Chapter 3
Antibacterial, Antiviral and Antifungal Activity of Essential Oils: Mechanisms and Applications

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Abstract Essential oils are natural products which combine antimicrobial and antioxidant activity, thus providing natural protection against microbial pathogens and other undesirable agents. Among the essential oils extracted from aromatic plants, oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) oils have been proposed for different biomedical and industrial applications. The antimicrobial mechanisms found in these essential oils have been explained on the basis of their content in natural compounds such as carvacrol, thymol, p-cymene and c-terpinene, among others. Although these two essential oils have received much attention, scientists working in the fields of biomedicine and food science, among others, are paying increasing attention to a wider variety of aromatic natural oils in an effort to identify novel and natural applications for the inhibition of microbial pathogens. Accordingly, a detailed revision of the main essential oils and their applications in biomedicine, food science and other industrial fields is presented. The review not only focuses on the main antibacterial applications reported to date, but also in the current and future developments for the inhibition of virus and fungi.

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3.1 Introduction

Essential oils (EOs) are oily, aromatic and volatile liquids that can be harvested from plant material. Usually, EOs are formed in specialised cells or groups within stems or leaves and are concentrated in particular regions of the plant, such as the bark, leaves or fruit. The composition of EOs is very complex, and many EOs contain 20–60 individual volatile compounds. The main components are hydrocarbons (pinene, limonene and bisabolene), alcohols (linalool and santalol), acids (benzoic acid and geranic acid), aldehydes (citral), cyclic aldehydes (cuminal), ketones (camphor), lactones (bergapten), phenols (eugenol), phenolic ethers (anethole), oxides (1,8 cineole) and esters (geranyl acetate) (Sell 2006; Miguel 2010).

Essential oils are multifunctional and exhibit a wide spectrum of activities, such as antiphlogistic, spasmolytic, antinociceptive, immunomodulatory, psychotropic, acaricidal, expectorative and cancer-suppressing activities. Furthermore, EOs and their components possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. These activities can be mediated by single compounds or groups of compounds, and these secondary metabolites have biological functions in the plants from which they originate, such as protection against predators and microbial pathogens, as well as involvement in defence mechanisms against abiotic stress (Bassolé and Juliani 2012; Lang and Buchbauer 2012).

Due to the broad range of antimicrobial and other beneficial effects, EO-producing plants have been used as medicinal plants over thousands of years. In total, 3,000 EOs are known, of which approximately 300 are commercially important, primarily in the flavouring and fragrances industries. In medicine, only a few EOs are used in aromatherapies and some components of EOs are used for flavouring in the food industry. However, the recent trend of using natural compounds in medicines and food preservation has led to an increasing interest in EOs in both research and industrial settings.

Food safety and foodborne illnesses are an increasingly important public health issues. Thus, the growth and metabolism of microorganisms in food products present a global problem. Microbial toxins cause serious foodborne illnesses, and each year millions of people die from diarrhoeal disease caused by the consumption of contaminated food (WHO 2002). Additionally, food represents a good growth medium for microorganisms, and microbial spoilage of food products result in high economic losses for the food industry. Thus, effective methods of reducing or eliminating foodborne pathogens or microbes that cause food spoilage are needed.

At the same time, an increasing demand from the consumer side for products with fewer synthetic food additives exists, and thus, a strong interest in using natural substances for food preservation as an alternative for the broad spectrum of synthetic chemical additives has appeared. Several methods have been suggested

for the biopreservation of food products, such as the use of bifidobacteria or natural plant extracts. In addition, new methods of applying antimicrobial substances to foods, such as on packaging films, have been implemented in order to apply high concentrations of preservatives to food surfaces and increase the shelf life.

Thus, plants like oregano, thyme, garlic, bay leaf, rosemary and clove or their extracts, known as EOs, can be used alone or in combination with other preservation methods, such as irradiation or modified atmosphere packaging (MAP), to improve the shelf life of food products (Mejlholm and Dalgaard 2002; Mahmoud et al. 2004; Miguel et al. 2004; Wong and Kitts 2006; Kykkidou et al. 2009; Yerlikaya and Gokoglu 2010). In many studies, the application of EOs resulted in a considerable extension of the food's shelf life. For example, a new active packaging, consisting of a label containing cinnamon EO attached to plastic packaging, was used to extend the shelf life of late-maturing peaches.

An important consideration when using EOs as preservation agents in food is the impact on sensory acceptability. In general, a relatively high concentration of EOs is required to achieve a significant antimicrobial and/or antioxidant activity. However, the high aromatic potential of EOs can affect the organoleptic quality of the corresponding food, resulting in unacceptable odour and taste during consumption (Gutierrez et al. 2008). Therefore, research should focus on optimising EO formulations and their applications to food products to facilitate the successful use of EOs in the food industry. The main objective is to obtain an effective antimicrobial and antioxidant activity at low concentrations without negatively affecting the organoleptic quality of food.

However, the antioxidant and antimicrobial activities of EOs have been shown to depend not only on the plant species but also other factors such as the number of samples, the method used to extract active compounds and the method employed for measuring antioxidant and antimicrobial capacity. In addition, the chemical composition of EOs can vary according to geo-climatic location, growing conditions (season, soil type, amount of water) and the plant's genetics. Consequently, the antioxidant activities and antimicrobial activities of EOs from the same plant can vary considerably according to numerous studies, and realistic comparisons between different types and sources of EOs can be difficult (Sangwan et al. 2001; Burt 2004). Additionally, most research focuses on the EO in its entirety, while information concerning the antioxidant activity of individual major constituents is scarce.

In the following sections, the antioxidant, antibacterial, antifungal and antiviral activities of several EOs are reviewed. Additionally, some applications of EOs in the medical field are discussed with a special focus given to the application of EOs as preservative agents in food products. Studies concerning the determination of the antioxidant and antimicrobial activity of some EOs in food products are summarised. Table 3.1 gives an overview of some commercially important EOs, their main active compounds and known antimicrobial and/or antioxidant activity.

Table 3.1 Plants whose essential oils contain bioactive compounds

Plant	Bioactive compound	Activity
Nutmeg (*Myristica fragrans*)	Sabinene, 4-terpineol, myristicin	Antifungal, antioxidant
Cedar (*Cedrus libani*)	Limonene	Antifungal
Garlic (*Allium sativum*)	Diallylle disulphide	Antibacterial, antiviral, antifungal, antioxidant
Clove (*Syzygium aromaticus*)	Eugenol and eugenyle acetate	Antiviral, antibacterial, antifungal
Coriander (*Coriandrum sativum*)	Linalool, E-2-decanal	Antifungal, antibacterial
Cinnamon (*Cinnamomum cassia*)	Cinnamaldehyde	Antibacterial, antiviral, antifungal, antioxidant
Eucalyptus (*Eucalyptus globulus*)	1,8-cineole	Antibacterial, antiviral
Peppermint (*Mentha piperita*)	Menthol, menthone	Antiviral, antioxidant
Lavender (*Lavandula officinalis*)	Linalool, linalyle acetate	Antibacterial, antiviral
Tea tree (*Melaleuca alternifolia*)	Terpinen-1-ol-4	Antibacterial, antifungal, antiviral
Lemon (*Citrus limonum*)	Limonene	Antibacterial, antiviral, antifungal, antioxidant
Oregano (*Origanum vulgare*)	Carvacrol, thymol, terpinene, cymene	Antibacterial, antifungal, antiviral, antioxidant
Rosemary (*Rosmarinus officinalis*)	Pinene, bornyl acetate, camphor, 1,8-cineole	Antibacterial, antifungal, antiviral, antioxidant
Sage (*Salvia officinalis*)	Camphor, pinene, 1,8-cineole, tujone	Antibacterial, antifungal, antiviral, antioxidant
Thyme (*Thymus vulgaris*)	Thymol, carvacrol, terpinene, cymene	Antibacterial, antifungal, antiviral, antioxidant

3.2 Antioxidant Effects of Essential Oils

3.2.1 General Aspects and Antioxidant Behaviour of EOs

An antioxidant is a substance that is capable of inhibiting specific oxidising enzymes, reacting with oxidising agents prior to the damage of other molecules, sequestering metal ions or repairing components of antioxidant systems such as iron transport proteins. Antioxidants (namely, vitamins, enzymes or Fe^{+2}) have the ability to protect cells from free radical damage and serve as chemopreventive agents by inhibiting free radical generation and play important roles in neutralising oxidative damage caused by these free radicals. The presence of antioxidants in the human diet has generated great interest due to their positive effects on human health due to their ability to neutralise free radicals and protect cells from oxidant damage.

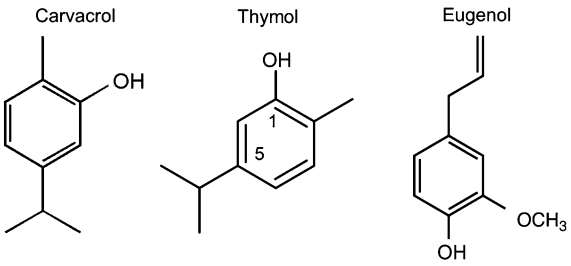

Fig. 3.1 Chemical structure of carvacrol, thymol and eugenol bioactive components of essential oils

Recently, interest in research into the role of plant-derived antioxidants in food and human health has grown. Plants are known to produce a wide variety of molecules with strong antioxidant effects such as vitamins (ascorbic acid, vitamin C; α-tocopherol, vitamin E; β-carotene, vitamin A precursor) and phenolic compounds. Among the main phenolic compounds identified in plant extracts, phenolic acids (e.g., p-coumaric acid, caffeic acid, rosmarinic acid and gallic acid), phenolic diterpenes (e.g., carnosic acid and epirosmanol) and flavonoids (e.g., aromatic compounds) possess antioxidant activity (Shan et al. 2005).

A large pool of studies describing the antioxidant activity of EOs exists (Adorjan and Buchbauer 2010; Miguel 2010). In preliminary studies, EOs from *Rosmarinus officinalis, Salvia fruticosa*, *Foeniculum dulce*, *Thymus vulgaris* and *Laurus nobilis* inhibited lipid oxidation; these results can be explained by the presence of phenolic compounds (carvacrol, thymol and eugenol) (Zygadlo et al. 1995; Özcan et al. 2001) (Fig. 3.1).

In 2007, the antioxidant and free radical scavenging activities of EOs from flowers and fruits of *Otostegia persica* have been investigated (Sharififar et al. 2007). By using GC–MS analysis, α-pinene, 1-octen-3-ol and cubenol were identified as the major constituents of the EO in flowers (EOFLs), while the most prominent in EO in the fruit (EOFR) was hexadecanoic acid. These results show that EOFLs possessed greater antioxidant and radical scavenging activity attributed to the high amount of oxygenated monoterpenes.

The antioxidant properties of some popular and commercially available EOs from lemon (*Citrus limon*), pink grapefruit (*Citrus paradisi*), coriander (*Coriandrum sativum*), clove (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), lavender (*Lavandula angustifolia*), peppermint (*Mentha. piperita*) and frankincense (*Boswellia carteri*) have been reported. The results show that clove EO exhibited the highest amount of total phenols (eugenol, eugenyl acetate, β-caryophyllene and 2-heptanone) and had the highest antioxidant activity, as well as the highest DPPH radical scavenging activity and the highest FRAP value. Lavender EO and limonene also exhibited a high DPPH radical scavenging activity. Furthermore, peppermint EO possessed the highest radical scavenging activity against ABTS radicals, while lavender oil was most effective for inhibiting linoleic acid peroxidation after 10 d. It has been shown that all EOs tested were capable of chelating iron (II), but the greatest effect was achieved by the rosemary EO (Chaieb et al. 2007; Misharina and Samusenko 2008; Viuda-Martos et al. 2010; Yang et al. 2010b).

In another study, the chemical composition and antioxidant effects of the EO from *Mentha piperita* were investigated. The main constituents found were menthol, menthone, menthyl acetate, 1,8-cineole, limonene, β-pinene and β-caryophyllene (Schmidt et al. 2009). As a result, *M. piperita* exhibited anti-radical activity on DPPH and hydroxyl radicals. Likewise, EO and methanol extracts of *Psammogeton canescens* were tested in vitro and showed significant antioxidant activity. The EO was a more effective radical reducer than the methanol extract at all concentrations tested. The chemical composition analysed by GC–MS showed that the main constituents of the oil were β-bisabolene, apiole, α-pinene and dill apiole (Gholivand et al. 2010).

A separate study demonstrated that antioxidant activity can differ significantly depending on the location where the plant was collected, as shown for Tunisian cultivated sage (*Salvia officinalis*) EO (Ben Farhat et al. 2009). Furthermore, the antioxidant potential of *Ruta montana* EO possessed anti-radical activity in a concentration-dependent manner (Kambouche et al. 2008).

3.2.2 Antioxidant Behaviour of EOs and Health Advantages

Free radical-mediated oxidation is an important energy-producing biological process in all living organisms. When oxygen-derived free radicals are overproduced, they can induce oxidative damage to biomolecules such as lipids, proteins and nucleic acids that produce oxidative stress. This oxidative stress may lead to atherosclerosis, ageing, cancer, diabetes mellitus, inflammation and several degenerative diseases in humans (Adorjan and Buchbauer 2010; Miguel 2010).

Including antioxidants in the diet has been found to have beneficial effects on human health because they protect biologically important cellular components, such as DNA, proteins and membrane lipids, from reactive oxygen species attacks. The use of naturally occurring antioxidants in daily life has been regarded as an effective way of promoting human health. Synthetic antioxidants have fallen out of favour because of their carcinogenicity, and thus natural antioxidants from plant products have become an increasingly promising alternative.

Among plant-derived products, EOs have been reported to have a strong potential for antioxidant, therapeutic and pharmaceutical purposes. EOs are known to scavenge free radicals, and this property makes them important in health maintenance and disease protection. Thus, volatile phenolic compounds found in EOs have been determined to be the main active ingredients in most herbs, e.g., menthol (in mint), carvacrol (in oregano and rosemary), thymol (in thyme) and eugenol (in clove). Meanwhile, the main components of EOs such as terpenoids, specifically monoterpenes (C10) and sesquiterpenes (C15), as well as a variety of low molecular weight compounds, have been found to be profitable (Anthony et al. 2012).

3.2.3 Antioxidant Effects of EOs on Food Preservation

Lipid oxidation is very important in the food industry. In addition to the effects on human health in the form of oxidative stress or oxidative damage, major concerns about lipid oxidation used in food technology exist because of the formation of oxidation products such as fatty acid hydroperoxides and secondary degradation products. These autoxidation products of fats and oils are responsible for off-flavours and characteristic rancid odours and are responsible for the decrease of both the nutritional quality and the safety of foods (Fasseas et al. 2008; Anthony et al. 2012).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been used to retard or minimise the oxidative deterioration of foods. Recently, consumers have rejected synthetic antioxidants because of their carcinogenicity, and in recent decades, increasing interest in natural antioxidants, especially plant-derived antioxidant compounds, has appeared. Many herbs, spices and their extracts have been added to a variety of foods to improve their sensory characteristics and extend shelf life. Herbs of the Lamiaceae family, mainly oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*), have been reported to have significant antioxidant capacity (Shan et al. 2005). Thus, numerous studies have shown that EOs are an abundant source of compounds exhibiting strong antioxidant activity and that these compounds can be used as natural antioxidants in the food industry. The effect of oregano EO on meat quality has been studied the most, whereas less information about other plants is available. Additionally, EO use has been especially important in meat products, although they have also been useful in seafood that has high polyunsaturated fatty acid content. Select applications of EOs as antioxidants on different foods will now be described.

3.2.3.1 Meats

The short shelf life of refrigerated, packed meat makes its commercialisation more difficult than other types of foods. Most related research advocates the addition of EOs to meat products in post-slaughter stages or their inclusion in active packaging.

Oregano (*Origanum vulgare*)-based films have been shown to stabilise lipid oxidation in beef muscle slices for 7 days at 4 °C (Oussalah et al. 2004). Likewise, oregano EO added to chitosan coating successfully protected dry fermented sausages from lipid oxidation (Krkić et al. 2012). Differences in the fatty acid profiles (myristic, oleic and linoleic acids) between coated and control sausages were observed after 2 months of storage, but after 7 months of storage, there was no difference. The content of most aldehydes was significantly lower in coated sausage than in the control after 7 months of storage, and odour and flavour were better for the coated sausage.

The use of basil (*Ocimum basilicum*) and coriander (*Coriandrum sativum*) EOs as antioxidants in Italian salami was investigated. The formulation containing 0.75 mg/g basil EO exhibited antioxidant activity on lipids but not the protein in the Italian salami during processing and after 30 days of storage at 18–25 °C (Cichoski et al. 2011). Likewise, salami with coriander EO exhibited reduced lipid oxidation, increasing the shelf life of the product. In addition, salami treated with the commercial antioxidant BHT had less antioxidant activity than those treated with coriander EO (Marangoni and Moura 2011).

De Oliveira et al. (2011) have shown the possible benefits of combining EOs and minimal amounts of sodium nitrite in cured meat products. Winter savoury (*Satureja montana* L.) EO was added at concentrations of 7.80, 15.60 and 31.25 μl/g in mortadella sausages formulated with different sodium nitrite levels (0, 100 and 200 mg/kg) and stored at 25 °C for 30 days. The effect on colour development and lipid oxidation (TBARS) was analysed, and the 100 mg/kg nitrite concentration appeared to be sufficient for the formation of the characteristic red colour. The use of EO at concentrations exceeding 15.60 μl/g adversely affected the colour of the product by reducing redness and increasing yellowness. A significant effect on lipid oxidation was observed in samples containing EO plus reduced amounts of sodium nitrite (De Oliveira et al. 2011).

The effects of thyme EO on the lipid stability of vacuum-packaged (VP) and refrigerated (4 °C) chicken liver were studied by (Papazoglou et al. 2012). Lipid oxidation was low, as determined by malondialdehyde (MDA) values, during the entire storage period (>12 d).

Incorporating EOs and other natural antioxidants in animal diets is another strategy to obtain lipid stability in food products. Several studies have been carried out to evaluate this strategy. The antioxidant effects of oregano EO and vitamin E were evaluated in chicken (Botsoglou et al. 2003a; Avila-Ramos et al. 2012). The results showed that α-tocopheryl acetate supplementation was more effective than dietary incorporation of oregano EO to extend the lipid stability of chicken. However, there was a synergistic effect between dietary EO and α-tocopheryl acetate supplementation in retarding lipid oxidation in raw and cooked turkey during refrigeration (Botsoglou et al. 2003b). Feeding swine with oregano EO also had positive effects, resulting in a lower oxidation of pork lipids than that of control samples throughout storage (Alarcon-Rojo et al. 2013). However, further studies did not find an effect with EO compounds (carvacrol and cinnamaldehyde) that were added to the diet of growing lambs on the sensory characteristics of the meat product (Chaves et al. 2008). Likewise, the studies on the effects of dietary oregano EO supplementation on finishing pig meat suggested a lack of an antioxidant effect (Simitzis et al. 2010).

3.2.3.2 Seafood

The effect of different EOs (bay leaf, thyme, rosemary, black seed, sage, grape seed, flax seed or lemon oils) on lipid oxidation of chub mackerel (*Scomber*

japonicus) was studied during 11 months of frozen storage at −20 °C (Erkan 2012). Thyme oil treatment was found to be particularly effective in delaying lipid oxidation. However, the other EO treatments resulted in lower TBA and free fatty acid values in the fish than those of control samples throughout storage. The addition of oregano EO at 0.2 % to fresh salted, packaged rainbow trout (*Oncorhynchus mykiss*) fillets stored for a period of 21 days at 4 °C also showed that lipid oxidation, as determined by thiobarbituric acid values (TBA), did not occur during the refrigerated storage period (Pyrgotou et al. 2010).

The efficacy of oregano and thyme EOs in quality retention of refrigerated (4 °C) squid (*Loligo vulgaris*) ring ready-to-eat (RTE) product was studied by (Sanjuás-Rey et al. 2012). Both EOs had inhibitory effects on lipid oxidation, as determined by the presence of peroxide and thiobarbituric acid-reactive substances and the formation of interaction compounds, was observed, with oregano oil being more effective at lower concentrations.

Successful inhibition of lipid oxidation was also achieved using *Zataria multiflora* EO, either separately (0.2/0.4 %) or in combination with a coating, on fresh silver carp (*Hypophthalmichthys molitrix*) fillets during storage at 4 °C (Zabol 2012). Likewise, laurel (*Laurus nobilis*) and cumin (*Cuminum cyminum*) EOs induced a decrease in lipid oxidation by ca. 40 % of TBA value on fresh vacuum-packed (VP) wild and farmed sea bream (*Sparus aurata*) fillets evaluated during storage on ice (Attouchi and Sadok 2010).

A mixture of 0.25 % turmeric and 0.25 % lemongrass EOs showed synergistic effects on the retardation of lipid oxidation of green mussel (*Perna canalicula*) stored at 4 °C (Masniyom et al. 2012). However, samples treated with 0.5 % lemongrass oil exhibited a higher likeness score for odour and flavour compared to samples treated with other EOs, making lemongrass EO the most promising agent to prevent deterioration and maintain the odour and flavour attributes of mussel during prolonged refrigerator storage.

Another strategy to delay lipid oxidation of fish products is the reduction of stress during transportation. *Lippia alba* EO has shown to be effective for reducing the formation of peroxides and thiobarbituric reactive substances compared to control fillets of silver catfish (*Rhamdia quelen*). It can be concluded that *L. alba* EO used as a sedative in the water to transport silver catfish can delay the lipid oxidation of fillets during frozen storage (Veeck et al. 2012).

3.2.3.3 Other Foods

Oregano and laurel EOs have been shown to protect fried-salted peanuts against lipid oxidation, considerably increasing their shelf life, whereas rosemary EO was less effective (Olmedo et al. 2008). It was concluded that these EOs could be used as natural antioxidants in foods with high lipid contents. In another study, these authors evaluated the antioxidant effect of aguaribay (*Schinus areira*) and cedron (*Aloysia triphylla*) EOs on fried-salted peanuts and found that these EOs were also

effective against lipid oxidation. However, these EOs could affect the sensory profile and consumer acceptance of the product (Olmedo et al. 2012).

Asensio et al. (2011) evaluated the preservative effect of 0.05 % oregano EO on extra virgin olive oil during storage. Chemical indicators of lipid oxidation were measured, and in general, olive oil samples with added oregano EO had lower peroxide, conjugated dienes and p-anisidine values and higher chlorophyll and carotenoid contents during storage (Asensio et al. 2011). Likewise, the antioxidant effects of EOs from rosemary, clove and cinnamon were determined on hazelnut and poppy oils (Özcan and Arslan 2011). These EOs were added at concentrations of 0.25 and 0.5 % to the oils and stored at 50 °C in the dark for 14 days. All tested EOs showed an antioxidant effect compared to control groups, and cinnamon oil was the most effective at retarding lipid oxidation of crude oils followed by clove and rosemary oils. Microwave heating induces severe quality and composition losses in soybean oil. To protect the oil, spike lavender (*Lavandula latifolia*) EO has been added and was shown to counteract the oxidation compared to control oils (Rodrigues et al. 2012).

In a further study, coriander EO (0.05, 0.10 and 0.15 %) was successfully applied as a natural antioxidant in cake during 60 days of storage at room temperature. Interestingly, the sensory properties of cakes containing 0.05 % CEO were not different from those of controls ($P < 0.01$) (Darughe et al. 2012).

3.3 Antibacterial Effects of Essential Oils

3.3.1 General Aspects

Antibacterial activity can be either bacteriostatic (inhibition of the bacterial growth) or bactericidal (destruction of bacterial cells). However, differentiating between these two actions is sometimes difficult. Antibacterial activity in relation to these two different modes of action is measured as the minimum inhibitory concentration (MIC) or the minimum bactericidal concentration (MBC) (Burt 2004). The antibacterial activity of EOs has been evaluated by adapting standard tests for antibiotic resistance. This process is especially difficult for EOs due to the volatility of some active substances, their high viscosity and their water insolubility. However, most studies that focus on the antibacterial activity of EOs are aimed at the designation of a possible inhibitory effect against bacteria, with special interest on health, environment and food. To rapidly screen for antibacterial activities of many EOs against a broad range of bacteria, the agar diffusion method is the most widely used. The EOs are applied to the inoculated agar using reservoirs, such as filter paper disks or holes punched in the agar. After incubation, the diameters of a possible inhibition zone are measured (Faleiro 2011).

The inhibitory action of EOs against bacteria is linked to the high hydrophobicity that causes higher cell permeability and results in the loss of cellular

components, such as ions, ATP, glucose, etc. Depending on the quantity and strength of the EO, an irreversible damage of the cell membrane induces the lysis of the bacterial cells (cytolysis) and therefore death. The difference in the cell wall structure of Gram-negative and Gram-positive bacteria explain the differences in antibacterial action, with Gram-positive strains much more sensitive to EOs. Another suggested mechanism is the inhibition of amylase and protease production, which stops toxin production and electron flow and results in coagulation of the cell content (Smith-Palmer et al. 2004; Di Pasqua et al. 2007; de Souza et al. 2010).

Essential oils and their components are known to exhibit inhibitory activity against a variety of bacteria. The oils of plants of the family *Lamiacae* are among the EOs that have been demonstrated to have a strong antimicrobial activity. Oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) oils are the most studied EOs exhibiting antibacterial activity against a broad range of Gram-positive and Gram-negative bacterial species. Additionally, a number of further *Origanum*, *Salvia* and *Mentha* species showed effective inhibition of bacterial growth (Hammer et al. 1999; Dorman and Deans 2000; Moreira et al. 2005; Gutierrez et al. 2008).

The antibacterial effect of EOs is attributed to a small variety of phenolic compounds and terpenoids, which have antibacterial activity in their pure form (Aureli et al. 1992). Carvacrol and thymol, the main components of EOs from *Lamiacae* family plants, have the most well-studied effect against bacteria. Other components with antibacterial activity are 1,8-cineole, citral, eugenol, geraniol, α-pinene, perillaldehyde and terpinen-4-ol (Kim et al. 1995b; Cosentino et al. 1999; Lambert et al. 2001). When applying all substances present in an EO, the antimicrobial effect of the individual compounds can be combined (additive effect), reduced (antagonist effect) or enhanced (synergetic effect).

The increasing interest in EOs as antibacterial agents resulted in a huge number of studies that analysed the antibacterial potential of many EOs against a broad range of bacterial species. Nevertheless, the great variability of plants that produce EOs, the differences in composition of EOs of even closely related plant species and the diversity of testing methods make generalisation very difficult. The antibacterial activities of a number of EOs against a huge number of bacterial species are listed in the book "Handbook of Essential Oils: Science, Technology, and Applications" (Baser 2010). Furthermore, reviews covering the antimicrobial activity of EOs summarise the large amount of screens performed to determine the antibacterial activity of specific EOs against a number of bacterial species, mainly pathogens (Kalemba and Kunicka 2003; Burt 2004; Lang and Buchbauer 2012). As mentioned before, some oils inhibit only Gram-positive strains, whereas EOs with a stronger antibacterial activity can inhibit both Gram-positive and Gram-negative strains due to the higher sensitivity of Gram-positive bacterial strains to EOs. The two Gram-negative species *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, both human pathogens that cause serious infections in clinics, exhibited a special robustness against EOs. Nevertheless, the oils of *Rosmarinus officinalis*, *Nepeta cataria*, *Mentha longifolia*, *Mentha viridis* and *Monticalia*

andicola showed a great inhibitory effect against *K. pneumoniae,* and oils from *Salvia rubifolia*, *M. andicola*, *Eugenia beaurepaireana* and *Pituranthos chlorantus* strongly affected *P. aeruginosa*. In contrast, *Aeromonas* species were very susceptible to most EOs.

3.3.2 Antibacterial Effects of EOs and Biomedical Applications

EO-producing plants have always been used as medicinal plants and antimicrobial agents in traditional medicine long before the discovery of microorganisms. Currently, the popularity of EOs is increasing because of the rejection of synthetic drugs whenever possible due to their unwanted side effects. Furthermore, the increasing drug resistance of clinically relevant bacterial strains represents a global problem. Thus, one of the main research aims in this field is the search for new antibacterial agents, especially natural compounds.

>EOs from organisms such as *Cleistocalyx operculatus*, *Eucalyptus globulus*, *Lavandula stoechas*, *Lavandula angustifolia*, *Lavandula luisieri*, *Melaleuca alternifolia*, *Salvia rosaefolia*, *Tanacetum parthenium*, *Thymus vulgaris* and *Zataria multiflora* have shown an inhibitory effect against methicillin-resistant *Staphylococcus aureus* strains (MRSA) (Dung et al. 2008; Roller et al. 2009; Polatoglu et al. 2010). *Z. multiflora* showed the highest antibacterial activity against MRSA, with an MIC of 0.25–1.0 μl/ml (Mahboubi and Ghazian Bidgoli 2010), followed by *T. vulgaris* that had an MIC of 18.5 μg/ml (Tohidpour et al. 2010). The activity of these two EOs is linked to their high thymol contents. The other mentioned active EOs contain mainly 1,8-cineole and terpinen-4-ol. *Helichrysum italicum* exhibited antibacterial activity against a number of Gram-negative drug-resistant strains, such as *Acinetobacter baumanii*, *Enterobacter aerogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* (Lorenzi et al. 2009). McMahon et al. (2008) made the important observation that some staphylococci have adapted to the EOs of *M. alternifolia*, indicating that bacterial strains can develop resistance to EOs similarly to antibiotics (McMahon et al. 2008). Thus, it is important to apply the EOs in a high concentration to achieve the irreversible damage of cells and avoid the evolution of resistance.

Limonene-containing EOs, such as those from several *Citrus* species, *Abies koreana* and *Fortunella japonica*, showed a high efficacy against *Propionibacterium acnes* and *Staphylococcus epidermidis*, both of which cause serious skin infections. The MICs were between 0.3 and 10 μl/ml, and these EOs could be applied in the treatment of skin infections, as well as in cosmetics to prevent infections (Baik et al. 2008; Kim et al. 2008; Yoon et al. 2009; Yang et al. 2010a). In general, EOs are suitable for use as bio-preservatives in cosmetic formulations, inhibiting bacterial growth and at the same time providing further beneficial effects and aroma.

The EOs of *Achillea ligustica*, *Mentha longifolia*, *Hyptis pectinata*, *Mentha piperita* and *Rosmarinus officinalis* effectively inhibit the growth of the dental

bacteria *Streptococcus pyogenes* and *Streptococcus mutans* more than chlorohexidine, thus making them possible substrates for dental formulations to avoid caries (Nascimento et al. 2008; Maggi et al. 2009).

The bacterium *Helicobacter pylori* colonises the human stomach and can cause ulcers and gastritis, thus making it a major target of antibacterial studies. However, few EOs have been tested against this bacterial species, although inhibitory effects on *H. pylori* have been shown for the EOs of *Apium nodiflorum*, *Plinia cerrocampanensis* and *Thymus caramanicus*, which have MICs of 12–60 μg/ml (Menghini et al. 2010; Vila et al. 2010). The EO of *Dittrichia viscosa* subsp. *revoluta* also has high antibacterial activity with an MIC of 0.33 μl/ml and could be applied for the treatment or prevention of *H. pylori* infections (Miguel et al. 2008).

3.3.3 Antibacterial Effects of EOs and Applications in Food Preservation

Historically, EOs have been used for flavour in foods and beverages. Due to their variable antimicrobial compound contents, they have a great potential as natural agents for food preservation.

Different EOs have been tested against foodborne pathogenic and spoilage bacteria. The oil from *Artemisia incana* showed considerable inhibitory effects against 26 foodborne pathogenic bacteria (Cetin et al. 2009). Likewise, *Magnolia liliflora* and *Allium schoenoprasum* (chives) EOs inhibited a number of food spoilage and foodborne pathogenic bacteria (Bajpai et al. 2008; Rattanachaikunsopon and Phumkhachorn 2008). *Artemisia echegarayi* EO exhibited antibacterial activity against the seven foodborne pathogens (*Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* serovar e*nteritidis* and *Salmonella enterica* serovar *typhimurium)*, but not against *Proteus mirabilis.* Two terpenes, thujone and camphor, were identified from this essential oil as the principal constituents responsible for antibacterial activity (Laciar et al. 2009). The leaf EOs from seven Himalayan Lauraceae species (*Neolitsea pallens*, *Lindera pulcherrima*, *Dodecadenia grandiflora*, *Persea duthiei*, *Persea odoratissima*, *Persea gamblei* and *Phoebe lanceolata*) had potent antibacterial activities against three Gram negative (*E. coli*, *S. enterica* and *Pasteurella multocida*) and one Gram positive (*S. aureus*) foodborne bacterial species, with MIC values between 3.90 and 31.25 μl/ml (Joshi et al. 2010). Additionally, laurel oil (*Laurus nobilis*) inhibited the microbial growth of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Enterococcus faecalis* and *Listeria monocytogenes* at concentrations from 0.02 to 2.5 % (vol/vol) but was inactive against *Escherichia coli* and *Salmonella typhimurium* (Erkmen and Özcan 2008). In a further study, the effects of garlic, bay, black pepper, origanum, orange, thyme, tea tree, mint, clove and cumin EOs on *L. monocytogenes*, *E. coli*, *Salmonella enteritidis*, *P. mirabilis* and *B. cereus* were analysed, showing that thyme, origanum, clove and orange EOs have strong

antibacterial activities against these bacterial species (Irkin and Korukluoglu 2009). The effect of EOs on the important foodborne pathogenic species *Salmonella* spp. has been evaluated in several studies. Citrus EOs had antibacterial activity against 11 serotypes/strains of *Salmonella*, with the most active compound being terpenes from orange essence (MIC = 0.125 % − 0.5 %) that is composed principally of d-limonene (94 %) and myrcene (3 %) (O'Bryan et al. 2008). Carvacrol, citral and geraniol showed potent antibacterial activities against *Salmonella typhimurium* and its rifampicin-resistant strain. The most potent compound in this EO was carvacrol, with an MIC value of 250 μg/ml for both strains (Kim et al. 1995a). Similarly, 63 *Campylobacter jejuni* isolates were screened for their resistance and susceptibility to cinnamaldehyde and carvacrol, the main constituents of plant-derived cinnamon and oregano EOs. The results showed that both substances exhibited a rapid antimicrobial activity against both antibiotic-resistant and non-resistant *C. jejuni* strains at concentrations of ~0.1 % and higher (Ravishankar et al. 2008).

However, even if a number of EOs showed inhibitory effects of the growth of a number of food pathogenic and spoilage species, it should be mentioned that these studies were carried out with pure bacterial cell cultures. The antibacterial action of EOs for the use in a complex food matrix can be very different and requires separate studies in the corresponding food of interest. In general, a much higher concentration is needed in food compared to the MIC determined for pure bacterial cultures. This is most likely due to the higher nutrient content in foods that enables the bacterial cells to repair cell damage (Gill et al. 2002). In addition, the high fat and protein content protects the bacterial flora against the EO activity. This assumption has been confirmed by various studies because EOs had a higher antibacterial effect in low-fat food than in high-fat food (Tassou et al. 1995; Smith-Palmer et al. 2001; Burt 2004).

It should be mentioned that for an effective application in the food sector, only EOs with a strong antibacterial potential are suitable, enabling application of very low concentrations that do not greatly influence the organoleptic properties of the corresponding food product. Additionally, for the safe application as preservative agents in foods, the activity against probiotic bacteria of the intestinal flora should be low to prevent negative side effects (Cetin et al. 2010). EOs or compounds isolated from EOs are applied directly to the food product or combined with further preservation methods, such as vacuum or MAP (Irkin and Esmer 2010). Thus, the preservative effects of increasing the salt content and decreasing the pH, storage temperature and amount of oxygen has a synergetic effect on the antibacterial activity of EOs (Skandamis and Nychas 2001). To achieve a thorough interaction of the EOs with the product, layers or films of the EOs are being applied.

Because the antibacterial effect and influence on the odour and flavour can vary significantly between the different food matrices, every EO has to be tested in the corresponding food product. In a number of studies, EOs have been tested in specific foods or in food models for their antibacterial activity in studies that are reviewed in the following sections.

3.3.3.1 Meats

In various meats, oils from coriander, clove, oregano and thyme exhibited elevated inhibitory effects against *L. monocytogenes* and *A. hydrophila* at concentrations of 5–20 μl/g (Tsigarida et al. 2000; Menon and Garg 2001; Burt 2004). When screening the alcohol extracts of angelica root, banana purée, bay, caraway seed, carrot root, clove (eugenol), marjoram, pimento leaf and thyme, only clove and pimento extract significantly inhibited the growth of *A. hydrophila* and *L. monocytogenes* in refrigerated, cooked, ready-to-eat meat (cooked chicken, refrigerated cooked beef and beef slices prepared from roasted whole sirloin tips). *A. hydrophila* was more sensitive to the EOs (Hao et al. 1998a, b). Cilantro EO was not suitable to inhibit the growth of *L. monocytogenes* strains on vacuum-packed ham. Ham disks were inoculated with a cocktail of five *L. monocytogenes* strains, treated with 0.1, 0.5 and 6 % cilantro oil diluted in sterile canola oil or incorporated into gelatine that included lecithin to enhance the incorporation of the cilantro oil, then vacuum-packed and stored at 10 °C. An inhibitory effect was observed only for the highest concentration of cilantro oil (Gill et al. 2002).

The inhibitory effect of oregano EO on autochthonous spoilage microflora on minced meat packed under aerobic or modified atmosphere (MA) and stored at 5 °C has also been studied. In all packaging conditions, only concentrations of 0.5 and 1 % oregano oil were effective. In addition to the microbial growth analysis, microbial metabolite formation has been evaluated. The results indicated that oregano EO delayed glucose and lactate consumption under aerobic and MA conditions. Furthermore, under aerobic storage, proteolysis was significantly inhibited, and the production of acetate under the MA was inhibited (Skandamis and Nychas 2001). The oil of mustard reduced the growth of aerobic mesophilic bacteria, as well as lactic acid bacteria, in an acidified chicken meat model stored for 2 weeks at 22 °C (Lemay et al. 2002).

An increase of the preservative effect of MAP and vacuum packaging with bay (*Laurus nobilis*) EO has been shown in ground chicken breast meat stored at 4 °C. Total viable counts (TVC), as well as the growth of two important foodborne pathogens, *L. monocytogenes* and *E. coli,* were notably reduced (Irkin and Esmer 2010).

In further studies, a synergetic effect has been observed for EOs combined with nisin against foodborne pathogens in mince meat products during storage at 4 and 10 °C for 12 days. Thus, the bactericidal effect of oregano EO against Salmonella *enteritidis* in minced sheep meat and the antibacterial effect of thyme EO against *L. monocytogenes* and *E. coli* in minced beef meat were enhanced by the addition of nisin. In all of these studies, the inhibitory effect was higher when stored at 10 °C than at 4 °C (Solomakos et al. 2008a, b; Govaris et al. 2010).

The combination of thyme oil and chitosan was applied to ready-to-cook chicken-pepper kebab stored under aerobic conditions at 4 °C for a period of 12 days. Treatments reduced the microbial growth, with lactic acid bacteria (LAB), *Brochothrix thermosphacta* and Enterobacteriaceae being highly sensitive against this combination, while Pseudomonads were the most resistant. The products' shelf lives were extended by ca. 4 and 6 days (Giatrakou et al. 2010).

The use of antibacterial films as wrappings has been demonstrated to be an effective technology for controlling surface contamination by foodborne pathogenic microorganisms in meat and poultry products. Edible films containing cinnamaldehyde or carvacrol (0.5, 1.5, 3 %) showed antibacterial effects against *S. enterica* and *E. coli* O157:H7 artificially inoculated onto chicken breasts and *L. monocytogenes* artificially inoculated onto ham. Carvacrol films were more active against all three pathogens than cinnamaldehyde films (Ravishankar et al. 2009).

3.3.3.2 Seafood

Food products of marine origin are known to spoil rapidly due to the growth and metabolism of bacteria because fish and other seafood represent an optimal growth medium for spoilage bacteria. Thus, the enhancement of seafood shelf life has special interest in the fishing and food industries. However, only a few studies have examined the application of EOs to avoid microbial spoilage of seafood. The oregano EO is the most studied EO in this context and exhibits the best antibacterial activity against seafood spoilage bacterial species. As in meat products, the antibacterial effects of EOs are influenced by fat content, with high fat content causing less efficacy (Burt 2004). The application of EO-coatings and spreading the EOs on the surface of fish has been demonstrated to be effective in enhancing the shelf life of seafood products (Ouattara et al. 2001; Harpaz et al. 2003).

Thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) were added at 0.05 % (vol/vol) as preservatives for the cold storage of Asian sea bass (*Lates calcarifer*). Both EOs considerably slow the spoilage process, with the fish treated with these oils being adequate for consumption after 33 days of storage (Harpaz et al. 2003).

Photobacterium phosphoreum is one of the main bacterial spoilage species in vacuum-packed or MAP fish products. Of nine tested EOs, oregano and cinnamon EOs had the strongest antimicrobial activity on *P. phosphoreum,* and oregano oil (0.05 %, v/w) reduced the growth of *P. phosphoreum* in naturally contaminated MAP cod (*Gadus morhua*) fillets and extended shelf life from 11–12 to 21–26 d at 2 °C (Mejlholm and Dalgaard 2002).

The combination of low-dose gamma irradiation and EO coating on the shelf life of pre-cooked shrimp (*Penaeus* spp.) stored at 4/−1 °C has been studied. Antimicrobial coatings were prepared by incorporating various concentrations of thyme oil and trans-cinnamaldehyde in coating formulations prepared from soy or whey protein isolates. The results showed that gamma irradiation and EO coating had synergistic effects, reducing the total aerobe counts and inhibiting the growth of *P. putida*, leading to an extension of shelf life of at least 12 days (Ouattara et al. 2001).

The antimicrobial effect of oregano and thyme EOs on a refrigerated (4 °C) squid (*Loligo vulgaris*) ring ready-to-eat (RTE) product has been studied by Sanjuás-Rey et al. (2012). Oregano EO exhibited an inhibitory effect on the microbial activity of aerobic, anaerobic and psychrotrophic bacterial species, as

well as of Enterobacteriaceae. EOs were added at different concentrations to the coating medium during processing. Oregano EO showed a more pronounced effect at higher concentrations. The application of oregano EO was combined with MAP, resulting in an enhancement of the quality of the squid rings (Sanjuás-Rey et al. 2012). Likewise, oregano EO combined with MAP and storage at 4 °C inhibited the microbial growth on salted sea bream (*Sparus aurata*) (Goulas and Kontominas 2007), sword fish (*Xiphias gladius*) fillets (Giatrakou et al. 2008) and salted rainbow trout (*Oncorhynchus mykiss*) fillets (Pyrgotou et al. 2010). The studies in rainbow trout showed significantly reduced growth of Lactic acid bacteria (LAB), followed by H_2S-producing bacteria (including *Shewanella putrefaciens*), *Pseudomonas* spp. and *Enterobacteriaceae* when treated with salt and oregano EO and stored under MAP conditions (Frangos et al. 2010).

However, the addition of thyme EO did not lead to any inhibitory effects on the microbial activity of the refrigerated RTE squid (Sanjuás-Rey et al. 2012). In contrast, the application of thyme EO extended the shelf life of fresh fish, such as gilthead sea bream (*Sparus aurata*) (Attouchi and Sadok 2010) and sea bass (*Dicentrarchus labrax*) fillets when combined with MAP and swordfish (*Xiphias gladius*) (Kykkidou et al. 2009). Two further studies compared the antibacterial effects of oregano and thyme EO on cod (*Gadus morhua*) fillets (Mejlholm and Dalgaard 2002) and Asian sea bass (*Lates calcarifer*) (Harpaz et al. 2003). The oregano EO exhibited a stronger antibacterial activity in the first study, but no differences between both types of oils were observed in slowing the spoilage in the second study.

The effect of *Zataria multiflora* EO on the quality of fresh silver carp (*Hypophthalmichthys molitrix*) fillets has been studied, showing an inhibition of microbial growth when used alone or in combination with coating (Zabol 2012). Furthermore, turmeric and lemongrass EOs lowered the microbial deterioration of green mussels (*Perna canaliculus*). The best results were obtained by applying a mixture of both EOs: 0.25 % turmeric EO and 0.25 % lemongrass EO (Masniyom et al. 2012).

The preservative effect of laurel EO (*Laurus nobilis*) and cumin EO (*Cuminum cyminum*) has also been demonstrated on fresh vacuum-packed sea bream (*S. aurata*) fillets. The treatment of wild and farmed sea bream fillets with laurel or cumin EOs induced a decrease in bacterial growth by ca. 0.5–1 log cfu/g, extending the shelf life of fish fillets by approximately 5 days of ice storage (Attouchi and Sadok 2010).

3.3.3.3 Dairy Foods

Most studies of the application of EOs in dairy products were aimed at the inhibition of the human pathogens *L. monocytogenes*, *S. enteritidis* and *E. coli O157:H7*.

In cheese, the growth of *L. monocytogenes* and *S. enteritidis* was restricted by clove, bay, cinnamon and thyme EOs when they were applied at concentrations of 1 % and stored at 4 and 10 °C. Only clove oil was effective against these two pathogens in cheese (Smith-Palmer et al. 2001; Vrinda Menon and Garg 2001).

In a subsequent study, feta cheese was inoculated with E. coli O157:H7 or *L. monocytogenes* and was then stored under MAP at 4 °C. The addition of oregano and thyme EOs at doses of 0.2 ml/100 g resulted in a decrease of the bacterial populations of both species (Govaris et al. 2011).

A synergetic inhibitory effect on *L. monocytogenes* was observed in skimmed milk, when HHP treatment was combined with carvacrol (Karatzas et al. 2001).

The inhibitory effect of a mixture of plant EOs (DMC) against a broad range of foodborne pathogens and spoilage species was tested in Spanish soft cheese. The EO mixture showed inhibitory activity against Gram-positive species such as *L. monocytogenes*, *Listeria innocua*, *Staphylococcus aureus*, *Lactobacillus brevis* and *Micrococcus luteus.* The EOs were less effective against Gram-negative species, such as *Salmonella choleraesuis*, *E. coli* O157:H7, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. No inhibitory effects were observed on *Pseudomonas fluorescens* (Mendoza-Yepes et al. 1997).

3.3.3.4 Other Foods

The antibacterial effects of natural volatile compounds against *Salmonella* have been studied on alfalfa seeds and sprouts. Only acetic acid, cinnamic aldehyde and thymol caused significant reductions in *Salmonella* populations after treatment for 7 h (Weissinger et al. 2001).

Likewise, the antibacterial activity of carvacrol on the foodborne pathogen *Bacillus cereus* was studied on rice. Carvacrol caused a growth inhibition of this pathogen at concentrations of 0.15 mg/g and higher. Due to the intense smell and taste of carvacrol at high concentrations, the treatment was combined with cymene, resulting in a synergistic effect (Ultee et al. 2000).

In a subsequent study, the effects of carvacrol and cinnamic acid on microbial spoilage of fresh-cut fruit were studied. Fresh-cut kiwifruits and fresh-cut honeydew were dipped in carvacrol or cinnamic acid solutions at 1 mM and resulted in a reduction of TVC during storage for 5 days at 4 and 8 °C, without adverse sensory consequences (Roller and Seedhar 2002).

Additionally, a positive inhibitory effect of thyme oil against *L. monocytogenes* was found in apple–carrot juice stored at 4 °C, at a concentration of 0.5 % and against E. coli O157:H7 on shredded lettuce and baby carrots stored at 4 °C after washing with a suspension of the EO (1.0 ml/l for 5 min) (Singh et al. 2002).

3.4 Antifungal Activity of Essential Oils

3.4.1 General Aspects

When analysing the chemical composition of EOs that exhibit strong antifungal activity against moulds, no obvious patterns become apparent. Some EOs contained predominantly non-phenolic terpenes, while others contained a high

percentage of phenolic monoterpenes, such as thymol and carvacrol. The phenylpropanoid eugenol was detected often in EOs with strong activity against moulds. In particular cases, the non-phenolic bicyclic monoterpenes camphor and α-pinene exerted noteworthy antifungal activities. EOs that exert strong antimicrobial activity against yeasts possess high thymol, carvacrol, cymene, linalool or α-pinene contents. Remarkably, many EOs with inhibitory effects against yeasts were found in genera of the Lamiaceae family including *Thymus* spp., *Origanum* spp., *R. officinalis, O. sanctum* and *Z. multiflora*. All of these EOs had at least one of the previously mentioned substances as the primary component.

Fungicidal mechanisms can involve the destruction of existing mycelia, as well as the inhibition of new mycelia development. Therefore, *Citrus sinensis* EO, which was noticed to be rich in limonene (84.2 %), exerted a marked antifungal activity against *Aspergillus niger* by destroying its mycelial cell walls (Sharma and Tripathi 2008). Moreover, EOs have been found to be capable of inhibiting the formation of spores. Thus, chamazulene was found to be the lead molecule in *Achillea millefolium* EO constituting 42.2 % of the whole oil. This EO was found to exert genotoxic effects against the fungal cells and suppress the development of spores (Sant'Anna et al. 2009).

Many moulds are able to produce toxic molecules, so-called mycotoxins, which represent a threat to human health because some of them (e.g., aflatoxin) act as carcinogens. In a preliminary study, the inhibitory effect of various concentrations of ground mint, sage, bay leaves, thyme, aniseed and red pepper and citrus peel oils on the growth and toxin production of an aflatoxin-producing strain of *Aspergillus parasiticus* was studied (Karapinar 1985). Of the different herbs tested, thyme was found to be a highly effective antifungal agent. Growth and aflatoxin formation were depressed by a 10-day incubation with orange and lemon EOs at a 1.6 % concentration. In the same manner, EOs of dill, coriander, basil, marjoram, rosemary, mint and thyme demonstrated antifungal activities against *Aspergillus flavus* and inhibited aflatoxin B1 production in vitro (El-Habib 2012). Dill EO was the most effective against aflatoxin production, while the EO of thyme and basil delayed the growth of *A. flavus*. In a recent study, the antifungal activity of cinnamon EO was evaluated on *A. flavus,* and the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) were determined in direct contact with the mould by macrodilution. A strong activity was observed with an MIC of 0.05–0.1 mg/ml and an MFC of 0.05–0.2 mg/ml. Furthermore, polyethylene terephthalate films containing cinnamon EO were tested in vapour phase, without direct contact with the mould, and produced total inhibition at 4 % CIN EO (Becerril et al. 2013).

In another study, the antifungal properties of 16 EO constituents were tested against five *Aspergillus* spp., four *Penicillium* spp. and two *Fusarium* spp. that are widely reported to contaminate foods. The strongest antifungal activity was caused by o- or p-alkyl substituted phenols; most potent compounds were isoeugenol, cinnamaldehyde, carvacrol, eugenol and thymol. The most resistant organism was *Penicillium verrucosum* var. *cyclopium* followed by *Penicillium roqueforti*, whereas *Penicillium. viridicatum* was the most sensitive (Knobloch et al. 1989).

Oregano (*Origanum hypericifolium*) EO was active against 14 fungi isolated from hazelnut and walnut and completely inhibited (100 %) hyphal growth when in direct contact and in headspace assays after 3 and 6 days, respectively. This high inhibitory effect correlates with the presence of aromatic components, such as monoterpenes, carvacrol, thymol and p-cymene (Ocak et al. 2012).

3.4.2 Inhibition of Fungal Activity and Health Aspects

Essential oils have exhibit marked antifungal activities. These inhibitory effects have been observed on different types of fungi such as dermatophytic fungi, moulds, phytopathogenic fungi and yeasts (Lang and Buchbauer 2012). Various dermatophytic fungi are responsible for the generation of fungal infections on human skin, nails and hair. Dermatophytes such as *Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum* are more susceptible to EOs compared to other types of fungi such as *Aspergillus* species and yeasts. The prevalent substances in the EOs that have been reported to have activity against dermatophytic fungi cannot be assigned to one particular chemical group, but it seems that the presence of phenylpropanoids (e.g., estragole, eugenol) and the monocyclic sesquiterpene alcohol α-bisabolol correlates with strong antifungal effects against fungi that cause skin infections.

Moreover, thymol, carvacrol and geraniol were shown to inhibit the development of *Candida* biofilms.

3.4.3 Inhibition of Fungal Activity and Food Preservation

In a preliminary attempt, the fungistatic properties of EOs from lavender, marjoram, mint, basil, sage, savoury, thyme and verbena were tested against a number of food spoilage and pathogenic fungi. The greatest fungistatic activities were shown by thyme, savoury and verbena oil (Pellecuer et al. 1979).

Several EOs that exhibited antimicrobial activity against phytopathogenic fungi belong to the Lamiaceae family. Furthermore, the existence of carvacrol, α-pinene and p-cymene in the EOs was related to a high antifungal effect. Additionally, the phenylpropane derivate eugenol is of a particular importance because it showed antifungal activity against a wide range of different phytopathogenic fungi. EO from cymbopogon, ajowan and dill seed exhibited high antifungal activity against plant and food roots. Cymbopogon oil was proposed to serve as a broad-spectrum fungistatic compound for the control of phytopathogenic fungi. The bioactive ingredients were identified as the simple monoterpenes geraniol, thymol and carvone, which have minimum inhibitory concentrations of 160, 200 and 225 mug/ml, respectively. It has been suggested that synthetic modifications can be applied to these molecules, especially to geraniol, to obtain an optimal antifungal product with lipophilicity (Sridhar et al. 2003). Amiri et al. (2008) isolated eugenol from *Syzygium*

aromaticum EO, and an eugenol-lethicin combination was subsequently tested for antifungal activity against phytopathogens. The presence of eugenol effectively diminished fungal infections in stored apples, underscoring their potential use as biofungicide of this combination (Amiri et al. 2008).

Antifungal activities of EOs from thyme, summer savoury and clove were evaluated in culture medium and tomato paste (Omidbeygi et al. 2007). The results showed that all EOs inhibited the growth of *A. flavus*, while the thyme oil and summer savoury showed the strongest inhibition at 350 and 500 ppm, respectively. Taste panel evaluations were carried out in a tomato ketchup base, and the 500 ppm thyme oil sample was accepted by panellists. The growth of *A. flavus* was also entirely impeded by applying savoury EO (thymol and carvacrol) on lemons 1 week before they were exposed to pathogens (Dikbas et al. 2008). In addition, this EO effectively suppressed the growth and the aflatoxin B1 and G1 synthesis of *A. parasiticus* (Razzaghi-Abyaneh et al. 2008). Likewise, the EOs from rosemary and ajowan inhibited the growth of *A. parasiticus* and its aflatoxin production. Ajowan EO was more effective in inhibiting growth, and rosemary EO was more effective against aflatoxin production. It was concluded that both EOs could be safely used as preservatives on some types of foods to protect them from toxigenic fungal infections (Rasooli et al. 2008). In a further study, antifungal effects of thyme and rosemary EOs on two aflatoxigenic *A. flavus* strains previously isolated from hazelnuts were investigated (Ozcakmak et al. 2012). Fungal growth was almost completely inhibited after a 90 min application of thyme EO concentration (250 and 125 μl/ml), while rosemary EO caused only a slight growth inhibition. Similarly, the prevalent substances in *Lippia alba* EO (neral, geranial), as well as the entire EO, were shown to inhibit both aflatoxin B1 production and *A. flavus* growth (Shukla et al. 2009). Moreover, the growth of other *Aspergillus* species and *Fusarium* strains was greatly impaired; thus, this EO was considered suitable for food preservation.

The effects of the EOs from lemon (*Citrus lemon*), Mandarin orange (*Citrus reticulata*), grapefruit (*Citrus paradisi*) and orange (*Citrus sinensis*) on the growth of moulds commonly associated with food spoilage (*A. niger*, *A. flavus*, *Penicillium chrysogenum* and *Penicillium verrucosum*) were studied (Viuda-Martos et al. 2008). All the oils had antifungal activity against all the moulds. Orange EO was the most effective against *A. niger*, and mandarin EO was most effective at reducing the growth of *A. flavus*, while grapefruit was the best inhibitor of the *P. chrysogenum* and *P. verrucosum* mould. Thus, citrus EOs can be considered a suitable alternative to chemical additives for use in the food industry.

The antifungal activity of cinnamon EO on pathogens such as *Rhizopus nigricans*, *A. flavus* and *Penicillium expansum* was also investigated. The results revealed that cinnamon oil had the potential to be employed as a natural antifungal agent for fruit applications, as cinnamaldehyde is its main constituent (Xing et al. 2010).

Despite its high antibacterial activity, oregano EO was also demonstrated to be a very competent antifungal agent. The EO of oregano has strong anti-*Aspergillus* activity and completely inhibited radial mycelial growth of *A. flavus*, *A. fumigatus*

and *A. niger*, during 14 days of interaction with MIC values between 80 and 20 μl/ml. In addition, this EO had a significant inhibitory effect on further assayed *Aspergillus* spp. and was able to inhibit fungal spore germination (Carmo et al. 2008; Mitchell et al. 2010).

Furthermore, oregano EO also showed inhibited yeast growth with MIC values of 20 and 0.6 μl/ml (Souza et al. 2007).

In further studies, a comparison of the antifungal activities of various EOs against a variety of food-spoiling yeasts was carried out in has been reviewed (Kunicka Styczyńska 2011). EOs from basil, garlic, marjoram, onion, peppermint, thyme, lemon and grapefruit were tested against yeasts that frequently cause food spoilage (*Candida rugosa, Debaryomyces hansenii, Kluyveromyces marxianus, Rhodotorula glutinis, Rhodotorula minuta, Saccharomyces cerevisiae, Trichosporon cutaneum, Yarrowia lipolytica* and *Zygosaccharomyces rouxii*) because they are the most popular spices and flavouring agents used in the food industry. The ranking of oils by their fungistatic activity against all these yeasts was as follows: thyme > marjoram > peppermint > basil > lemon > grapefruit > onion, garlic oils.

In recent studies, EOs were combined with coating and the ability to inhibit fungal growth was tested. Inhibition by vapour contact with *A. niger* and *Penicillium digitatum* by Mexican oregano (*Lippia berlandieri*), cinnamon (*Cinnamomum verum*) or lemongrass (*Cymbopogon citratus*) EOs added to amaranth, chitosan, or starch edible films was studied. It was postulated that edible chitosan edible that incorporate Mexican oregano or cinnamon EO could improve the quality of foods by the action of the volatile compounds on surface growth of moulds (Avila-Sosa et al. 2012). In another study, chitosan coatings, containing bergamot, thyme and tea tree EOs were applied to oranges (cv. Navel Powell), after inoculation with *Penicillium italicum* (Cháfer et al. 2012). Preventive antimicrobial treatments with coatings containing tea tree EO were the most effective with a reduction of the microbial growth of 50 %, compared to the uncoated samples. The results of this study provided a useful tool for the development of new environmentally friendly and healthier commercial applications in the control of the main postharvest fungal decay of citrus fruits.

3.5 Antiviral Activity of Essential Oils

A virus is a small infectious particle (20–300 nm), which is able to infect cells of another living organism and replicate itself. Viruses cannot reproduce on their own because they are composed only of genes and a protein coat and are sometimes surrounded by a lipid envelope. Viral infections provoke an immune response that usually eliminates the infecting virus.

Many EOs have been used for centuries in folk medicine, and in recent years, the biological properties of various EOs have been demonstrated by a number of studies (Adorjan and Buchbauer 2010; Elizaquível et al. 2012). To combat viral infections with EOs or their constituents seems to be a promising treatment

considering that many viral infections cannot be counteracted so easily by administering only a lozenge. Although more studies are necessary to analyse the biological properties of EOs and elucidate their mechanism of action, EO use in the treatment of viral diseases has been confirmed by many studies throughout the most recent decades.

In a preliminary attempt, antiviral activity was investigated in different fractions of rosemary extracts. An ethanol extract of steam-distilled rosemary EO was further fractionated by dichloromethane and hexane extraction and dried extracts were tested for activity against herpes simplex virus (HSV) propagated in human embryo lung fibroblast (HELF) cell cultures. The hexane extract was shown to have activity against HSV-2 and was not cytotoxic to HELF cells.

In later research, the antimicrobial activity of *Salvia fruticosa* EO exhibited cytotoxic activity against African green monkey kidney (Vero) cells and high levels of virucidal activity against HSV-1, a ubiquitous human virus (Sivropoulou et al. 1997).

The EO and extracts obtained from *Origanum acutidens* and methanol extracts from callus cultures were evaluated for their antiviral activities. Inhibitory effects of the methanol extracts from herbal parts on the reproduction of HSV-1 and a slight antiherpetic effect of callus cultures were observed. In contrast, none of the extracts inhibited the reproduction of influenza A/Aichi virus (Sökmen et al. 2004).

EOs from *Artemisia arborescens*, *Eugenia caryophyllus*, *Cedrus libani* and *Melissa officinalis* were tested against HSV-1 and HSV-2 and showed the ability to inhibit both viruses and cell-to-cell virus diffusion (Saddi et al. 2007; Loizzo et al. 2008; Schnitzler et al. 2008). The most promising oil, which had the highest activity against HSV-1, was *Juniperus oxycedrus* oil with an IC50 value of 200 μg/ml. The major constituents of this oil were found to be α-pinene and β-myrcene.

In a comparative study, the antifungal effects of various EOs (anise, hyssop, thyme, ginger, chamomile and sandalwood) against HSV-2 were tested. The screen identified chamomile as the most promising EO due to its higher selectivity index (Koch et al. 2008). In another study, the EO of star anise (*Illicium verum*) was also shown to be a promising antiviral candidate against HSV-1. Later on, the same authors reported antiviral activities of the EOs from eucalyptus, tea tree and thyme and of their major monoterpene compounds against HSV-1 (Astani et al. 2010). The highest selectivity index was shown by α-pinene and α-terpineol. Moreover, the mixtures of different monoterpenes, which are present in natural tea tree EO, revealed a 10-fold higher selectivity index and a lower toxicity than their isolated single monoterpenes.

Tea tree EO has also been tested against polio type 1, ECHO 9, Coxsackie B1, adeno type 2, HSV-1 and HSV-2 (Garozzo et al. 2009). As a result, tea tree EO showed no virucidal activity against polio 1, adeno 2, ECHO 9, Coxsackie B1, HSV-1 and HSV-2 but exhibited a slight virucidal effect against HSV-1 and HSV-2. The results of this study showed that tea tree oil can be considered a promising drug in the treatment of influenza virus infection.

The inhibitory effect of EOs of *Lippia alba*, *Lippia origanoides*, *Origanum vulgare* and *Artemisia vulgaris* on yellow fever virus (YFV) replication was investigated, showing antiviral activity against YFV through direct virus inactivation (Meneses et al. 2009). A subsequent study investigated the antiviral activity of Mexican oregano (*Lippia graveolens*) EO and its major component, carvacrol, against different human and animal viruses. An antiviral effect was detected against acyclovir-resistant herpes simplex virus type 1 (ACVR-HSV-1), acyclovir-sensitive HSV-1, human respiratory syncytial virus (HRSV), bovine herpesvirus type 2 (BoHV-2) and bovine viral diarrhoea virus (BVDV). The human rotavirus (RV) and BoHV-1 and 5 were not inhibited by the EO. Carvacrol alone exhibited high antiviral activity against RV, but it was less efficient than its effect on the other viruses.

The application of hyssop (*Hyssopus officinalis*) and marjoram EOs was evaluated for inactivation of non-enveloped viruses using murine norovirus and human adenovirus as models. A significant reduction of virus titres (TCID 50) was observed when EOs were used at different temperatures and times (Kovač et al. 2012).

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