India. The plant has been naturalized in North and South America, North and West Asia, New Zealand, the Azores etc. *Artemisia absinthium* naturally grows in uncultivated fields and roadsides, on rocky slopes, preferring arid, non-acidic ground, loam or sandy loam, rich in nitrogen, phosphorus and potassium, often forms colonies.

Cultivated plantations of wormwood can be exploited up to 10 years, and raw material can be harvested twice a year. Production of wormwood EOs is available from the steam distillation (with yields around 0.4%) mainly from Morocco, China, India, and Bulgaria (usually less than 20 tons annually).

USAGE AND APPLICATIONS

Wormwood is significant in medicine, cosmetics (its EO is used as a fragrance component in soaps, cosmetics and perfumes, but not in aromatherapy), and the food industry.

Artemisia absinthium, due to the most important ingredients such as EOs, tannins, polyphenols, flavonoids, lactones, and other bitter substances have been known as medicinal plants since antiquity. Wormwood's application for healing is mentioned in written form (probably for the first time) in the ancient Egyptian medicinal document Ebers papyrus, dating to around 1550 BC. Data of the plant's use as a remedy was reported by a famous Persian scientist, physician, and philosopher Avicenna (Ibn Sina) as well as in many ancient European medicinal books. The *A. absinthium* plant has been widely used for medicinal purposes mainly due to their hepatoprotective, cholagogue, deodorant, digestive, emenagogue, cardiac stimulant, antiseptic, anthelmintic, antiparasitic, diuretic, antipyretic, tonic, antibacterial, antimicrobial, cytostatic, antitumor, antimalarial, etc. properties and antioxidant, antidepressant, and chemotherapeutic activities as well. Wormwood preparations are used to treat anorexia, insomnia, anemia, headache, sclerosis, indigestion, ache, fatigue, to calm down epileptic and hysteric attacks, and to heal wounds and insect bites. Wormwood (on its own or in combination with other medicinal herbs) preparations are mostly applied as a remedy for various digestive problems, and as an anthelmintic cure.

Ancient Egyptians and American Indians used wormwood herbs for their religious and magic ceremonies. The plant is used as an ingredient to the incenses. However, preparations of the plant help to withstand cold and other hardships of highlands to the people of Himalayan region. The plant is ornamental and dried wormwood herb material can be used in floristic compositions.

USAGE AND APPLICATIONS OF WORMWOOD IN FOOD SCIENCE

Wormwood EOs, obtained from aerial parts of the plant, have a dark green to orange or dark brown color (sensitive to ultraviolet/visible light), with strong odor. The most characteristic and important (in quantity) component in wormwood EOs is thujone (especially the *cis*-isomer). Among other principal constituents were determined such compounds as (Z)-epoxyocimene, sabinyl, bornyl or chrysanthenyl acetate, 1,8-cineole, chrysanthenol, caryophyllene, chamazulene, some monoterpenes, etc. It should be mentioned that EOs from *A. absinthium* roots (normal and transformed (hairy)) have a low quantity of thujone type monoterpenoids. For example, the root oil (yield 0.2%) of Serbian origin was characterized by α-fenchene (23.3%) and some esters, such as linalyl 3-methyl butanoate (21.1%), linalyl butanoate (14.4%), linalyl propanoate (8.2%), and linalyl acetate (7.7%) (Blagojević et al., 2006). Compositional variability of the EOs influences properties of oil and possibilities of the application. Compositional changes depend on the origin, soil characteristics, seasonal variations, kind of raw material (leaves or flowering tops, fresh or dried), extraction methods, storage conditions, etc. (see Tables 1–3 indicating the most predominant constituents in wormwood oils from various countries).

Wormwood aerial parts are characteristic of their pesticidal and herbicidal nature, and are used successfully to protect seeds, fields, stored vegetables, fruits, and crops. Various organic or aqueous extracts and EOs of the plant exhibit antifeedant and toxic effects on pests, which cause a huge damage direct to the plants and reduce yields of the agricultural products. It has been reported, that wormwood extracts or EOs are active against the Colorado potato beetle (*Leptinotarsa decemlineata*) (Ertürk and Uslu, 2007), the granary weevil (*Sitophilus granarius*) (Kordali et al., 2006) (Lawrence, 2003 and references therein cited), and some moths (the African cotton leafworm (*Spodoptera littoralis*)) (Bailen et al., 2013). The oils are very effective against some aphids: the peach potato (*Myzus persicae*) (Bailen et al., 2013), the bird cherry-oat (*Rhopalosiphum padi*) (Bailen et al., 2013), and the corn leaf (*Rhopalosiphum maidis*) (Halbert et al., 2009). Also, wormwood repels some ticks (*Ixodes ricinus*) (Jaenson et al., 2005), two spotted spider mites, house flies, fleas, ants, caterpillars, insect larvae, and mosquitoes. The EOs obtained from wormwood plants grown in Poland are strongly toxic to the lesser grain borer (*Rhyzopertha dominica*) and mildly toxic to the darkling grain beetle (*Trobolium confusum*) (Lawrence, 2003 and references therein cited).

TABLE 1 Three Main Constituents (at Least over 5%) of Wormwood Essential Oils from Western European Countries (Listed in Alphabetic Order) Published since 2000

Plant Material	EO Yield (%)	Main Components (%)	Origin	References
Commercial samples from retail pharmacy, aerial parts(*)	0.7	Sabinyl acetate (18.6), sabinene (9.3), myrcene (5.4)	Belgium	Orav et al. (2006)
Different parts, during various growing stages: vegetative, before anthesis, full flowering, and seeding(**)	Steam distillation 2 h, 0.4–1.5	β-Thujone (14.0–48.6), (Z)-6,7-epoxyocimene (6.1–38.2), sabinyl acetate (≤18.1)	Croatia	Juteau et al. (2003)
**	Steam distillation 2 h, 0.4–1.0	(Z)-6,7-Epoxyocimene (22.6–49.7), *cis*-chrysanthenyl acetate (11.4–36.7), bicyclo [2,2,1]-hept-2-en-7-ol (≤18.9)	France	Juteau et al. (2003)
*	0.7	Linalool + α-thujone (10.3), neryl butanoate (13.9), compounds with curcumene structure (11.3)	France	Orav et al. (2006)
*	0.3	α-Bisabolol (7.5)	Germany	Orav et al. (2006)
*	0.3	β-Thujone (38.7)	Greece	Orav et al. (2006)
Aerial parts at flowering	Hydrodistillation 3 h, 0.3	Caryophyllene oxide (25.3), *p*-cymene (16.8), 1,8-cineole (8.9)	Greece	Basta et al. (2007)
*	0.2	Carvone (18.5), β-thujone (12.3), sabinyl acetate (11.4)	Italy	Orav et al. (2006)
*	0.8	Sabinene (30.1), myrcene (18.0)	Scotland	Orav et al. (2006)
Aerial parts	Hydrodistillation 2.5 h, 0.3	Different chemotypes: β-thujone (≤63.4), *cis*-β-epoxyocimene (10.7), *trans*-sabinyl acetate (8.8), sabinene (10.8), *p*-cymene (6.8)	Serbia	Blagojević et al. (2006)
*	0.1	1,8-Cineole (18.0), linalool + α-thujone (10.9), β-thujone (6.2)	Spain	Orav et al. (2006)
Different aerial parts, seasonal variations	Hydrodistillation 1.5 h	Different chemotypes: *cis*-epoxyocimene (≤70.9), chrysanthenyl acetate (≤58.1)	Spain	Molina et al. (2008)
Plants from different origins cultivated under different conditions, during flowering		Different chemotypes: *cis*-epoxyocimene (≤75.1), (Z)-2,6-dimethylocta-5, 7-dien-2,3-diol (≤54.6), *cis*-linalool oxide (≤54.1), chrysanthenyl acetate (≤32.2), chrysanthenol (≤21.4), bornyl acetate (≤15.1), unknown diterpenes (≤10.6), thymol (≤10.0)	Spain	Bailen et al. (2013)
Leaves and flowers, cultivated in field (from seeds of wild populations) and aeroponically	Supercritical fluid extraction, ≤2.7; hydrodistillation 4 h, 0.5	(Z)-Epoxyocimene (≤39.5), chrysanthenol (≤15.7), chrysanthenyl acetate (≤13.5)	Spain	Martín et al. (2011)

Essential oils (EOs) were obtained from air-dried aerial parts of wormwood by hydrodistillation (duration of the procedure was indicated in some references).
*Commercial samples from retail pharmacy, aerial parts
**Different parts, during various growing stages: vegetative, before anthesis, full flowering and seeding

TABLE 2 Five Main Constituents (or at Least over 5%) of Wormwood Essential Oils from Central and Eastern European Countries

Plant Material	EO Yield, %	Main Components (%)	Origin	References
Commercial samples obtained from retail pharmacy(*)	0.4–1.1	Sabinyl acetate (70.5), β-thujone (≤64.6), epoxyocimenes (59.7), sabinene (≤25.3), myrcene (≤29.9)	Estonia	Orav et al. (2006)
*	0.3	Sabinene (18.1), myrcene (17.7)	Hungary	Orav et al. (2006)
*	0.4	Sabinyl acetate (23.6), *trans*-verbenol (9.2), compounds with curcumene structure (9.0), β-thujone (6.2) linalool + α-thujone (5.8)	Latvia	Orav et al. (2006)
*	0.2	Sabinyl acetate (13.7), *trans*-verbenol (11.7), compounds with curcumene structure (6.3)	Lithuania	Orav et al. (2006)
Leaves and inflorescences, at flowering	Hydrodistillation 2 h, 1.3–3.6	*trans*-Sabinyl acetate (≤55.2), thujones (≤36.3), *trans*-sabinene hydrate (11.0), myrcene (≤9.2), β-pinene (≤10.4), 1,8-cineole (≤7.1), sabinol (6.4)	Lithuania (Vilnius city surroundings)	Judzentiene et al. (2009)
Leaves and flowers, during different phenological stages (first leaves, full flowering and fruiting)	Hydrodistillation 2 h, 1.2–2.9	Different chemotypes: *trans*-sabinyl acetate (≤51.3), thujones (≤71.7), sabinene (≤23.4), epoxyocimenes (≤14.2), *cis*-chrysanthenol (13.6), β-pinene (≤12.9), linalool (≤12.2), *trans*-chrysanthenyl acetate (11.6), *trans*-sabinol (10.3), 1,8-cineole (6.2)	Lithuania (over all the country)	Judzentiene and Budiene (2010); Judzentiene et al. (2012)
*	Hydrodistillation 2 h, 0.2	Myrcene (38.9), sabinene (9.2), sabinyl acetate (5.7)	Moldova	Orav et al. (2006)
At blooming	0.4–0.9	Different chemotypes with predominant compounds: Sabinyl acetate, Chrysanthenyl acetate, sabinene, β-thujone, sabinene, β-myrcene, 1,8-cineole	Poland (central and north-eastern)	Geszprych et al. (2011)
At preflowering, full flowers and after flowering		Sabinene + myrcene (≤21.1), β-thujone (≤16.9), 1,8-cineole (≤12.7), sabinyl acetate (≤20.1)	Poland	Lawrence (2003) and therein cited
*	0.3	Epoxyocimenes (22.1), sabinene (9.3), neryl-3-methyl-butanoate (5.5)	Russia	Orav et al. (2006)
*	0.3	Myrcene (21.5), thujyl alcohol (18.9), sabinene (17.3), α-thujone (7.4), camphor (5.5)	Russia	Lawrence (2006) and therein cited
*	0.4	Curcumene derivative (7.8), neryl-3-methyl-butanoate (7.3), β-thujone (6.3)	Ukraine	Orav et al. (2006)

Essential oils (EOs) were obtained from air-dried aerial parts of wormwood by hydrodistillation (duration of the procedure was indicated in some cases).
*Commercial samples obtained from retail pharmacy

TABLE 3 Main Constituents (over 5%) of Wormwood Essential Oils from Asian, African, and American Countries

Plant Material	EO Yield, %	Main Components (%)	Origin	References
Commercial sample obtained from retail pharmacy	0.1	Sabinyl acetate (34.2), chamazulene (6.6), linalool + α-thujone (6.1)	Armenia	Orav et al. (2006)
	0.5	Myrcene (10.8), β-thujone (10.1), *trans*-sabinyl acetate (26.4)	Canada (Western)	Lopes-Lutz et al. (2008)
		Bornyl acetate (23.0), terpinen-4-ol (8.2), 1,8-cineole (5.1)	Cuba	Lawrence (2003)
Experimentally grown		α-Phellandrene (50.5), terpinen-4-ol (12.0), linalool (10.5)	Egypt	Lawrence (2006)
Fresh leaves	Hydrodistillation 3 h, 1.4	Camphor (27.4), davanone (16.4), ethyl-(E)-cinnamate (5.8)	Ethiopia	Tariku et al. (2011)
		β-Pinene (34.0), *p*-cymene (14.6), myrcene (8.9), β-thujone (6.9)	Iran (Ashkhaneh region, Northern Khorasan province)	Lawrence (2006)
Harvested at full blooming	Hydrodistillation 2.5 h, 1.3	β-Pinene (23.8) β-thujone (18.6), sabinene (8.9)	Iran (Guilan province)	Rezaeinodehi and Khangholi (2008)
At full flowering	Hydrodistillation 3.5 h, 0.9	β-Pinene (25.8), α-phellandrene (25.5), sabinene (14.4), *p*-cymene (7.6), β-thujone (5.1)	Iran (Northern, Gilan Province)	Lawrence (2006) and references therein cited
		β-Thujone (29.8), phellandrene (20.2)	Iran	Gandomi Nasrabadi et al. (2012)
At flowering stage	Hydrodistillation 3 h, 0.5–0.8.	Myrcene (8.6–22.7), *cis*-chrysanthenyl acetate (7.7–17.9), dihydrochamazulene isomer (5.5–11.6), germacrene D (≤8.0), β-thujone (≤7.3), linalool acetate (≤7.0), α-phellandrene (≤5.3), linalool (5.3–7.0)	Tajikistan (Central and Southern, 1200–1300 m altitude)	Sharopov et al. (2012)
	Hydrodistillation 3 h, 0.8	Chamazulene (28.6), spathulenol (7.4), phellandrene (6.1), β-calacorene (5.6), sabinene (5.5)	Turkey (East Anatolian region)	Bagci et al. (2010)
Harvested at flowering	Hydrodistillation 4 h, 0.9	Chamazulene (17.8), nuciferol butanoate (8.2), nuciferol propionate (5.1)	Turkey	Kordali et al. (2005a,b)
At flowering stage	Hydrodistillation 4 h, 1.1	Sabinene (17.6), myrcene (11.0), chrysanthenyl acetate (11.0), myroxide (5.8), *trans*-sabinyl acetate (7.7)	Turkey (Western and Southwestern)	Baykan Erel et al. (2012)
Fresh material	0.3–0.7	Percentage as an average mean: β-thujone (56.3), (Z)-epoxyocimene (14.8)	Uruguay (Sauce-Canelones)	Umpiérrez et al. (2012)
Commercial sample		Average means: β-thujone (33.2), *cis*-sabinyl acetate (32.8)	USA	Lawrence (2003)

Essential oils (EOs) were mostly obtained from air-dried aerial parts of wormwood by hydrodistillation (duration of the procedure was indicated in some cases).

Furthermore, extracts of the plant obtained by organic and supercritical fluid (CO_2) extraction methods showed significant activity over the insect pests *Spodoptera littoralis*, *M. persicae*, and *Rhopalosiphum padi* (Martín et al., 2011, 2013).

Allelopathic properties of wormwood extracts (including and EOs) were elucidated against some herbs (as the wheat caryopses, ryegrass, brome grass, etc.). However, the extracts (obtained by hydrodistillation, extraction by water and organic solvents, and by supercritical CO_2) were phytotoxic to lettuce (*Lactuca sativa*) and English ryegrass (*Lolium perenne*) (Martín et al., 2011). Also, phytotoxic effects of the EOs (major composition is presented in Table 1) were tested also against lettuce and ryegrass by Bailen et al. (2013).

Wormwood EOs have shown high inhibitory effects on bacterial and fungal growth. Oils containing different chemical composition display potential antifungal activity on the growth of agricultural pathogenic fungi (Juteau et al., 2003; Kordali et al., 2005a,b; Lopes-Lutz et al., 2008; Baykan Erel et al., 2012). Antimicrobial activity of the EOs (see Table 3 for its principal composition) was tested against 64 bacterial strains by Kordali et al. (2005b). The results of the antifungal assays of wormwood EO (see Table 1) against *Fusarium oxysporum* and *Fusarium solani* were presented by Bailen et al. (2013). Antimicrobial activity of the oils was tested against *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergilus niger* (Blagojević et al., 2006). Wormwood is very effective against many food-borne pathogens. EO (see Table 3) activity against *Salmonella typhimurium*, *Listeria monocytogenes*, *E. coli* O157: H7, *Bacillus cereus*, and *S. aureus* were evaluated by Gandomi Nasrabadi et al. (2012). Lopes-Lutz et al. (2008) reported, that the EO (the main composition is in Table 3) had inhibitory effects not only on the growth of bacteria (*E. coli*, *S. aureus*, and *Staphylococcus epidermidis*), but also some yeasts (*C. albicans*, *Cryptococcus neoformans*) and dermatophytes (*Trichophyton rubrum*, *Microsporum canis*, and *Microsporum gypseum*), as well as on *Fonsecaea pedrosoi* and *A. niger*. Umpiérrez et al. (2012) concluded that the wormwood EO (see Table 3) exhibited dual activity against some of the main insects and fungi of tomato crops, making it possible to manage both mischiefs with only one kind of treatment. Effective insecticidal properties were elucidated (both by direct contact and by vapor) against two of the major tomato insects: tomato leafminer (*Tuta absoluta*) and whitefly (*Trialeurodes vaporariorum*), while fungicidal activity was observed against the fungi *Alternaria* spp. and *Botrytis cinerea* (Umpiérrez et al., 2012).

Specifically, wormwood oils are very effective against some parasites and worms, which cause serious ailments and diseases not only in humans, but in domestic animals as well. Application of wormwood preparations plays an important role in ethno-veterinary and also is very promising in modern veterinary, in order to reduce damage in stockbreeding domain. Anthelmintic properties against ovine nematodes were evaluated by Tariq et al. (2009), while antiprotozoal and anti-trichinellosis effects by Caner et al. (2008). The oils (see Tables 1 and 3) from the wormwood plants exhibited antiparasitic effects against several *Leishmania* strains (Bailen et al., 2013; Tariku et al., 2011).

In addition to the many activities mentioned above, wormwood possesses antioxidant properties as well. It means that compounds from the plant are able to inhibit oxidation of lipids or other molecules, to adsorb or neutralize free radicals, to decompose peroxides, or to block propagation of oxidizing chain reactions. Application of phytochemical antioxidants in the food industry is of great interest. Antioxidant activity of different wormwood extracts (containing mainly flavonoids, sesquiterpene lactones, polyphenols, terpenes, and other volatile constituents, acids, etc.) is well documented in many research studies. In particular, antioxidant and radical scavenging activities of EOs (see Table 3) from the areal parts of *A. absinthium* were evaluated (Kordali et al., 2005a,b; Lopes-Lutz et al., 2008; Baykan Erel et al., 2012).

Nowadays and notably in the past, aerial parts of *A. absinthium* (wormwood) have been used as a culinary herb (but in very limited amounts) due to it specific odor, bitter taste, preservative, digestive, and tonic properties. Usually, only flowering tops, fresh or dried young shoots or nonwoody parts of the plant are used as a spice in roasted and boiled fatty meats (of goose, duck, mutton, and pork), in vegetable salads, meat and fish stews, some soups, omelettes, and other dishes prepared with eggs. However, wormwood has been applied as a flavoring ingredient in beer production before the common application of hops (*Humulus lupulus*). Some gourmands like cheese use seasoning with a mixture of wormwood, thyme, and rosemary (at ratio 4:2:1). *Artemisia absinthium* is a minor ingredient in many herbal tea mixtures. For example, in Lithuania, small farmers still are often using the herbs of wormwood and tansy (*Tanacetum vulgare*) for preservative purposes (to protect smoked meats from flies blow, other pests, and spoilage) as well as for flavoring (it gives a specific pleasant smell to the meat).

Aerial parts of *A. absinthium* are largely used in industry of alcoholic drinks. Wormwood EO is an important ingredient for flavoring some beverages and wines, such as vermouths, various liqueurs, and aperitifs, but the plant is mostly known as a raw material used in the manufacture of absinthe, a bitter spirit. This alcoholic beverage is obtained by macerating dry wormwood and other traditional spice herbs (mostly aniseed and fennel) in ethyl alcohol, and then distilling the liquor. The drink is usually emerald green in color, turning to cloudy, opalescent white (mostly because of anise) when mixed with water. The green color of the beverage is due to the presence of chlorophyll obtained from plants during the second

maceration. Generally, absinthe contains high level of alcohol (60–80%) and content of EOs is up to 20%. The history of the drink is long and complicated. The bitter spirit originated in the Val de Travers region in Switzerland at the end of eighteenth century, where, in around 1792, physician Pierre Ordinaire patented absinthe as a medical remedy (mainly for digestive disorders). For the first time, in 1797 absinthe was produced commercially. A little later, absinthe was imported from Switzerland to France. The alcoholic drink with the nickname "*La Fée Verte*" (the green fairy) was very popular in late ninteenth and early twentieth centuries (days of the Belle Époque) in France, especially among many Parisian painters and writers. Absinthe was a significant part of bohemian culture. In America, New Orleans was the center of absinthe culture until the ban in the United States. By 1915, absinthe had been banned due to the toxic and hallucinogenic properties (abuse of absinthe and extra severe crimes performed by absinthe drinkers) in most of Europe, including Switzerland, France, the Netherlands, the Austro–Hungarian Empire, and Belgium.

A controversial discussion about the impact of the characteristic wormwood EO constituent thujone on the illness absinthism (including the symptoms of convulsions, hallucinations, and insomnia) appeared in the scientific society. Some research studies even claim that thujone contribution (especially the β isomer) is minor and principally the high content of alcohol in the drink was the cause of the chronic intoxication problems. The beverage itself was acquitted, but maximum of acceptable levels of thujone in final products for consumption were placed under control. Levels of thujone in flavourings are limited by the European Union (European Council Directive 88/388/EEC) and the United Kingdom (SI 1992, No. 1971). The maximum levels of the natural toxin thujone in foodstuffs and beverages are 0.5 mg/kg with some exceptions (35 mg/kg in bitter drinks).

Meanwhile, it should be also mentioned about the toxic effects of other constituents that are present in wormwood EOs, such as artemisia ketone, sabinene, sabinol, sabinyl acetate, 1,8-cienole, etc. Many research studies have confirmed strong toxicity (abortifacient, neurotoxic, a skin irritant, embryotoxic, and antiimplantation effects) of sabinyl acetate to mammalians. Furthermore, wormwood EO containing appreciable amount of *trans*-sabinyl acetate (45.2%) and only a moderate quantity of thujones (12.3%, sum of *cis*- and *trans*-isomers), has showed high toxic activity (Judzentiene et al., 2012).

Many brands (around 200) of absinthe are produced in the USA, Australia, various European countries, such as Switzerland, France, Germany, Spain, Portugal, Belgium, Netherlands, England, Italy, Czech Republic, etc. Two major types could be distinguished according to the color of absinthe "*La Verte*" (green) and "*La Bleue*" (blue), but also are produced absinthe yellow (of mead base) or even red. Absinthe "*Rouge*" (an extremely rare kind) has a cranberry red color because of the hibiscus flowers used for its production. The taste of the flavored drink could vary according to the different medicinal and finest herbs used for its manufacturing. As well as common wormwood (*A.absinthium*), more than 10 different traditional plants, Roman wormwood (*Artemisia pontica*), green anise (*Pimpinella anisum*), star aniseed (*Illicium verum*), lemon balm (*Melissa officinalis*), peppermint (*Mentha×piperita*, also known as *Mentha balsamea* Willd), liquorice (roots of *Glycyrrhiza glabra*), hyssop (*Hyssopus officinalis*), coriander (*Coriandrum sativum*), sweet fennel (*Foeniculum vulgare*), roots of angelica (*Angelica archangelica*), sweet calamus (*Acorus calamus*), etc., are used as ingredients in absinthe beverage.

SUMMARY POINTS

- Chemical variability of wormwood essential oils depending on origin has been shown.
- Wormwood essential oils play a significant role in preservation of agricultural fields, seeds, vegetables, fruits, grains, and other stored products against many pests, fungi, and microbes.
- Anthelmintic, antiprotozoal, antitrichinellosis, leishmanicidal, etc. properties are well documented for the oils.
- Antioxidant and radical scavenging activities of the oils from areal parts of *A. absinthium* were evaluated.
- Wormwood is used for flavoring many alcoholic beverages, different liqueurs, aperitifs, wines, and vermouths.
- The plant is a principal flavoring component in production of bitter spirit, absinthe, whose numerous brands are produced in various countries.

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Chapter 98

Yellow or White Mustard (*Sinapis alba* L.) Oils

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List of Abbreviations

p-HBITC *para*–hydroxybenzyl isothiocyanate
WMEO White mustard essential oil
cfu Colony forming unit

INTRODUCTION

The essential oil precursors of mustard seeds are glucosinolates, and sinalbin is the precursor for the active component, *para*–hydroxybenzyl isothiocyanate (p-HBITC) in white mustard essential oil (WMEO). WMEO is an essential oil with very low volatility. Synthetic p-HBITC has no perceptible odor while WMEO has a mustard smell and a burning taste sensation on the tongue. WMEO has to be extracted using solvents or supercritical carbon dioxide, because of its instability in aqueous environments. The history of mustard goes back many centuries to the time of the Gautama Buddha (~600 BC), when Kisa Gotami, a young mother who had lost her young son, was asked by him to get a handful of mustard seeds from a house where no relative had died, in order to see reality (Rhys Davids and Norman, 1989). Mustard was also used by the Romans in more recent history (Man and Weir, 2010).

WMEO is not commercially available currently and has been investigated for applications in foods in a limited manner. This chapter includes some of its chemical and antimicrobial properties in broth and actual foods.

BOTANICAL ASPECTS

Three commercial mustard species are commonly cultivated today. They are yellow or white mustard (*Sinapis alba* L.), black mustard (*Brassica nigra*), and oriental mustard (*Brassica juncea*), as shown in Figure 1. Mustard was one of the earliest domesticated crops and spread into Asia, North Africa, and Europe over thousands of years. With shipping restrictions imposed on crops during the Second World War, North America became a major grower and exporter. Mustard is a cool season crop, seed maturity is attained in 80–90 days; shorter for the white or yellow mustard and longer for the oriental and black mustard (http://www.hort.purdue.edu/newcrop/afcm/mustard.html). This short cropping cycle and the relatively low level of pest damage, has allowed widespread commercial cultivation of mustard, the pattern of which has changed substantially over the past years. For example, in 2004 Canada produced 286,700 tonnes or 43% of the supply, while in 2011 it produced only 124,800 tonnes. Myanmar and Nepal produced 49,000 and 132,865 tonnes in 2004 and went on to produce 88,520 and 176,186 tonnes, respectively, in 2011 (http://faostat3.fao.org). This may be an indication of the higher use of fixed oil of mustard as a human food in those countries, despite the concerns regarding erucic acid found in mustard oil (Rastogi et al., 2004).

USAGE AND APPLICATIONS

The precursor sinalbin is hydrolyzed to *p*-HBITC, a nonvolatile sharp tasting oily compound, on grinding and moistening white mustard seed. This reaction is catalyzed by the endogenous enzyme myrosinase (E.C. 3.2.3.1 thioglucoside glucohydrolase) and is further activated by low concentrations of ascorbic acid (Björkman, 1976).

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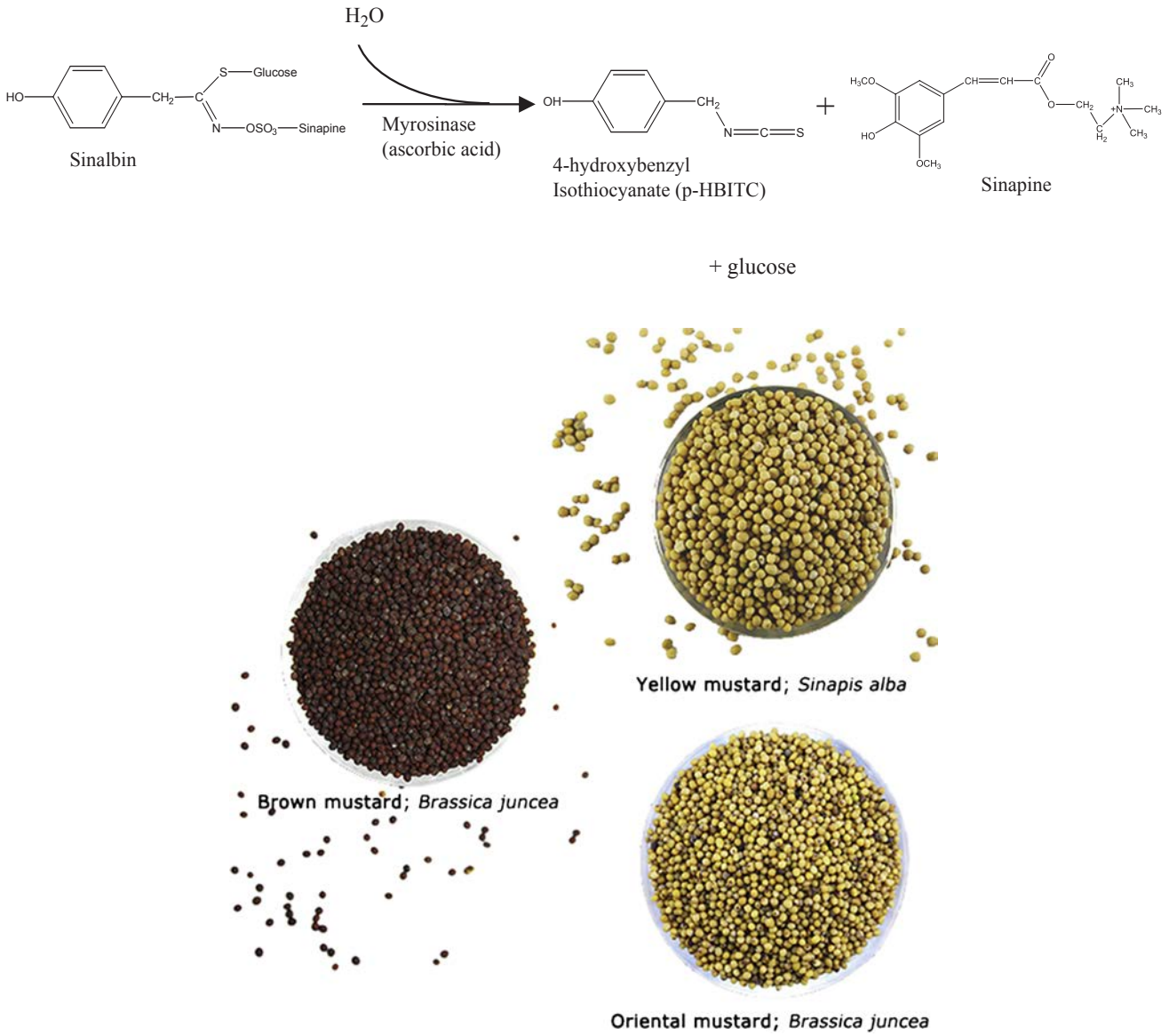

FIGURE 1 Three main types of mustard seed in cultivation today. Yellow or white mustard (*Sinapis alba* L.), black or brown mustard (*Brassica nigra* or *B. juncea*), and oriental mustard (*Brassica juncea*) (http://www.grainscanada.gc.ca/mustard-moutarde/harvest-recolte/2010/hqm10-qrm10-1-eng.htm).

To produce WMEO, mustard seeds are first cold pressed to remove most of the fixed oil and the partially defatted mustard press cake is moistened in the presence of ethyl acetate and ascorbic acid. After the reaction period, the ethyl acetate extract which contains p-HBITC and the fixed oil found in the starting press cake, is removed by centrifugation. Evaporation of ethyl acetate under reduced pressure yields WMEO: a brown-colored oil that has mustard-like smell and a sharp burning taste.

The p-HBITC concentration in WMEO is dependent on the extent of oil pressing from mustard seed. For WMEO to be cost-effective, other uses have to be found for fixed mustard oil and seed proteins which are of high quality (Table 1). Rastogi et al. (2004) showed that erucic acid (C22:1) in mustard oil does not counterbalance the beneficial effects of linolenic acid but in fact it is beneficial in that it shows an inverse risk for ischemic heart disease in Indian populations. Thus, mustard oil can be widely used as a cooking oil.

The chemistry of WMEO has been previously reported (Ekanayake et al., 2012). When cold pressed oil and WMEO were compared by high performance liquid chromatography (HPLC)/high resolution mass spectrometry (MS) the variations of the observed chromatographic peak areas of the triglyceride portions of the oils varied within about 10%. The nonlipid, small molecule components (e.g., sinalbin) could be traced to hydrolytic products originating from components found in *Brassica* species or simple metabolic variants (e.g., *O*-methylation). The hydrolysis of p-HBITC was pH-dependent, being much faster at neutral and alkaline pH values and slower under acidic conditions. The hydrolytic

TABLE 1 Composition of Defatted and Deheated White Mustard Flour

Constituent	% by Weight
Calories	374 kCal/100 g
Moisture	6.4
Fat	4.8
Total carbohydrates (by difference)	40.2
Ash	5.69
Protein	43.3
Amino acid composition of protein	mg/100 g
Aspartic acid	3020
Threonine	1670
Serine	1470
Glutamic acid	6260
Proline	2410
Glycine	2250
Alanine	1760
Valine	2020
Isoleucine	1680
Leucine	2810
Tyrosine	1390
Phenylalanine	1660
Lysine	2270
Histidine	1040
Arginine	2510
Cystine	564
Methionine	638
Tryptophan	536

Gross composition of defatted and deheated mustard meal resulting after cold pressing mustard seed, generating and extracting WMEO, and drying the resulting residue. Amino acid distribution of the mustard protein. From Ekanayake et al. (2012).

products were essentially the same with p-hydroxybenzyl alcohol being the major product. Nonfood applications to do with the "heating" property of mustards have been described by Bisset and Wichtl (2001) including the use of mustard plasters for localized skin heating.

Synthesis of p-HBITC was reported by Ekanayake et al. (2006). p-HBITC was also prepared by purifying from WMEO as described in Ekanayake et al. (2012). The structure of p-HBITC was confirmed by ^{1}H nuclear magnetic resonance (NMR) in deuterated chloroform ($CDCl_3$) and C^{13}-NMR in $CDCl_3$.

Characterization of WMEO

HPLC/MS analysis of WMEO resulted in the chromatogram given in Figure 2. The reaction chemistry of p-HBITC as described by Kawakishi and Muramatsu (1966), Kawakishi et al. (1967) and Soledade et al. (1997) was taken into account in interpreting the identity of the peaks as described in detail in Ekanayake et al. (2012).

Normal phase HPLC analysis of p-HBITC was used to quantify it after the hydrolysis experiments. This method showed that p-HBITC is stable for at least 24 h in dry 20% ethyl acetate; 80% n-hexane enabling HPLC analysis using an autosampler. Recoveries and relative standard deviations were very good and ranged from 84% to 99% and 3% to 6%, respectively.

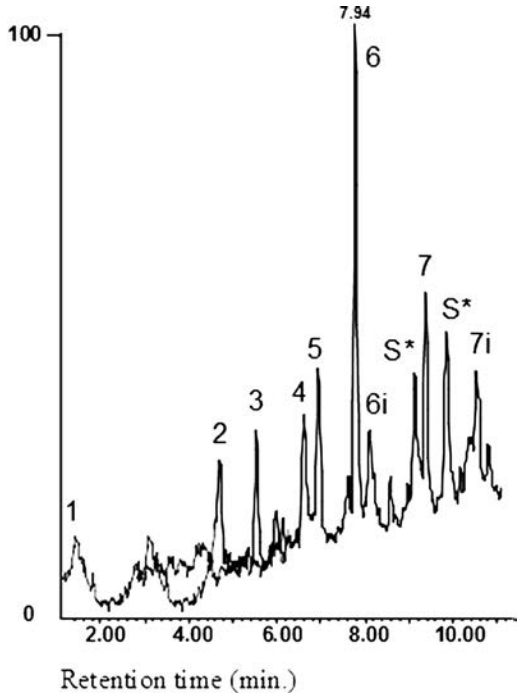

FIGURE 2 HPLC/MS analysis of white mustard essential oil. HPLC/positive ion electrospray MS analysis of WMEO extract, % relative intensity. Peak 4 is *p*-HBITC. Other peaks identified in Ekanayake et al. (2012).

USAGE AND APPLICATIONS IN FOOD SCIENCE

WMEO is a new natural product, previously unused in commercial food products. The work toward developing an economically attractive commercial supply was completed only in 2010–2011 and the active component p-HBITC is still not available commercially in catalogs.

Attempts at pairing a specific food matrix in need of a secondary barrier for food protection with a potential antimicrobial is very rarely a linear or straight forward exercise. In the food industry, several competing factors need to be reviewed and cooptimized to meet predetermined technical success criteria and business decision criteria (David et al., 2013a). The key factors that must be considered include (1) efficacy against target microorganisms in the food matrix during processing, (2) sensory impact, (3) business case and justification, (4) cost-in-use, (5) storage, (6) end use by consumers, (7) toxicology, regulatory, and labeling considerations, and (8) sustainable supply.

In utilizing essential oils as antimicrobials, their characteristic sensory properties need to be first recognized during the formulation phase of a targeted food or beverage product. Often times this may not be the case where late stage addition of an essential oil may prove to be an insurmountable formulation challenge, especially in the fast paced formulation stage of food product development.

Beyond the generalizations in the literature (Delaquis and Mazza, 1995), the first antimicrobial property of WMEO was observed in beverage products containing potassium sorbate. The beverage was inoculated with mixed groups of acid and preservative resistant yeasts containing at least four yeast isolates, including *Zygosaccharomyces bailii*, and acid-tolerant bacteria. All yeast and bacteria utilized in the inoculum were previously isolated from spoiled or suspect fruit juice beverages. Inoculated beverage products were stored at 73 °F for at least 60 days and aerobic plate counts of both yeast and bacteria populations were enumerated as per standard methods.

At 12.5 mg/L or 12.5 ppm p-HBITC, there was growth in all the yeast and bacteria challenged beverage containing tubes, while at 25.0 mg/L (25 ppm), there was no detectable growth for up to about 35 days. When calcium fortified (1.54 g/L calcium^{++}) beverages were challenged in a similar manner at 60 mg/L (60 ppm) p-HBITC, there was growth observed in all the yeast and bacteria challenged beverages, while no detectable growth was observed at 80 mg/L (80 ppm) *p*-HBITC. This lead us to believe that p-HBITC in combination with potassium sorbate was able to exert a cidal effect on preservative resistant yeast and bacteria that simulated a low level postprocess challenge (10–15 colony forming units (cfu)/mL) due to recontamination by adventitious organisms.

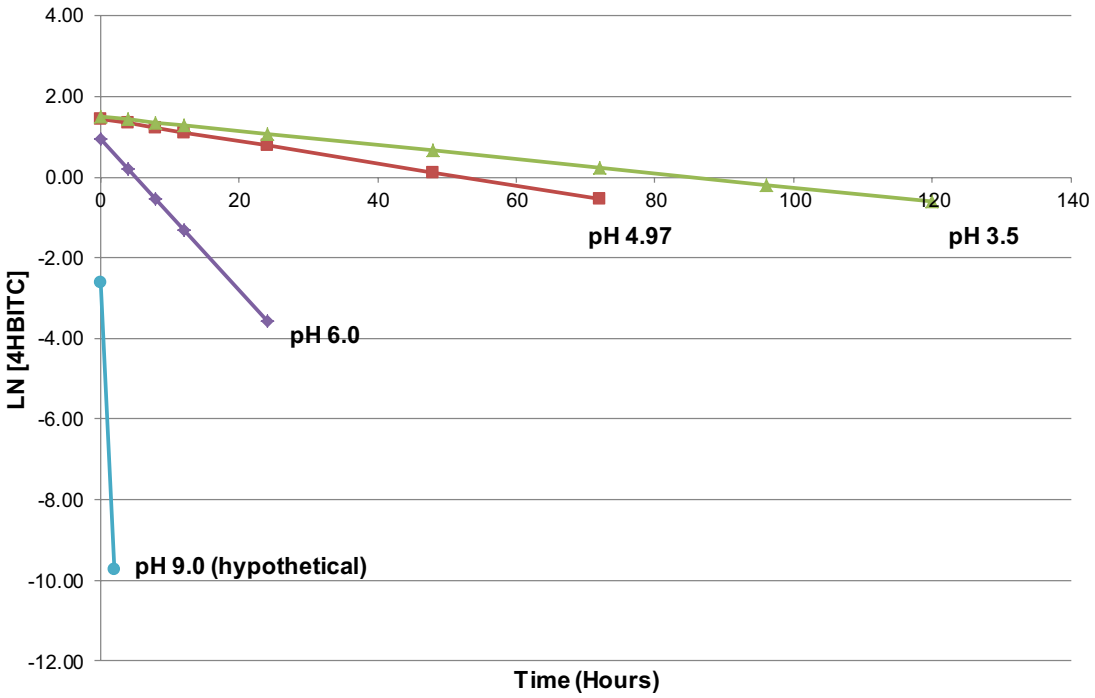

FIGURE 3 Hydrolysis of p-HBITC in aqueous media. Comparison of the calculated regression lines at pH 3.5, pH 4.97 (roasted red bell pepper puree), pH 6.0 and pH 9.0 (hypothetical), pH 3.5, 21 °C: LN [*p*-HBITC] = −0.017588 × time + 1.502754. R^2 = 0.98, pH 6.0, 21°C: LN [*p*-HBITC] = −0.188270 × time + 0.95759. R^2 = 0.91, LN-Natural log.

The instability of p-HBITC in aqueous media was quantified by tracking its hydrolysis in pH 3.5, 6.0, and 9.0 buffer and a roasted red bell pepper puree (pH 4.9) using HPLC (Ekanayake et al., 2012). A summary of the linear regression lines resulting from that experiment is provided in Figure 3. At alkaline pH its hydrolysis is almost instantaneous as seen by the nearly vertical line in Figure 3.

Recognizing the instability of *p*-HBITC in aqueous media, a challenge study was conducted in broth where a range of typical food pathogens at 1×10^7 cfu/mL were mixed with p-HBITC in maltodextrin at p-HBITC concentrations of 60, 120, and 360 mg/L. Log count reductions of 1×10^6 cfu/mL were observed with all organisms on incubation at 6.5 °C for 5 days (Ekanayake et al., 2006).

David et al. (2013b) evaluated the effect of WMEO on inoculated *Salmonella* sp. in a sauce with particulates. Sauce with particulates was made by mixing a starch-based sauce, which served as the carrier for WMEO, with frozen vegetable and chicken particulates inoculated with *Salmonella* sp. WMEO at 250–750 ppm p-HBITC, was able to reduce inoculated *Salmonella* counts by 0.8–2.7 log (cfu/g) in the frozen sauce with particulates in a dose-dependent manner, starting from the point of formulating the sauce through the microwave cooking step (Figure 4). The potential beneficial role of WMEO in frozen finished products subjected to unintentional abuse conditions was also evaluated. When WMEO (p-HBITC at 250–750 ppm) containing frozen sauce with particulates was kept at room temperature for 5 h, it was able to reduce inoculated *Salmonella* counts from the point of first contact and up to 5 h by 0.7–2.4 log (cfu/g). Figure 4 represents typical results, where a concentration-dependent decrease in the viable microorganism count occurred over the complete cycle.

The p-HBITC concentration in an identical sauce with particulates was tracked throughout the process from the initial mixing to the final microwave heating step. After the initial reduction in p-HBITC concentration when WMEO was added to the sauces, the p-HBITC concentration was almost constant until the beginning of the microwave heating step. Complete hydrolysis took place at the end of the heating step at all three levels of addition.

Based upon the above data generated in both broth and beverage and food models, WMEO can be potentially used as a secondary barrier for food protection against low level microbial recontamination. The time to achieve complete hydrolysis of p-HBITC in the food matrix is pH-dependent and the hydrolytic data presented above can be used to predict the time of complete breakdown of p-HBITC. While these data are suggestive of the usefulness of WMEO, the effectiveness of WMEO under conditions of commercial food production and consumer use needs further evaluation.

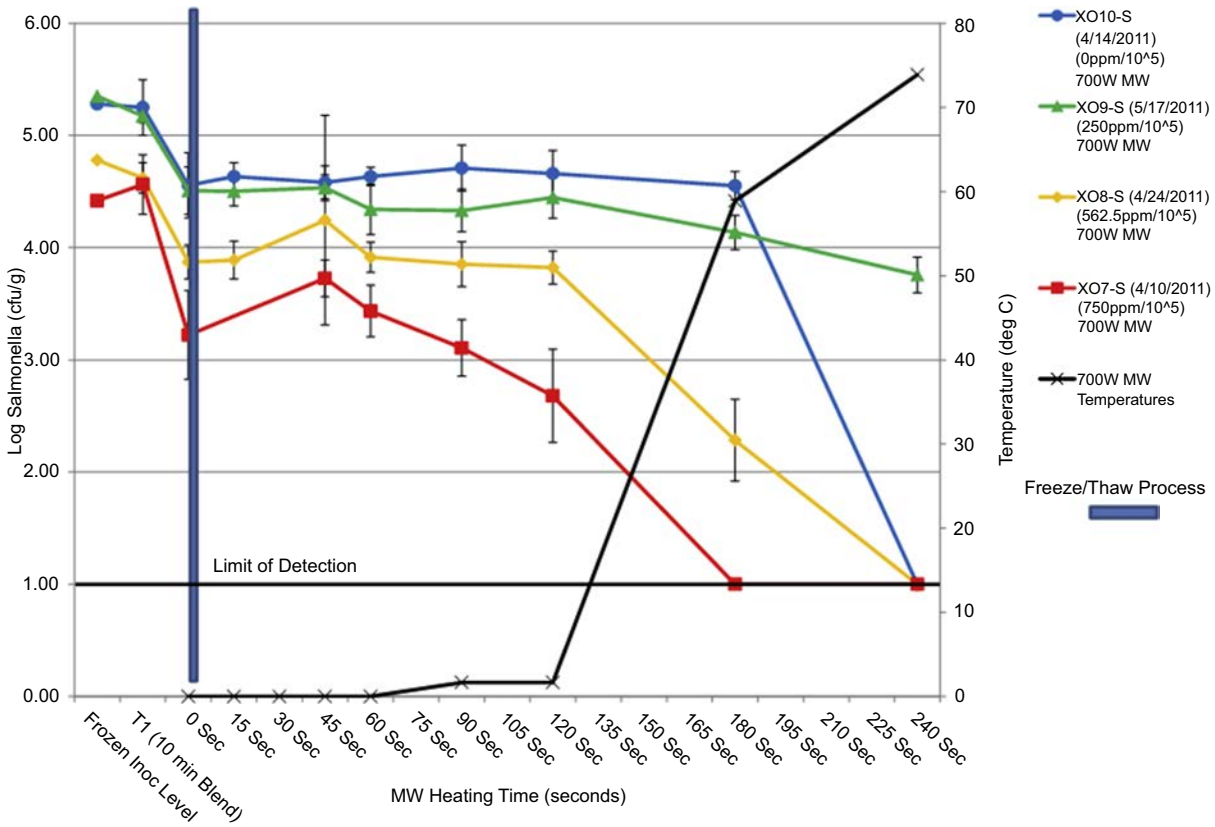

FIGURE 4 Time–kill curves for cooking sauce in 700 W microwave oven, high inoculum. Time–kill curves for WMEO in sauce with inoculated-particulates with high inoculum level (10^6 CFU/g) *Salmonellae* cooked in a 700 W microwave oven (100% power level with a carousel). Log *Salmonellae* counts in sauce with particulates from the point of contact in WMEO (0, 250, 562.5, and 750 ppm 4-HBITC) containing gravy: blending for 10 min, storing in freezer for 48 h, cooking in microwave oven for 4 min. Mean and standard deviation of nine determinations at each point arising from three different 25 g samples. Solid vertical line indicates 48 h gap for freezing. From David et al. (2013b).

The available chemistry and microbiological data should be accompanied by an appropriate toxicological study to show that the final remnant low levels of p-HBITC in foods do not pose a health risk to the consuming public. The long history of safe use of mustard-based products should help in the overall assessment of its safety in the food supply.

SUMMARY POINTS

- WMEO containing the active component p-HBITC was produced in large quantity.
- WMEO was characterized using HPLC/MS and the triacylglycerol part was shown to be unchanged from the cold pressed mustard oil.
- p-HBITC is not stable in aqueous media but is able to exert an antimicrobial effect during its lifetime.
- p-HBITC exerts its antimicrobial effect in actual food and beverage systems.
- WMEO is not commercially available at this time.

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Chapter 99

Ylang–Ylang (*Cananga odorata*) Oils

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List of Abbreviations

FEMA Flavor and Extract Manufacturers Association of USA
GC Gas chromatography
GC–MS Gas chromatography–mass spectrometry
GRAS Generally regarded as safe
US FDA United States Food and Drug Administration

INTRODUCTION

Ylang–ylang essential oil is obtained by steam or hydrodistilling the mature flowers of *Cananga odorata* Hook.f. & Thomson (family: Annonaceae). It is a highly fragrant oil of great value in the perfumery and food industries. The plant is native to Indonesia and Malaysia, but was naturalized by cultivation in several other countries such as the Pacific Islands, Northern Australia, Thailand, Vietnam, and Madagascar (Manner and Elevitz, 2006). It is one of the few essential oils from fragrant flowers which enjoy the reputation as an important essential oil widely used by perfumery and cosmetic industries for the production of high-grade perfumes. The essential oil finds application in the manufacture of soaps, in aroma therapy, and most importantly as a food ingredient (Burdock and Carabin, 2008; Benini et al., 2012). At present, ylang–ylang essential oil is mainly produced in the Comoros islands, Madagascar, and Mayotte (Benini et al., 2012).

In trade, four grades of ylang–ylang oils are recognized (Guenther, 1952; Arctander, 1960; Gaydou et al., 1988; Anonymous, 1992). These are ylang–ylang extra, ylang–ylang first, ylang–ylang second, and ylang–ylang third grades. Ylang–ylang extra super has also been added (Benini et al., 2012). These grades of oil are obtained by collecting the fractions at different time intervals during steam or hydrodistillation of the mature flowers. The physicochemical and odor characteristics of these grades vary slightly. The oils of extra super, extra, and first grades which are collected early during distillation are superior in odor as they contain more volatile components such as alcohols, ethers, and esters than the other grades which are rich in less volatile components (sesquiterpenes). The oils of extra super and extra grades possess desirable top notes and are therefore rated high.

BOTANICAL ASPECTS

Cananga odorata is a tall tree growing typically up to a height of 30–70 feet but rarely up to a height of 100 feet. The entire, simple, 20 cm-long leaf is dark green, alternate, elliptic-oblong, slightly pubescent with a prominent midrib. The fruit is greenish-black in color initially turning to yellow on maturity. The highly fragrant flower is greenish-yellow initially turning to deep yellow/yellowish-brown on maturity (Figure 1). The flowers are axillary and umbellate in shape with three sepals and six petals of about 8 cm in length. They appear in hanging clusters with 4–12 flowers. For cultivation, the plant is pruned to a height of about 10 feet. In India, it is grown in gardens and is known by different local names such as *Karumugai*, *Chettu sampangi*, and *Apurvachampaka* (Anonymous, 1992). Two types of *C. odorata* flower oils are produced: one is the ylang–ylang oil from *C. odorata* Hook. forma *genuina* and the other is the cananga oil from *C. odorata* forma *macrophylla* which is a semiwild species. For a long time, it was believed that both species were not different. Subsequent taxonomic studies classified the cananga tree as *C. odorata* Baill.f. forma *macrophylla* and ylang–ylang tree as *C. odorata* Hook.f. & Thomson forma *genuina*. The name ylang–ylang is a translation of the Philippine expression "Along-Ilang" which means hanging or fluttering item. As the flowers of this tree droop, the tree was named ylang–ylang.

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FIGURE 1 Ylang–ylang flower.

TABLE 1 Production of Ylang–Ylang Oil in Comoros and Madagascar

	Production (Tonnes)	
Year/Years	Comoros	Madagascar
1970/1980	107–110	20
1990	76	16
2000	58.5	12
2006	39.5	15

Bontin (2006).

Cananga tree is mostly confined to Java where its flowers are available throughout the year. The odor of cananga flower oil is considered inferior to ylang–ylang oil. Ylang–ylang oil is mainly produced in Madagascar, Indonesia, Mayotte, and Comoros islands where ylang–ylang trees are commercially cultivated. For cultivation acidic soils in rainforest areas are preferred. Plants are raised through seeds which are collected in June/July. Sowing of seeds is done in nursery beds in June/July usually 2 months before rainy season. Two months after germination, the plants attain a height of about 50 cm and the seedlings are transplanted to the field. Planting is done at a spacing of about 5–6 m so as to allow the tree grow in umbrella shape. The trees yield flowers from fifth year onwards and up to about 30 years. Flowering is generally low during the periods December to March and July to September and high during April to June and October to December (Bontin, 2006). The plant was reported to flower throughout the year in the Pacific Islands (Manner and Elevitz, 2006). The yield of flowers varies depending on the location where it is cultivated. In the Comoro Islands, a yield of 900–1500 kg flowers from 1 ha (2.5 acres) plantation producing approximately 18–30 kg essential oil was reported. In Madagascar, the yield is approximately 1600 kg/ha equivalent to 40 kg of essential oil (Manner and Elevitz, 2006). In recent years, ylang–ylang oil production also started in countries like Brazil and the Reunion Islands. As per the available information, approximately 84 metric tons of the oil was produced in the year 1984 (Srinivas, 1986). From 1970 to 1980 the total production of the oil in the Comoro Islands and Madagascar was around 127–130 metric tons, the Comoros Republic being the main producer (107–110 metric tons). The production, however, declined in subsequent years. In the year 2006, the production of ylang–ylang oil was 54.5 metric tons (Bontin, 2006; Table 1). Though the correct figures are not available, the current world production of ylang–ylang oil is estimated at 50 metric tons per annum.

USAGE AND APPLICATIONS

Ylang–ylang oil is used in aromatherapy as sedative, antiseptic, aphrodisiac, and for treating trauma, acute anxiety, and phobias. It is reported to have relaxing effect when used for massaging humans (Hongratanaworakit and Buchbauer, 2004). In some countries, the flowers are used for ceremonial purposes. Other uses of the oil include scenting of coconut oil in some countries. The oils of extra and first grades are used in high-grade perfumes as they contain high amounts of aromatic ethers, esters, as well as monoterpene esters. The oils of other grades which are rich in less volatile compounds are used in soaps, detergents, and cosmetics. The reported usage levels of ylang–ylang oil in these products are shown in Table 2.

TABLE 2 Usage Levels of Ylang–Ylang Oil in Soaps, Detergents, and Cosmetics

	Usage Level (%)	
Item	Normal	Maximum
Soaps	0.020	0.30
Detergents	0.002	0.03
Creams and lotions	0.010	0.10
Perfumes	0.300	1.00

Burdock and Carabin (2008).

The oil is also used in talcum powder perfumes and for the fixation of floral odors. Mixed with other flower oils like jasmine, neroli, and iris, ylang–ylang oil finds application in the preparation of bouquets (Anonymous, 1992).

USAGE AND APPLICATION IN FOOD SCIENCE

Aroma and taste are the main factors for determining the flavor of food items which are contributed by compounds with organoleptic properties. Essential oils contain monoterpenes and sesquiterpenes, phenyl propanoids, aromatic alcohols, esters, and ethers which possess characteristic organoleptic properties. They are therefore widely used as flavoring agents in food products. Ylang–ylang oil is one of the essential oils which have been in use as flavoring agent for a very long time. As many food materials are perishable with time, they require protection from spoilage during storage and distribution. The major causes of quality loss and shelf-life reduction of food products are the microbial contamination and lipid oxidation. For use as flavoring agent in food products, the essential oil must be rich in odorants. As the composition of the oils from different geographical locations vary, it is better to analyze the oil and see that the oils of different grades are rich in compounds having characteristic odor. Studies on the composition of ylang–ylang essential oils from different countries and their biological activities as well as toxicity studies are briefly presented in this section.

Over the last six decades, chemical compositions of ylang–ylang oils produced in different countries such as China, Laos, Thailand, Madagascar, Comoros islands, and Fiji have been the subject of several studies (Duve et al., 1975; Srinivas, 1986; Lawrence, 1986, 1989, 1995; Gaydou et al., 1986, 1988; Stashenko et al., 1993, 1995, 1996; Benini et al., 2012; Brokl et al., 2013). These studies revealed the presence of aliphatic alcohols, aldehydes, acids, esters, phenols, monoterpenes, sesquiterpenes, and their oxygenated derivatives, phenyl propanoids, nitrogen containing compounds as well as benzenoid compounds in the flower essential oil. More than 100 compounds have been identified in the ylang–ylang flower oils of these countries. Composition of the oils of grades extra, first, second, and third ylang–ylang oils were also investigated and compared. The main components of ylang–ylang oils are *p*-cresyl methyl ether (*p*-methyl anisole), linalool, methyl benzoate, benzyl acetate, geranyl acetate, (E)-caryophyllene, germacrene D, benzyl benzoate, and methyl salicylate (Table 3). There is wide variation in the compositions of ylang–ylang oils and their different grades depending upon the origin and the grade. The oil produced in Yunnan (China) was characterized by the presence of large amounts of sesquiterpenes and oxygenated sesquiterpenes. The oils of the flowers of ylang–ylang plants transported from Laos and Thailand and grown in Xishuangbanna (Yunnan) also contained high amounts of sesquiterpenes and oxygenated sesquiterpenes (Lawrence, 1986). Srinivas (1986) investigated the four grades of ylang–ylang oils produced in the Comoros Islands. The main components of the extra and first grade were *p*-methyl anisole, linalool, benzyl acetate, *m*-cresyl acetate, and *p*-cresyl acetate. Subsequently, Benveniste and Azzo (1992) reported the composition of the four grades of ylang–ylang oils produced in the Comoros Islands in the Kato technical bulletin and newsletter. According to this report, the oils of extra and first grade ylang–ylang oils contained high concentration of *p*-methyl anisole, methyl benzoate, linalool, benzyl acetate, geranyl acetate, and (E)-cinnamyl acetate than in the oils of second and third grades. The oils of second and third grades had high concentration of sesquiterpenes α-copaene, (E)-caryophyllene, α-humulene, germacrene D, α-farnesene, δ-cadinene, and (E,E)-α-farnesyl acetate (Lawrence, 1995). Samples of first, second, and third grade ylang–ylang oils of Madagascan origin were analyzed and their compositions compared (Gaydou et al., 1988). The compounds *p*-methyl anisole, methyl benzoate, linalool, benzyl acetate, methyl salicylate, geranyl acetate, and the minor components 3-methyl-2-butenyl acetate, 2-methyl-3-butene-2-ol, 3-methyl-2-buten-1-ol, bis(3-methyl-2-buten-1-ol), 1,8-cineole, geraniol, and (E)-cinnamyl acetate were found in higher concentration in first grade than in second and third grade ylang–ylang oils. To find out

TABLE 3 Main Components of the Oils of Extra, First, Second, and Third Grades of Ylang–Ylang Oil

	Grade			
Compound	Extra	First	Second	Third
p-Methyl anisole	8.40–16.50	5.75–7.60	1.19–1.80	0.39–1.85
Methyl benzoate	4.97–8.70	3.35–6.40	0.70–2.30	0.20–1.00
Linalool	8.60–13.60	7.27–18.60	2.56–2.80	0.76–1.00
Benzyl acetate	12.36–25.10	7.68–17.40	1.68–7.00	0.69–3.70
Geranyl acetate	4.46–5.30	4.26–5.90	2.96–4.10	1.21–5.30
(E)-cinnamyl acetate	0.00–3.97	0.57–2.76	0.27–1.85	0.01–0.88
(E)-caryophyllene	1.70–5.18	6.00–8.04	7.50–12.90	9.00–12.39
Germacrene D	0.00–17.07	0.10–20.11	1.51–21.71	15.13–25.13
(E,E)-α-Farnesene	8.17–18.00	0.30–10.69	1.66–15.73	6.45–23.75
Benzyl benzoate	2.20–4.83	4.34–14.85	4.70–12.29	4.30–12.80
Benzyl salicylate	1.88–2.37	0.32–3.41	1.02–3.86	0.70–2.81

which compounds played a role in the differentiation of the three grades, multivariate statistical analysis was carried out. It was found that the percentage composition of the compounds 3-methyl-2-butenyl acetate, 1,8-cineole, methyl benzoate, (E)-cinnamyl acetate, germacrene D, ϒ-muurolene, α-copaene, (E)-caryophyllene, (E,E)-farnesyl acetate, ϒ-cadinol, and benzyl benzoate could be used to determine the correct grade of ylang–ylang oil from Madagascar.

Ylang–ylang oils obtained from the mature yellow flowers, petals, and ovaries collected in Bucaramunga, Santander (Columbia) were investigated by Stashenko et al. (1995) for their chemical composition. Oil samples obtained by steam distillation of the materials for 1 and 2 h were analyzed by high-resolution gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The oil of mature yellow flower obtained by distillation for 1 h was found to have major components *p*-methyl anisole, methyl benzoate, linalool, benzyl acetate, geranyl acetate, germacrene D, and benzyl benzoate. The same material when steam distilled for 2 h gave an oil containing germacrene D, (E,E)-farnesene, (E,E)-farnesol, and benzyl benzoate in higher concentration. The oil of petals after 1 h distillation contained methyl benzoate, linalool, benzyl acetate, (E)-cinnamyl acetate, (E)-caryophyllene, germacrene D, and benzyl benzoate as the main compounds. The oil of the ovaries after 2 h steam distillation contained α-pinene, linalool, geranyl acetate, (E)-cinnamyl acetate, (E)-caryophyllene, germacrene D, and benzyl benzoate as the main components. It is clear that steam distillation of ylang–ylang flowers for 2 h duration yields oils having higher concentration of sequiterpenes, whereas distillation for 1 h yields oils containing *p*-methyl anisole, methyl benzoate, linalool, benzyl acetate, geranyl acetate, and the minor components (*Z*)-3-hexenol, 2-methyl-3-buten-2-ol, etc. in higher concentration. Study of the variation in essential oil composition during flower development revealed that the oil composition changed both qualitatively and quantitatively from undeveloped green flower stage to fully matured deep yellow flower stage (Stashenko et al., 1995). Though there was no appreciable change in the mono- and sesquiterpene content, the oil of fully matured yellow flowers contained 16 times more of light oxygenated compounds (*p*-cresyl methyl ether, benzyl alcohol, methyl benzoate, linalool, benzyl acetate, cinnamyl acetate, etc.), and 4 times more of heavy oxygenated compounds (sesquiterpene alcohols, sesquiterpene esters, benzyl benzoate, benzyl salicylate, etc.) than the oil from undeveloped green flowers. The oil of fully matured yellow flowers was the material of choice for the extra grade ylang–ylang oil.

There appears to be no report on the composition of ylang–ylang oil from India. The authors have studied the composition of the oils of ylang–ylang flowers collected from the trees of *Cananga odorata* Hook.f. & Thomson forma *genuina* grown in Mysore, Bangalore, and Visakhapatnam (India) by GC–MS analysis. The major components of the three oils are presented in Table 4. The main components of the oil from Bangalore were *p*-methyl anisole (10.9%), geraniol (5.4%), geranyl acetate (8.0%), (E)-caryophyllene (14.8%), α-humulene (4.4%), germacrene D (12.8%), (E,E)-α-farnesene (2.7%), (E,E)-farnesol (7.7%), and benzyl benzoate (11.6%). The major components of the oil from Mysore were: *p*-methyl anisole (10.0%), linalool (4.2%), benzyl acetate (17.3%), geranyl acetate (11.3%), (E)-cinnamyl acetate (3.2%), benzyl benzoate (23.0%), and benzyl salicylate (14.3%). Ylang–ylang oil from Visakhapatnam was found to contain *p*-methyl anisole

TABLE 4 Composition (%) of the Main Components of Ylang–Ylang Flower Essential Oils from Three Locations in India

Compound	Bangalore	Mysore	Visakhapatnam
p-Methyl anisole	10.9	1.0	4.5
Methyl benzoate	2.3	1.5	2.0
Linalool	0.8	4.2	16.6
Benzyl acetate	0.2	17.3	21.4
Geraniol	5.4	0.5	0.8
Geranyl acetate	8.0	11.3	24.3
(E)-cinnamyl acetate	0.1	3.2	3.9
(E)-caryophyllene	14.8	0.3	1.3
α-Humulene	4.4	0.3	0.1
Germacrene D	12.8	0.1	3.3
(E,E)-α-Farnesene	2.7	0.1	1.4
δ-Cadinene	1.3	0.2	0.2
δ-Cadinol	1.8	0.6	0.3
α-Cadinol	2.1	0.8	0.4
(*Z*,*Z*)-Farnesol	7.7	0.8	0.4
Benzyl benzoate	11.6	23.0	6.6
Benzyl salicylate	0.6	14.8	5.5

(4.5%), linalool (16.6%), benzyl acetate (21.4%), geranyl acetate (24.3), (E)-cinnamyl acetate (3.9%), benzyl benzoate (6.6%), and benzyl salicylate (5.5%) as the major components. The oil from Bangalore is rich in sesquiterpenes and is similar to ylang–ylang oil from Yunnan (China). The high content of benzyl salicylate in the oil from Mysore is noteworthy. The structures of the main components of ylang–ylang oil are presented in Figure 2. A typical gas chromatogram of ylang–ylang oil (from Visakhapatnam) obtained on a nonpolar BP-1 column is shown Figure 3.

Ylang–ylang oils have pale to deep yellow color with a harsh floral fragrance. The physicochemical characteristics of the four grades of this oil will help in their differentiation and for this reason; quality standards for these grades were given by different organizations and suppliers. The physicochemical properties of these grades reported by the Grasse Essential Oil Association and Afnor are presented in Table 5.

Ylang–ylang oil has been used in food products as food additive and flavoring agent for more than 100 years. The oil is used for flavoring alcoholic and nonalcoholic beverages, baked goods, chewing gum, gelatin, pudding, ice cream, and hard and soft candies. The reported usage levels of ylang–ylang oil in these products are provided in Table 6. The oil has been approved for use in foods by United States Food and Drug Administration (FDA), Flavor and Extract Manufacturers' Association (FEMA). The international organization of flavor industries vouched its safety. Ylang–ylang essential oil is generally regarded as safe (GRAS). Based on the consumption data, it was estimated that the individual consumption of ylang–ylang oil is 0.006 mg per day (Burdock and Carabin, 2008). Among the ylang–ylang oils from the three locations in India, the oil from Visakhapatnam appears suitable for use in food products because of the presence of high amounts of fragrant components *p*-methyl anisole, methyl benzoate, linalool, benzyl acetate, and geranyl acetate in the oil. In view of the exotic flavor characteristics of the oil, use of this oil in different types of cuisine should be explored.

Secondary metabolites of plants have been exploited by humans to protect themselves, their livestock, and foods against diseases, pests, and bacterial infections. Interest in the use of essential oils in medicine, food preservation, and crop protection increased in recent times. Essential oils which are used in perfumery, cosmetics, and for food flavoring are preferred as antimicrobial agents and antioxidants in food products because they are natural materials, and are less toxic.

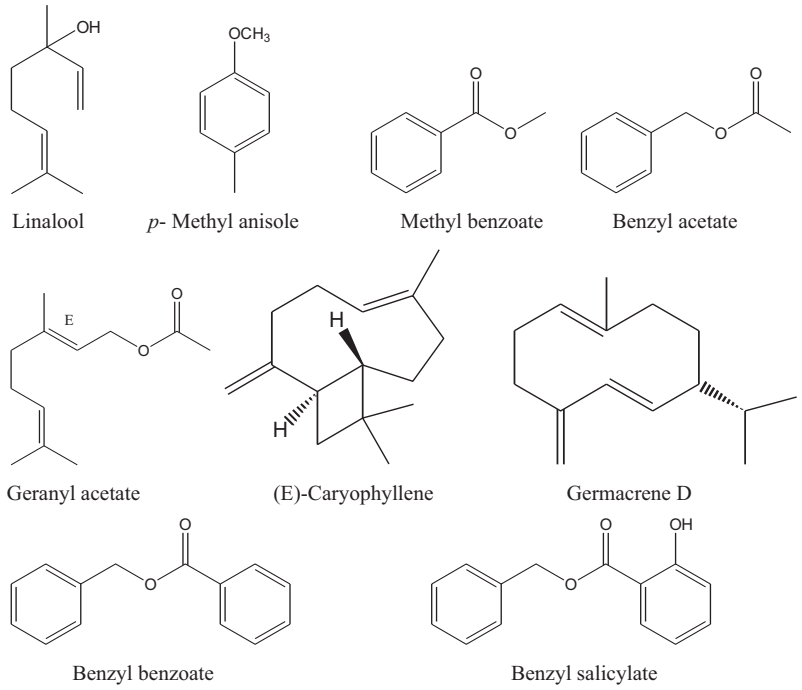

FIGURE 2 Structures of main components of ylang–ylang flower essential oil.

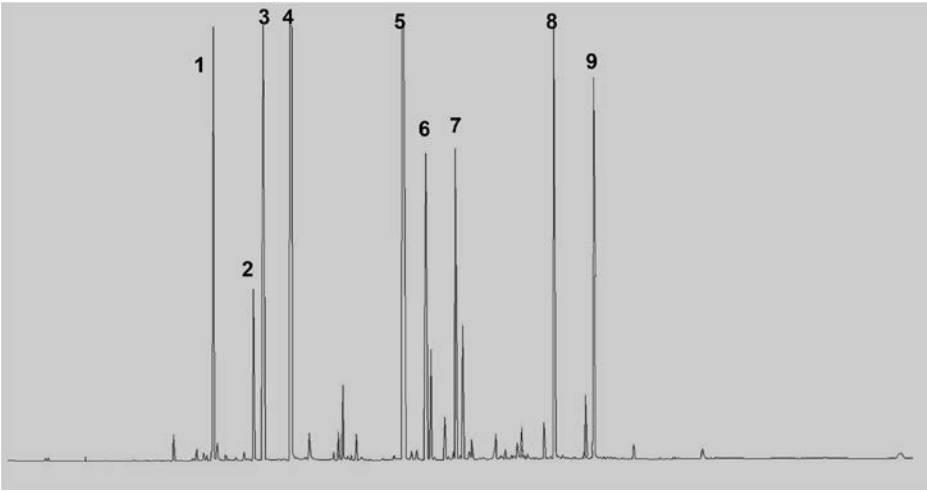

FIGURE 3 Gas chromatogram of ylang–ylang oil from Visakhapatnam, India. 1, *p*-Methyl anisole; 2, Methyl benzoate; 3, Linalool; 4, Benzyl acetate; 5, Geranyl acetate; 6, Cinnamyl acetate; 7, Germacrene D; 8, Benzyl acetate; 9, Benzyl salicylate.

Ylang–ylang oil is one such oil used in foods as a flavoring agent and as an adjuvant. Studies on biochemical and pharmacological effects and toxicity effects of ylang–ylang oil were reviewed by Burdock and Carabin (2008). The results of some of these studies are briefly presented. The oil was reported to have no antimicrobial activity when tested against two strains of *Streptococcus pneumoniae*, one nonencapsulated (R36A) and the other encapsulated (IC2) (Horne et al., 2001). However, ylang–ylang oil exhibited modest inhibitory activity against seven bacterial strains (coagulase negative *Streptococcus*, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Stenotrophomonas maltophilia*) and *Candida albicans* (Maudsley and Kerr, 1999). Ylang–ylang oil displayed no antifungal activity against *Malassezia furfur* (Lee and Lee, 2010). The methylene chloride and methanol extracts of ylang–ylang flowers collected from the trees in south-east Asia were shown to possess amoebicidal activity against three species of *Acanthamoeba* (Chu et al., 1998).

Assessment of the in vitro free radical scavenging capacity of ylang–ylang oil revealed that the oil exhibited minimal radical quenching activity (Mantle et al., 1998). Ylang–ylang oil was one of the 13 essential oils tested for antioxidant

TABLE 5 Published Standards of Ylang–Ylang Flower Essential Oil Grades

Property	Extra	First	Second	Third
Acid value				
Afnor	<3	<3	<3	<3
Grasse EOA	2.8	2.8	2.8	2.8
Ester value	130–182	89–130	56–89	34–56
Afnor	125–160	90–120	65–80	38–58
Grasse EOA	130–182	89–130	56–89	34–6
Optical rotation $[\alpha]_D$ at 20 °C				
Afnor	−36° to −45°	−28° to −44°	−40° to −55°	−43° to −63°
Grasse EOA	−25° to −40°	− 44° to −60°	−40° to −68°	−35° to −67°
Specific gravity at 20 °C				
Afnor	0.950–0.965	0.933–0.945	0.923–0.929	0.906–0.921
Grasse EOA	0.946–0.982	0.928–0.949	0.918–0.933	0.906–0.923
Refractive index at 20 °C				
Afnor	1.5010–1.5090	1.5000–1.5100	1.5050–1.5110	1.5070–1.5150
Grasse EOA	1.4980–1.5090	1.5000–1.5090	1.5057–1.5117	1.5070–1.5150

EOA, Essential Oil Association.
Gaydou et al. (1988).

TABLE 6 Uses of Ylang–Ylang Oil in Different Types of Foods and its Usage Levels

	Usage Level (ppm)	
Food Type	Normal	Maximum
Alcoholic beverages (FEMA)	0.99	2.91
Nonalcoholic beverages (FEMA)	0.98	2.85
Baked goods (FEMA)	3.03	5.03
Chewing gum (FEMA)	12.04	58.81
Chewing gum (NACGM)	11.63	12.31
Frozen dairy products (FEMA)	2.92	4.89
Gelatin, pudding (FEMA)	1.03	3.00
Hard candy (FEMA)	1.05	1.38
Hard candy (NACGM)	3.18	4.19
Soft candy (FEMA)	3.00	4.98

FEMA, Flavor and Extracts Manufacturer's Association; NACGM, National Association for Chewing Gum Manufacturers.
Burdock and Carabin (2008).

activity in three assay systems (Wei and Shibamoto, 2007). The oil was shown to exhibit significant antioxidant activity in aldehyde/carboxylic acid assay, 1,1-diphyenyl-2-prenylhydroxyl free radical scavenging assay, and malanaldehyde formation assay. The major component of the oil germacrene (most probably germacrene D) was shown to possess significant antioxidant activity during this investigation.

The limited number of studies performed on the toxicity of ylang–ylang oil indicated low order oral and dermal reactions caused by the oil in addition to irritation and sensitization reactions in animals and humans. The results of these studies are reviewed and summarized by Burdock and Carabin (2008). It is still not certain which of the components of ylang–ylang oil are responsible for these reactions. The phenylpropanoids eugenol and isoeugenol and the lignan dehydrodiisoeugenol present in ylang–ylang oil were reported to be responsible for contact hypersensitivity of the oil (Watanabe et al., 1985). A high concentration of linalool was reported to be a potential sensitizer (Cockayne and Gawkrodger, 1997). Studies conducted on subchronic inhalation of ylang–ylang oil on female rats showed no significant toxicological effects (Fukayama et al., 1999). In view of the fact that ylang–ylang oil has been in use as fragrance and food flavoring material for a long time and there are no reports of adverse health effects, it is concluded that the estimated level of consumption of the oil does not pose any risks to human health. The health hazards posed by the above mentioned three compounds to humans are considered negligible when used as food flavoring agents as they have been approved by US FDA, FEMA, and given GRAS status. Though ylang–ylang oil can be used safely as flavoring agent in foods, it is better to avoid direct contact of the oil with skin as a precautionary measure in view of the reported contact dermatitis.

SUMMARY POINTS

- Ylang–ylang flower essential oil has been used as flavoring agent in processed food products for over a century and is considered as a safe food ingredient.
- Chemical compositions of ylang–ylang flower oils of different countries were examined by several researchers. The major components of the flower essential oil are: *p*-methyl anisole, linalool, benzyl acetate, geranyl acetate, (E)-caryophyllene, germacrene D, benzyl benzoate, and benzyl salicylate.
- The essential oil exhibited limited antimicrobial and antioxidant activities.
- Among the three oils studied in India, the oil from Visakhapatnam (Andhra Pradesh province) is more suitable for use as food flavoring agent than the oils from Bangalore and Mysore (Karnataka province).
- Ylang–ylang oil has been approved by the US FDA and FEMA for use as a flavoring agent in processed food products and has been awarded GRAS status.

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Essential Oils in Food Preservation, Flavor and Safety

Edited by Victor R. Preedy

Essential Oils in Food Preservation, Flavor and Safety is an authoritative synopsis of many of the complex features of the essential oils as applied to food science; ranging from production and harvesting to the anti-spoilage properties of individual components. While much of essential oil usage is based on traditional practices or folk lore, modern rigorous scientific research has led to the discovery of novel lipid fractions and has identified the structural characteristics of key components, which allow them to be used as both external and internal agents.

This book embraces a holistic approach to these globally used lipid factions. Providing information on nearly 100 essential oils whose properties have been determined to have positive impact in food application, ***Essential Oils in Food Preservation*** is divided into two distinct Parts: General Aspects, which explores the broad discovery and application of these oils, and Named Essential Oils, in which individual oils are presented in sub-sections on *Botanical Aspects, Usage and Applications, and Usage and Applications in Food Science*.

This book will be a valuable resource for those seeking options for food preservation, flavor and safety purposes, as well as for inspiring additional research.

Key Features

- Explains how essential oils can be used in new or redesigned products
- Presents a unique subsection emphasizing how essential oils are frequently used to impart flavor and aroma
- Provides an exceptional range of information from general use insights to specific use and application information
- Explores geographically specific aspects and uses
- Examines traditional and evidence-based uses
- Includes methods and examples of investigation and application

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