Chapter 5
Antimicrobial Activities of Essential Oils

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Abstract Essential oils are one of the most important groups of plant constituents responsible for biological activity of herbs and spices, and especially for their medicinal and antimicrobial properties. Numerous *in vitro* studies have demonstrated activity of different essential oils against bacteria, moulds and yeast. The power of essential oils is connected with their main constituents. The oils containing phenols such as thymol, carvacrol, and eugenol, exhibit the most pronounced activity against all kinds of microorganisms. This chapter gives a literature review of recent *in vitro* investigations concerning antibacterial and antifungal activity of essential oils. The oils recognized as the most valuable antimicrobial agents and used as food ingredients will be presented, namely thyme oil, clove oil, different cinnamon, mint and citrus oils, and rosemary oil.

Keywords Essential oils • Antibacterial activity • Antifungal activity • Thyme oil • Clove oil • Cinnamon oils • Mint oils • Citrus oils • Rosemary oil

5.1 Introduction

Spices and herbs have been used as food additives since ancient times, both as flavoring agents and as natural food preservatives. Essential oils, odorous and volatile products derived from plants, have found a considerable range of applications. They are used mainly as flavors and fragrances in food and perfumery industries. However, due to their antimicrobial activity essentials oils are also important for food and cosmetic preservation and for the control of human, animal and plant diseases that are of microbial origin.

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With growing interest in the use of essential oils in the food, agricultural and pharmaceutical industries, examination of these natural products has become increasingly important. In recent years antibacterial and antifungal potential of essential oils have been extensively researched and reviewed (Kalemba and Kunicka 2003; Burt 2004; Edris 2007; Reichling et al. 2009; Tajkarimi et al. 2010). In this chapter *in vitro* antimicrobial potential of some essential oils will be presented in the light of the recent 15 years investigations. The focus will be directed to the essential oils commonly used as food ingredients which antibacterial and antifungal activity have been well documented.

5.2 Distribution of Essential Oils

Essential oils are complex mixtures of volatile compounds produced by plants as secondary metabolites. They are obtained of taxonomically defined plant material mainly by water or steam distillation with the exception of essential oils from citrus peels obtained by cold pressing. Fragrance- and flavor-producing substances can be isolated by many other methods. However, such products shall not be considered as essential oils (Franz and Novak 2010).

Essential oils are biosynthesized and accumulated in various plants including annual, biennial or perennial herbaceous plants, and deciduous or evergreen shrubs and trees. All parts of plants are used as sources of essential oils: leaves or leafy stems (e.g., mint, oregano, thyme, rosemary, sage); fruits (anise, coriander, fennel, cumin, citrus, peppers, star anise, juniper); seeds (cardamom, nutmeg); stems (citronella); roots (angelica); rhizomes (ginger, turmeric); flowers or blossoms (rose, orange); flower buds (clove); bark (cinnamon, cassia); wood (camphor); bulbs (onion, garlic). The majority of plants produce essential oils in different botanical parts. These oils can be similar in composition (e.g. oils of angelica root and seed, oils of clove buds and leaves) or entirely different (e.g. oils of cinnamon bark and leaves; bitter orange peels, flowers and leaves; coriander immature leaves and seeds).

Essential oils are stored in special organs: secretory cells, ducts and cavities located inside different plant tissues, or in glandular hairs situated in the outer cell layer, mainly of leaves or petals. Some plant species produce exudates such as resins and balsams that also can be used as sources of essential oils. The content of essential oil in resins achieves 30%, while the content in plant parts is lower and amounts to 0.02% for flowers, 1% for herbs and 3–5% for seeds and fruits. The exception is clove buds containing 15–20% of essential oil.

Essential oils are usually colorless or pale yellow liquids with strong odor resembling the source plant material. They are soluble in alcohol, plant oils and most organic solvents but they are immiscible with water. Their density is usually smaller than that of water, they are characterized by a high refractive index and most of them are optically active.

Essential oils, their fractions and isolates are utilized in food, perfumery, flavors and fragrances, cosmetics and toiletries, fine chemicals, pharmaceutical industries as well as in therapy and aromatherapy. They are also sources of aroma chemicals, particularly of enantiomers that are useful as chiral building blocks in syntheses. Essential oils derived from spices, aromatic or medicinal plants are used as food ingredients fulfilling two roles flavor and preservative. Majority of them is generally recognized as safe (GRAS). Twenty eight essential oils that are used in medicine have their monographs in the European Pharmacopoeia 5 (EP 5).

5.3 Antimicrobial Activity and Composition of Essential Oils

The antibacterial and antifungal properties of essential oils have been known and utilized for centuries. Each essential oil displays antimicrobial activity that is resultant of essential oil effectiveness and microorganism susceptibility. The power of any biological activity of essential oils is strictly connected with the oil composition and especially with the content of some highly active constituents. Essential oils are multicomponent mixtures containing usually more than 100 components. In some commercially important oils more than 300 constituents have been known so far. These are subdivided into two groups: hydrocarbons that are made up almost exclusively of terpenes (monoterpenes, sesquiterpenes and diterpenes) and oxygenated compounds that beside terpene have phenylpropanoid and aliphatic skeletons. Some compounds may also contain nitrogen or sulphur. Due to the wide variety of number of carbon atoms, constitutional isomers (acyclic, mono-, bi- and tricyclic) and stereoisomers combined with different functional groups (hydroxyl, carbonyl, carboxyl) a great diversity of structures can be found in each group of constituents. The compounds found the most frequently as essential oils constituents are monoterpenes.

The antimicrobial activity rank of essential oil components depends mainly on the functional group. On the basis of hundreds of previous investigations the order proposed by Kalemba and Kunicka (2003) is corrected to the following one:

phenols > cinnamic aldehyde > alcohols > aldehydes = ketones > ethers > hydrocarbons.

The highest activity was reported for phenols. Monoterpenes: thymol and carvacrol as well as phenylpropanoid – eugenol are the most frequent phenols found in essential oils. Essential oils with phenols as main compounds express the highest and broadest activity against both bacteria and fungi. These are thyme, oregano and savory oils containing thymol and carvacrol as well as clove and cinnamon leaf oils containing eugenol. The highest activity of phenols is explained by acidic character of the hydroxyl group forming hydrogen bound with an enzyme active center. Cinnamaldehyde – the main component of cinnamon bark oil, also falls into the group of essential oil constituents of the highest antimicrobial activity. Monoterpene aldehydes, e.g. citronellal, neral, geranial, that are major components of citronella

and lemon balm oils, show lower activity that is comparable to the activity of alcohols such as: linalol (coriander and lavender oils), menthol (peppermint oil), geraniol, nerol and citronellol (rose and geranium oils), α-terpineol and terpinen-4-ol (tea tree oil), borneol, farnesols. Other oxygenated terpenes include ketones: carvone (caraway oil), menthone (peppermint oil), pulegone, α- and β-thujones and camphor (sage oil); esters: mainly acetates of monoterpene alcohols, and oxides: 1,8-cineole (eucalyptus oil), anethole (anise oil, fennel oil), estragole, ascaridole, bisabolol oxides. Oct-1-en-3-ol, (*E*)- and (*Z*)-hex-3-enols belong to the most important aliphatic constituents. Monoterpene hydrocarbons that can be the most frequently found as essential oil constituents are: limonene, pinenes (α- and β-), phellandrenes (α- and β-), terpinenes (α- and γ-), sabinene, camphene and myrcene. β-Caryophyllene is the most common sesquiterpene hydrocarbon. Hydrocarbons are the main components of citrus and conifer oils. These general rules enable to predict to some extent the *in vitro* antimicrobial activity of essential oil with known chemical composition.

Chirality is an important aspect of essential oil compounds because enantiomers may possess different smell and taste, e.g. (*S*)-(+)-carvone which isomer is a main constituent of caraway oil has a caraway flavor while (*R*)-(−)-isomer being the major component of spearmint oil possesses a mint flavor. It is known that enantiomers of some compounds have such different biological activity that one of them is a drug and the other a poison, e.g. thalidomide. According to the recent research in case of antimicrobial activity of essential oil constituents chirality seems to be not significant point. Only a few research have been done on that score. Similar activity was shown for both linalool enantiomers against *Botrytis cinerea* (Özek et al. 2010), both carvone enantiomers and both limonene enantiomers against a wide spectrum of human pathogenic bacteria and fungi tested (Aggarwal et al. 2002; Jirovetz et al. 2004). On the other side, (*R*)-carvone and essential oils containing its high amount were more effective than those containing (*S*)-enantiomer against postharvest fungal pathogens of fruit (Combrinck et al. 2011). Similar observation were reported by Lis-Balchin et al. (1999) for α-pinene, 18 out of 25 different bacteria and 2 out of 3 filamentous fungi were found to be more affected by the (+)-α-pinene than by its (−)-enantiomer.

The biological activity of essential oils is strictly connected with their chemical composition. Since essential oils are natural products, their composition cannot be precisely quantified. Correct botanical description of the plant material is out of discussion. The genus *Mentha* L. comprises about 25 species and even 900 taxons, there are hundreds of eucalyptus species and varieties. Different thyme and oregano species or even genera are accepted universally as thyme or oregano, respectively. That is the main reason that oils marketed at the same name showed great variability between the antimicrobial action, e.g. eucalyptus or chamomile (Lis-Balchin et al. 1998).

Moreover, numerous species produce several subspecies, varieties or chemotypes with different dominant constituents. The most spectacular example is common thyme, *Thymus vulgaris* L. Thymol and carvacrol types are the most relevant but

other such as geraniol, cineol, or linalol types grow in different regions. Thyme oils derived from different chemotypes of *T. vulgaris* (Ferhout et al. 1999; Oussalah et al. 2006) and different *Thymus* species (Oussalah et al. 2007) varied not only in flavor but first of all in their antimicrobial properties. On the other side, several thyme species produce essential oil with thymol as the main constituent and according to EP 5 thyme oil is obtained from *T. vulgaris* L. and *T. zygis* Loefl.

This is also the case of two other very common pharmacopoeial essential oils that can be misleaded with others. Two varieties of *Foeniculum vulgare* Mill. are traded as spice: bitter fennel fruit and sweet fennel fruit. The later is usually used as flavoring and the former is listed in EP 5. Pharmacopoeial sweet orange oil is obtained by pressing from peels of *Citrus aurantium* L. var. *dulcis* (syn. *C. sinensis* (L.) Osbeck). Under the same name distilled orange oil, and terpeneless oils both distilled and pressed are traded. Moreover, bitter orange peel oil is also available. As far as fennel and orange peel oils concerned, the composition of different oils do not differ significantly. Entirely different is the case of two important essential oils produced from Ceylon cinnamon. Eugenol is the main constituent of cinnamon leaf oil while cinnamaldehyde of cinnamon bark oil. Strangely, some researchers – even in very recent reports – do not specify which of these two oils they tested. Other valid example is rosemary oil. Two pharmacopoeial types of this oil, Spanish as well as Marocco and Tunisian type differ significantly in chemical composition.

What is more, it should be stressed that even for the properly defined plant material and isolation method significant fluctuations in percentage composition of essential oil can be observed. They are mainly due to cultivation conditions (growing region, weather, climate, soil, etc.), harvesting time, methods of preparing material, distillation parameters. This is the reason that in pharmacopoeial or ISO requirements a rather broad range of the content of main oil constituent as well as minimal or maximal content of other important constituent is given. Chemical composition of essential oil under investigation has to be reported together with its antimicrobial activity. Although this rule is obvious it is not always comply, even in many of very recent investigations.

5.4 Methods for Antimicrobial Assessment of Essential Oils

For antimicrobial assessment of essential oils the conventional *in vitro* methods used for testing antibiotic effectiveness are usually applied. Since they have been critically reviewed (Kalemba and Kunicka 2003; Burt 2004), here only the most important aspects will be pointed. As the most suitable the serial dilution method in liquid broth or agar is widely accepted, with the microdilution on agar as the most common recently. Different ways of microorganism growth assessment are applied. Traditional counting of colonies has been replaced by turbidimetry, impedimetry or cytometry. The semi-quantitative agar diffusion method (disc or hole) is generally considered as inappropriate for essential oils that are volatile and likely to evaporate.

This method can be useful in screening of great number of essential oils against a broad range of microorganisms (Hili et al. 1997; Lis-Balchin et al. 1998). The mutation of agar diffusion method called microatmosphere method is used for the estimation of essential oil activity in vapor phase. It is especially suitable for defining the activity of essential oils which are to be employed as the atmospheric preservatives or drugs used by inhalation. It has been proved that activity of essential oils depends on the assay method used and method should correspond with the application mode (Tullio et al. 2007; Inouye et al. 2003; Suhr and Nielsen 2003).

Antimicrobial activity of essential oils have been assessed since the beginning of twentieth century and reviewed for at least 40 years. Although several authors have been stressed the need of standard, reproducible method for assessing essential oils, many methods are applied and developed. What is more, even for the same method used, the comparison of experimental results obtained by different authors still remains difficult. A number of factors influencing the results of antimicrobial activity can be standardized, e.g. culture conditions, solvents used to facilitate the oil dispersion. Some of factors are difficult to standardization such as susceptibility of microorganism strain even if they come from same collections. Only a few of researchers control the susceptibility of microorganisms toward approved antibiotics or antimycotics (positive control) and even fewer make a negative control. The next difficulty results from great diversity of ways of reporting the antimicrobial activity: various units of oil concentration (replaced sometimes by dilution), different definition of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, usage of such factors as BA_{50}, IC_{50}, IC_{80} instead of MIC. And last but not least, not always the composition of the essential oil is given and often the denomination of oil is ambiguous, especially in the case of commercial names.

The majority of a huge number of available results on antimicrobial activity of essential oils was obtained by different *in vitro* methods. These should be treated only as the first step in efficacy assessment. It has been many times proven and reviewed by Burt (2004) and Tajkarimi et al. (2010) that there is no rational relationship between *in vitro* and *in vivo* (as far as therapy of infectious diseases concerns) or *in situ* testing (in the case when essential oil is food or cosmetic preservative or functional ingredient). Effective doses of essential oils in food and cosmetics are much higher than MIC values established by *in vitro* methods. *In vivo* and *in situ* research are expensive and difficult. However, they are indispensable for proper evaluation of essential oils as drugs and preservatives. Such research are described in the other chapters. In this chapter the *in vitro* antibacterial and antifungal activity of dietary essential oils will be reviewed on the basis of last 15 years research. The first criterion of essential oil selection was their importance in food industry as well as in medicine. The second criterion was the power of antibacterial and antifungal activity, the oils are listed according to their effectiveness. The description of appearance and requirements for the content of important oil constituents are referred to European Pharmacopoeia 5 (2005) (EP 5), while the yield, flavor and applications of essential oils to Wright (2004).

5.5 Thyme Oil

Thyme oil is obtained from the fresh flowering aerial parts of *Thymus vulgaris* L. and *T. zygis* Loefl. (shrubs, Lamiaceae) with the yield of 0.5–1.2%. Two kinds of oil are known: red (dark reddish-brown with crude strong, aromatic flavor) and white (redistilled, yellow of milder odor). The oil is used as an agent in seasoning blends and in traces in many flavors, e.g. of mandarin and orange.

The major components of the oil according to EP 5 are: thymol (36.0–55.0%), p-cymene (15.0–28.0%), γ-terpinene (5.0–10.0%), linalol (4.0–6.5%), carvacrol (1.0–4.0%), myrcene (1.0–3.0%), terpinen-4-ol (0.2–2.5%) (Fig. 5.1).

5.5.1 Antibacterial Activity

Thyme oil has always been among these most frequently investigated against the antimicrobial activity and it was always placed among the most effective against both bacteria and fungi. It has often been investigated parallel with oregano oil and exhibited similar antimicrobial activity.

Thyme oil exhibits very high antibacterial activity. According to Biavati et al. (1997) a majority of bacteria strains from the genera *Bacillus* (8 spp.), *Clostridium* (8 spp.), *Bifidobacterium* (7 spp.), *Lactobacillus* (7 spp. and ssp.), *Pseudomonas* (6 spp. and ssp.), *Enterococcus* (2 spp.), *Lactococcus lactis* (2 ssp.), as well as *Streptococcus salivarius*, *Agrobacterium vitis*, *Xanthomonas pruni*, and *Erwinia carotovora* was inhibited at 400–600 ppm of thyme oil. The most resistant were three *Pseudomonas* species and three *Bifidobacterium* species with MIC 1,200–>2,000 ppm. Twenty oils were assessed in this research and only oregano oil showed as high activity as thyme oil. Similar activity of thyme oil was established against 3 bifidobacterias from dental caries MIC 600–>2,000 ppm (Crociani et al. 1997). Thyme oil was among the most effective out of 52 essential oils against *Acinetobacter baumanii*, *Aeromonas sobria*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Serratia marcescens* with MIC 0.12–0.5%. Likewise majority of oils tested in this report thyme oil was inactive even at 2% toward *Pseudomonas aeruginosa* and *Salmonella typhimurium* (Hammer et al. 1999). Thyme oil along

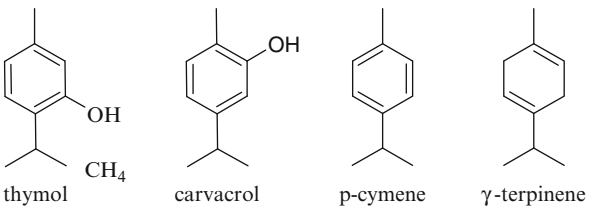


Fig. 5.1 Main components of thyme oil

with oregano and cinnamon bark oils were the most active out of 31 oils against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Streptococcus pyogenes* (625–1,250 mg/L), *Escherichia coli* (2,500 mg/l) and *P. aeruginosa* (5,000 mg/L) (Roengsumran et al. 1997). Significantly higher activity of thyme oil was observed against five food-borne bacteria: with MIC 0.02% for *S. aureus* and *Listeria monocytogenes*, 0.04–0.05% for *Salmonella enteritidis*, *Camphylobacter jejuni* and *E. coli*. Thyme oil was the most inhibitory from among 21 oils (Smith-Palmer et al. 1998). Similar BA_{50} values were established for *E. coli* and *Salmonella enterica* (0.05%), lower for *C. jejuni* (0.022%), and higher for two *L. monocytogenes* strains (0.091% and 0.22%, respectively). From 96 oils assessed in this research only three other (clove, cinnamon bark and leaf oils) showed equally high effectiveness (Friedman et al. 2002). The oil was effective against reference *Salmonella* Enteritidis (S. *enterica* subsp. *enterica* serotype Enteritidis) with MIC at 107 μg/ml as well as five clinical strains of this foodborne bacteria (MIC 67–320 μg/ml) (Rattanachaikunsopon and Phumkhachorn 2010). The same range of MIC values were reported for *S. aureus* (31.2 μg/ml) and *E. coli* (62.5 μg/ml) and for other bacteria, *Bacillus cereus* (15.6 μg/ml), *Proteus vulgaris* (31.2 μg/ml), *Proteus mirabilis* (62.5 μg/ml), *S. typhimurium* (125 μg/ml), *S. typhi* (250 μg/ml), *K. pneumoniae* and *P. aeruginosa* (both 500 μg/ml) (Al-Bayati 2008), as well as for antibiotic-resistant *Micrococcus luteus* (66.7 μg/ml) (Friedman 2006), and *Propionibacterium acnes* (0.016%) (Zu et al. 2010).

The antibacterial efficacy of thyme oil depended of the vegetation period of plant material and was highest for the oil of thyme in full flower that at 400 ppm caused total inactivation of five bacteria (e.g. *Sarcinia flava*, *Listeria innocua*) and at 800 ppm of all of other 15 strains of tested bacteria (e.g. *Pseudomonas fluorescens*, *Bacillus thuringiensis*) (Marino et al. 1999). The efficacy against seven food borne bacteria strains strongly depended on the origin of the oil. The biggest differences were observed toward *L. monocytogenes* with MIC 0.1–0.5 μg/ml for thyme oil from France and 2 μl/ml for oil from Spain (Rota et al. 2004).

Thyme oil was always one of the best in antimicrobial activity in comparison studies of some essential oils, irrespective of microorganism used. Thyme oil together with oregano oil showed the highest antibacterial activity against *Streptococcus pneumoniae* from among 73 essential oils (Horne et al. 2001), against *Pseudomonas putida* (MIC 0.05%) from among 60 oils tested (Oussalah et al. 2006), among 13 oils against 12 bacterial strains with *Lactobacillus sakei*, *Clostridium botulinum* and *Clostridium perfringens* as the most sensitive (Nevas et al. 2004), against *Lactobacillus* and *Staphylococcus* species commonly used in food industry and *Enterobacter* species related to food spoilage (Viuda-Martos et al. 2008). Thyme oil was the most active among 13 oils against *E. coli* O157:H7 (Burt and Reinders 2003), among 51 oils against 3 bacteria (*S. aureus*, *E. coli* and *P. aeruginosa*) (Hili et al. 1997), among 6 oils against 25 bacteria (Dorman and Deans 2000), among 14 oils against respiratory tract pathogens in gaseous contact with MID (Minimal Inhibitory Dose) 3.13–6.25 mg/l air (Inouye et al. 2001), and among five oils against two reference strains and nine isolated strains of *Vibrio alginolyticus* (MIC 0.078–0.31 mg/ml) (Hajlaoui et al. 2010).

It is worth to note that both bacteriostatic and bactericidal concentrations of white thyme oil against *E. coli* (625 and 1,250 μl/l, respectively) were two times lower than that of red thyme oil (Burt and Reinders 2003).

Essential oil of *T. zygis*, Spanish variety of *T. vulgaris*, was effective against poultry origin strains and pig origin strains from *Enterobacteriaceae* family showing MIC 0.5% against *Salmonella essen* and 2–4% against *Salmonella choleraesuis*, *S. enteridis*, *S. typhimurium*, *E. coli* (Penalver et al. 2005).

5.5.2 Antifungal Activity

Thyme oil at a concentration 1 μl/ml showed 92–100% inhibition of six pathogenic fungi, e.g. *Fusarium oxysporum*, *Penicillium brevicompactum*, and *Aspergillus fumigatus* (Zabka et al. 2009) and at 100 ppm 47–100% growth inhibition of 6 different fungi strains (Bourrel et al. 1995). The mycelial growth of *Aspergillus niger* and *Aspergillus flavus* was completely inhibited at the presence of thyme oil lower than 700 μg/ml (Paster et al. 1995; Viuda-Martos et al. 2007) and of A*spergillus parasiticus*, *A. ochraceus* and *Fusarium moniliforme* at 500 ppm (Soliman and Badeaa 2002). The oil applied against fungi colonising stored grain proved the possibility of using it as an alternative to chemicals in grain preservation (Paster et al. 1995; Soliman and Badeaa 2002). The oil effectively inhibited the growth of rye bread spoilage fungi both in agar test and in vapors at 250–270 μl/l (Suhr and Nielsen 2003). It controlled the growth of *Botrytis cinerea* and *Rhizopus stolonifer* and the decay of strawberries caused by these fungi (Reddy et al. 1998). Fungicidal effect of thyme oil against *B. cinerea* and *Mucor piriformis* in vapour phase was observed at significantly lower concentration (5 μl/l) than in liquid medium (600 μl/l) (Abdolahi et al. 2010).

Thyme oil exhibited high antifungal activity against some dermatophytes and *A. flavus* (MIC 156–625 ppm), as well as *Candida albicans* (MIC 1,250 ppm) (Amvam Zollo et al. 1998). Similar MIC toward *C. albicans* (0.12%) was established by Hammer et al. (1999) and lower one (0.31 μl/ml) by Donaldson et al. (2005). The fluconazole-resistant and fluconazole-susceptible strains of *Candida* species revealed similar susceptibility to thyme oil. *C. albicans, C. glabrata* and *C. crusei* were inhibited at MIC 0.32 mg/ml of thyme oil (Neves et al. 2009) while *C. albicans*, *C. dubliniensis*, *C. tropicali, C. krusei* and *C. glabrata* at MIC 400–3,200 μg/ml (Pozzatti et al. 2008). Thyme oil was the most active among 51 oils against and 4 yeast, e.g. *C. albicans* and *Torulopsis utilis* (Hili et al. 1997), as well as among 8 oils against plant pathogenic fungi with MIC 200–400 ppm (Giamperi et al. 2002).

Thyme red oil followed by clove oil showed the highest activity out of seven tested oils (e.g. fennel, sage, lavender) both by micro dilution method and in vapor contact against a total of 44 strains of environmental and clinically undesirable filamentous fungi, including *Microsporum canis*, *Epidermophyton floccosum*, *Aspergillus* sp. *Penicillium* sp. *Cladosporium cladosporioides* (Tullio et al. 2007).

5.6 Clove Oil and Cinnamon Leaf Oil

Clove oil and cinnamon leaf oil contain eugenol as the main constituent and are usually tested for their antimicrobial activity in the same investigations. Hence, they will be discussed together.

Clove oil is obtained from dried flower buds of *Syzygium aromaticum* (L.) Merill et LM Perry (*Eugenia caryophyllata* Thunb., Myrtaceae), yield between 15% and 20%. The oil is a clear yellow liquid which becomes brown when exposed to air, it has a characteristic clove-like aroma and burning, spicy flavor and is used in seasoning blends and in some natural flavors, especially banana, blackberry, cherry and smoke.

The major components of the oil are: eugenol (75.0–88.0%), eugenyl acetate (4.0–15.0%), β-caryophyllene (5.0–14.0%) (EP 5).

Cinnamon leaf oil is obtained from the leaves of Ceylon cinnamon tree *Cinnamomum zeylanicum* Blume (*C. verum* J. Presl, Lauraceae), yield of 1%. The oil is a reddish-brown to dark brown mobile liquid with spicy cinnamon, clove-like odor and taste. It is used as an alternative to clove oil in seasoning blends, and can be blended with cinnamaldehyde to approximate the character of cinnamon bark oil.

The major constituents of the oil are: eugenol (70.0–85.0%), β-caryophyllene (1.5–7.0%), linalol (1.5–3.5%), safrole (max. 3.0%), *trans*-cinnamic aldehyde (max. 3.0%), cinnamyl acetate (max. 2.0%), 1,8-cineole (max. 1.0%), coumarin (max. 1.0%) (EP 5) (Fig. 5.2).

5.6.1 Antibacterial Activity

Due to the similarity of essential oil composition, antimicrobial activities of clove oil and cinnamon leaf oil have been very often investigated parallel. Both oils acted strongly bacteriostatic at 0.03–0.05% and bactericidal at 0.04–0.1% concentrations toward *S. aureus*, *L. monocytogenes*, *S. enteridis*, *C. jejuni* and *E. coli* (Smith-Palmer et al. 1998), and with BA_{50} lower than 0.13% (Friedman et al. 2002). Although the same bacteria species were investigated in two research works with the exception of *S. aureus*, the susceptibility of *C. jejuni* to tested essential oils was the biggest according to Friedman et al. (2002) or the lowest one by Smith-Palmer et al. (1998). Higher MIC (0.25 ml/100 ml) and MBC (0.3 ml/100 ml) were established for clove oil against *E. coli* by Moreira et al. (2005). Clove and cinnamon oils in doses of 500 μg/ml caused 61–99% decrease in population growth of 3 bacteria (Hili et al. 1997). Cinnamon oil was slightly more effective (MIC 6.25–12.5 μg/ml) than clove oil (MIC 12.5 μg/ml for six strains and 50 μg/ml for *K. pneumoniae*) against seven major respiratory tract microorganism, e.g. *Haemophilus influenzae*, *Streptococcus agalactiae,* and *S. pyogenes* (Fabio et al. 2007). The effectiveness of both oils was similar in both solid diffusion and vapor diffusion test against four Gram-positive and four Gram-negative bacteria species. *Yersinia enterocolitica* was

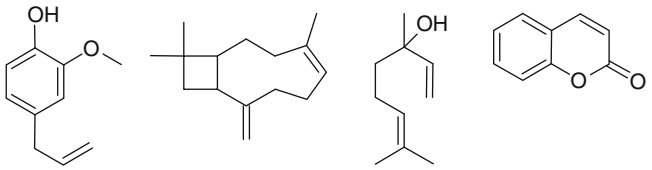


Fig. 5.2 Main components of clove oil and cinnamon leaf oil

the most susceptible and *P. aeruginosa* the least susceptible strain (Lopez et al. 2005; Goni et al. 2009).

Clove oil at the dilution 1/100 inhibited five of the six tested meat spoilage organisms (Ouattara et al. 1997), with MIC at 0.1% inhibited the growth of *P. putida* strain of meat (Oussalah et al. 2006), with MIC 500 μg/ml the growth of different *Aeromonas* isolates (Zaki et al. 2001), and with MIC 80–320 μg/ml the growth of a reference strain and five clinical strains of *Salmonella* Enteritidis (Rattanachaikunsopon and Phumkhachorn 2010). In disc diffusion test the oil was active against a large number of oral pathogenic bacteria and yeast (Kouidhi et al. 2010) and against 23 out of the 25 bacteria and all of 20 different isolates of *L. monocytogenes* in disc diffusion test (Deans et al. 1995).

5.6.2 Antifungal Activity

In comparative investigations with a number of essential oils, clove oil and cinnamon leaf oil always were on the top in activity, along with thyme oil, e.g.: among 51 oils against 3 bacteria and 4 yeast strains (Hili et al. 1997), among 20 oils against 45 bacteria and 8 yeast species (Biavati et al. 1997), among 45 oils against 7 bacteria and 3 fungi strains (Chao et al. 2000).

Clove oil and cinnamon leaf oil exhibited fungistatic and fungicidal activity against three postharvest pathogens of banana with inhibitory concentration 0.04–0.06% and lethal concentration (MLC) 0.06–0.11% (Ranasinghe et al. 2002) as well as were most active from among 49 essential oils tested against *B. cinerea* (Wilson et al. 1997). Clove and cinnamon oils in doses of 500 μg/ml caused significant decrease in population growth of 4 yeasts (59–100%) (Hili et al. 1997).

Clove oil exhibited fungistatic activity against four plant pathogens, e.g. *Aspergillus alternata* (MIC 0.05%), while above this concentration lysis of conidia and inhibition of mycelial growth were detected (Beg and Ahmad 2002). The oil is also effective in vapor phase against four fungal and four yeast species that are important food spoilage microorganisms (Matan et al. 2006). Mould and yeast strains isolated from ochnomycosis were inhibited by 2% of clove oil (Gayoso et al. 2005). The oil was active against 8 mould cellulolytic strains contaminated archive and museum reserves (Delespaul et al. 2000).

Cinnamon leaf oil showed antifungal activity (MIC 400 ppm) against *Stachybotrys chartarum* (Misra et al. 2000), and at 500 ppm completely inhibited spore production and germination as well as fungal colony development of five fungi such as *Cladosporium herbarum*, *R. stolonifer* and *B. cinerea* (Tzortzakis 2009). The oil is classified as the most effective against *Malassezia furfur* and *C. albicans* (Ferhout et al. 1999). The fluconazole-resistant and fluconazole-susceptible strains of *Candida* species (*C. albicans*, *C. dubliniensis*, *C. tropicali, C. krusei* and *C. glabrata*) were similarly susceptible to the oil with MIC 800–1,600 μg/ml (Pozzatti et al. 2008).

5.7 Cinnamon Bark Oil and Cassia Oil

Cinnamon bark oil and cassia oil have similar composition and will be presented together.

Cinnamon bark oil is obtained from the bark of Ceylon cinnamon tree *Cinnamomum zeylanicum* Blume (*C. verum* J.Presl, Lauraceae), yield of 0.5%. The oil is a light yellow liquid becoming reddish over time with spicy cinnamon odor and slightly bitter and pungent taste. It is used as an alternative to clove oil in seasoning blends, and can be blended with cinnamaldehyde to approximate the character of cinnamon bark oil. It is generally employed in cookery as a condiment and flavoring material.

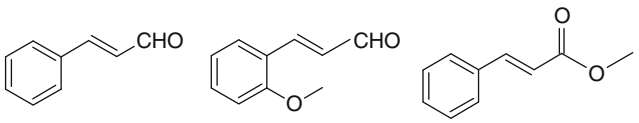
The main components of the oil are: *trans*-cinnamic aldehyde (55.0–75.0%), eugenol (max. 7.5%), linalol (1.0–6.0%), β-caryophyllene (1.0–4.0%), 1,8-cineole (max. 3.0%), benzyl benzoate (max. 1.0%), *trans*-2-methoxycinnamaldehyde (0.1–1.0%), coumarin (max. 0.5%), safrole (max. 0.5%) (EP 5). Coumarin and safrole are limited in food and beverages by EU.

Cassia oil is obtained from leaves, bark and young branches of *Cinnamomum cassia* Blume (Chinese cinnamon, a large tree, Lauraceae). The oil is a reddish-brown liquid and has odor reminiscent of cinnamon bark oil with the unique note of 2-methoxycinnamaldehyde which distinguishes cassia from cinnamon oil. Cassia oil is a major part of the traditional flavor of cola drinks. It is also used in confectionery and as ingredient in other natural flavors such as cherry, vanilla and some nut flavors.

The main components of the oil are: *trans*-cinnamaldehyde (70.0–90.0%), *trans*-2-methoxycinnamaldehyde (3.0–5.0%), cinnamyl acetate (1.0–6.0%), coumarin (1.5–4.0%), eugenol (max. 0.5%) (EP 5) (Fig. 5.3).

5.7.1 Antibacterial Activity

Antimicrobial activity of cinnamon bark oil was assessed many times while there are only a few reports on activity of cassia oil. The antibacterial activity of cinnamon bark oil was one of the highest among 45 essential oils (Chao et al. 2000) and higher



trans-cinnamaldehyde *trans*-2-methoxy-cinnamaldehyde cinnamyl acetate

Fig. 5.3 Main components of cinnamon bark oil and cassia oil

than that of 30 other oils investigated against *B. subtilis*, *S. typhi*, *S. pyogenes E. coli* and *P. aeruginosa* (Roengsumran et al. 1997). Low MIC values were established in this last research toward *P. aeruginosa* (1,250 mg/l) and higher toward *S. aureus* (2,500 mg/l) that were supported by activity of the main constituent of the oil – cinnamaldehyde, 625 mg/l for *P. aeruginosa* and 1,250 mg/l for *S. aureus*. The oil appeared to be strongly effective against 21 bacteria with *S. pneumoniae* and *Acinetobacter lwoffii* as the most sensitive at MIC <0.04 mg/ml and *S. aureus*, *S. pyogenes*, *Enterobacter aerogenes*, *B. cereus,* and five *Listeria* strains, as the most resistant at MIC 0.56 mg/ml (Unlu et al. 2010). In other research the oil showed similarly high activity with MIC at 20–25 μg/ml toward four Gram-positive (*B. subtilis*, *B. cereus*, *S. aureus*, *M. luteus*) and two Gram-negative bacteria (*K. pneumoniae* and *Serratia marcescens*) (El-Baroty et al. 2010). Cinnamon bark oil was the most active out of six oils against clinical isolates of six bacteria strains with MIC at 0.25 mg/ml (Sivamani and Sahul Hameed 2010). The oil was similarly active against six *S.* Enteritidis strains with MIC at 67–267 μg/ml (Rattanachaikunsopon and Phumkhachorn 2010) and less active against *E. coli* (MIC 4 μl/ml) and *P. aeruginosa* (11 μl/ml) (Pattnaik et al. 2010). High activity of the oil against five strains of respiratory tract pathogens (e.g. *S. pneumoniae*, *H. influenzae*) was also observed by gaseous contact at 1.56–6.25 mg/l air (Inouye et al. 2001).

Cassia oil and Ceylon cinnamon bark oil were equally effective in inhibiting the growth of various isolates of bacteria including Gram-positive and Gram-negative revealing MIC 75 μg/ml for *Vibrio parahaemolyticus*, MIC 150–300 μg/ml for *E. coli*, *P. vulgaris*, *P. aeruginosa*, *Vibrio cholerae*, and *S. typhymurium*, MIC 600 μg/ml for *S. aureus* and *E. aerogenes*, (Ooi et al. 2006) and MIC 0.03% for *C. jejuni* (Rossi et al. 2007).

Both cassia and cinnamon bark oil showed the highest activity out of 28 essential oils tested against four bacteria (*E. coli*, *S. typhimurium*, *L. monocytogenes* and *S. aureus*) being more effective than clove oil and cinnamon leaf oil with MIC 0.025–0.05% (Oussalah et al. 2007).

5.7.2 Antifungal Activity

Fungistatic and fungicidal activity of cinnamon bark oil against three postharvest pathogens of banana, e.g. *Fusarium proliferatum*, was higher than that of clove and cinnamon leaf oil, MIC 0.03–0.05%, MLC 0.04–0.8% (Ranasinghe et al. 2002).

Similar MIC values were established for three other plant pathogens, namely *Rhizopus nigricans* (0.64%), *A. niger* and *Penicillium expansum* (0.16%) (Xing et al. 2010). The oil inhibited the growth of *A. fumigatus* (MIC 25 μg/ml), *Trichophyton mentagrophytes* (MIC 12.5 μg/ml) and *C. albicans* (MIC 50 μg/ml). The effectiveness in gaseous contact was better than by solution contact (Inouye 2003; Inouye et al. 2003). The efficacy of cinnamon bark oil against two *C. albicans* strains was similar (MIC 0.07–0.12 mg/ml) and against *Candida parapsilosis* and *C. krusei* lower than 0.04 mg/ml (Unlu et al. 2010).

Both leaf and bark cinnamon oils were classified as the most effective against *Malassezia furfur* and *C. albicans* (Ferhout et al. 1999). These two oils were similarly efficient against six dermathophytes (e.g. *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum cannis* and *M. gypseum*) with MIC 0.08–0.16 μg/μl while bark oil revealed significantly higher effectiveness (MIC 0.08–0.16 μg/μl) than leaf oil (MIC 0.31–0.64 μg/μl) toward five strains of yeast (*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and *Cryptococcus neoformans*) and *A. niger* (MIC 0.16 vs. 0.31 μg/μl) (Jantan et al. 2008). In the report comparing anti candidal and anti dermatophytic properties of essential oils form different parts of 11 cinnamon species Ceylon cinnamon leaf oil (MIC 0.55–5.91 μg/μl) and wood oil (MIC 1.51–6.04 μg/μl) were among the most active (Mastura et al. 1999). Both cinnamon oils were highly effective against 8 pathogenic fungus (4 *Aspergillus*, 2 *Fusarium* and 2 *Penicillium* spp.) isolated from food materials (Singh et al. 2007) and against *A. niger, F. oxysporum*, *Penicillium notatum* and *Mucora heimalis* (MIC 100 μg/ml) (El-Baroty et al. 2010).

Cassia oil effectively inhibited fungi including yeast (*C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei*), MIC 100–450 μg/ml, filamentous molds (three *Aspergillus* spp. and one *Fusarium* sp.), MIC 75–150 μg/ml and three dermatophytes, MIC 18.8–37.5 μg/ml. (Ooi et al. 2006).

It is amazing that even in the latest reports about antimicrobial activity of cinnamon oil the authors were not clear as to whether the oil under investigation was leaf or bark oil. This is the case of strong activity of cinnamon oil against *E. coli*, MIC 300 ppm (Ceylan and Fung 2003), *Listeria monocytogenes* (Paparella et al. 2008) and three *Aspergillus* species (Carmo et al. 2008).

5.8 Peppermint Oil and Cornmint Oil

Peppermint oil is obtained from flowering tops and leaves of *Menta x piperita* L. (herbaceous perennial plant, Lamiaceae), yield of 0.3–0.7%. The oil is a colorless, pale yellow or pale greenish-yellow liquid. It has a characteristic mint odor and taste followed by the sensation of cold. It is used to give a peppermint flavor to a wide range of applications, at first as flavor additives in bubble gum and toothpaste. It is also used in mint and herbal blends and in liquor and sweets flavors.

Major components of peppermint oil are: menthol (30.0–55.0%), menthone (14.0–32.0%), methyl acetate (2.8–10.0%), isomenthone (1.5–10.0%), menthofuran

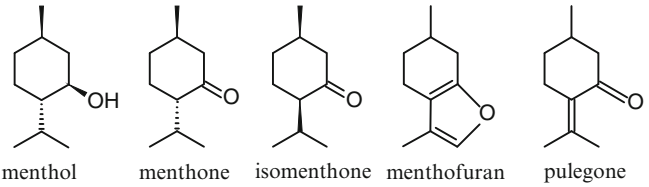


Fig. 5.4 Main components of peppermint oil and cornmint oil

(1.0–9.0%), pulegone (max. 4.0%), carvone (max. 1.0%), isopulegol (max. 0.2%) (EP 5).

Cornmint oil is obtained from flowering tops and leaves of *Mentha canadensis* L. (*M. arvensis* L., Japanese mint, Lamiaceae), yield of 0.5–2%. The content of menthol achieved 90% of the oil. Menthol is partly separated by crystallization and the remaining oil has appearance and odor resembling peppermint oil. The oil is used as a cheap alternative to peppermint oil, but easily recognized organoleptically because of its harsh flavor. Cornmint oil can be used in herbal blends and liquor flavors. Menthol obtained from the oil is mainly used in drug and cigarette production.

Major components of partly dementholized Cornmint oil are: menthol (30.0–50.0%), menthone (17.0–35.0%), isomenthone (5.0–13.0%), menthyl acetate (1.5–7.0%), limonene (1.5–7.0%), isopulegol (1.0–3.0%), pulegone (max. 2.5%), carvone (max. 2.0%), 1,8-cineole (max. 1.5%) (EP 5) (Fig. 5.4).

5.8.1 Antibacterial Activity

More than a half of mint oil production falls on peppermint oil and it is the most important because of its exceptional properties. Wide spectrum of therapeutic properties of this oil includes antibacterial and antifungal activities. Biological activity of mint oils is due to the content of their main constituent (1R, 3R, 4S)-(−)-menthol. Mint oils have shown high or middle activity against bacteria and fungi when compared with other essential oils. Peppermint oil was assessed more frequently than Japanese mint oil, that usually was tested as a raw but not dementholized oil.

Peppermint oil exhibited medium activity against *S. aureus*, *C. jejuni* and *L. monocytogenes* with MIC 0.03–0.1% being less active against *E. coli* and *S. enteridis* with MIC >1% (Smith-Palmer et al. 1998). Its BA_{50} was 0.3–0.7% against three tested bacteria and 0.07% against *C. jejuni* (Friedman et al. 2002). In research of activity of 20 essential oils action against 53 microbial strains peppermint oil was in the group of middle activity. From 45 bacteria the most susceptible to peppermint oil were seven *Clostridium* sp. and two *Lactococcus* sp. strains with MIC 400–600 ppm. The susceptibility of eight yeast species was similar. The activity of the oil against eight *Bacillus* sp. (MIC 400–1,800 ppm) and seven *Lactobacillus* sp. (MIC 1,400–2,000 ppm) was the lowest one (Biavati et al. 1997), being the same

against three bifidobacterias from dental caries (Crociani et al. 1997). Its MIC value against four *V. cholerae* strains varied from 0.27 to 0.80 μl/ml (Pattnaik et al. 1996) and against different *Aeromonas* isolates was of 1,250 μg/mL (Zaki et al. 2001). *E. coli* with MIC 5 μl/ml, and *S. aureus,* MIC 2.25 μl/ml appeared susceptible to peppermint oil while *P. aeruginosa* was resistant (MIC >20 μl/ml (Pattnaik et al. 2010). The same MIC value for *E. coli* as well as for *Staphylococcus epidermidis* (5.7 mg/ml) was reported by Schelz et al. (2006). The antibacterial study of four peppermint oils of different origin and composition has shown that for some out of 16 bacteria strains all oils were similarly active, e.g. against *K. pneumoniae* and *Yersinia enterocolitica* (both MIC 2.5 mg/ml) and for other strains quite different, e.g. *Pseudomonas syringae* (0.62–2.5 mg/ml) (Iscan et al. 2002). The oil inhibited also the growth of *S. aureus*, at the concentration <0.1% preventing from enterotoxin B formation (Tassou et al. 2000). Astonishingly low MIC values amounted to 1–3 μg/ml were determined for peppermint oil against 11 bacteria strains (e.g. *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*) by Sokovic et al. (2010).

Parallel with the changes in peppermint oil composition depending on the planting time and mineral fertilisation, the oils shown different degree of inhibition: the oils from spring planted and fertilised crops were more active against some bacteria (Hussain et al. 2010). Essential oil of *M. piperita* exhibited a stronger antibacterial activity than other mint oils, in particularly against *E. coli* strains and the multiresistant strains of *Shigella sonnei* and *Micrococcus flavus* (Mimica-Dukic et al. 2003). Bacteriostatic effect of peppermint oil at 800 ppm was the most pronounced toward *E. coli* O157:H7 out of nine strains of Gram-negative and *Listeria innocua* out of six strains of Gram-positive food spoilage bacteria (Marino et al. 2001). In other research MIC of this oil against *E. coli* was established at 2.0 ml/100 ml (Moreira et al. 2005).

In parallel evaluation of both mint oils raw Japanese mint oil (80% of menthol) appeared to be significantly more effective than peppermint (28% of menthone and only 4% of menthol) against *S. aureus* (MIC 30 vs. 120 μg/ml) and *B. subtilis* (20 vs. 123 μg/ml) while their low effectiveness against *E. coli* was similar (ca. 300 μg/ml) (Hussain et al. 2010). However, in other investigation both oils revealed similar activity toward 13 bacteria species (Nevas et al. 2004). In the work comparing peppermint oil and dementholized cornmint oil (both ca. 40% of menthol) tiny differences were observed in antibacterial activity by disc diffusion method and pronounced ones by dilution method, especially in the case of *K. pneumoniae* with MIC 600 ppm for peppermint and 6 ppm for cornmint oil (Jirovetz et al. 2009). Both those essential oils inhibited the proliferation of *Helicobacter pylori*, *S. enteritidis*, *E. coli* and both methicillin-resistant and methicillin-sensitive *S. aureus* strains (Imai et al. 2001). Japanese mint oil showed microbicidal activity against seven bacteria strains (Thoppil et al. 2001).

5.8.2 Antifungal Activity

Peppermint oil was in the group of 13 oils with the highest activity from among 51 researched, at the concentration 500 μg/ml showing the growth reduction from 8%

for *Torulopsis utilis* to 94% for *Schizosaccharomyces pombe* (Hili et al. 1997). A pronounced activity against *A. niger* (Chao et al. 2000) and phytopathogenic fungi (Zambonelli et al. 1996) was observed. It inhibited also the growth of opportunistic *C. albicans* strain (Ezzat 2001). Mimica-Dukic et al. (2003) also reported good activity toward *C. albicans* (MIC 8 μl/ml) as well as toward *Trichophyton tonsurans* (MIC 4 μl/ml).

Japanese mint oil showed microbicidal activity against 8 fungi strains (Thoppil et al. 2001) and antifungal activity against toxic *A. flavus* (Varma and Dubey 2001) and two other moulds (Panday 2003). The oil at 1,000 ppm completely inhibited fungal growth of *A. ochraceus* and ochratoxin A production (Basilico and Basilico 1999) and was effective against 5 different human pathogens: MIC 1–3.90 μl/ml against four fungi and 62.5 μl/mL against *T. rubrum* (Rath et al. 2001).

5.9 Citrus Oils

The most valuable citrus peel oils are isolated by cold pressing of the fresh peel from the evergreen trees' fruit. However, citrus peel oils produced by hydrodistillation, as well as terpeneless citrus oils are also available on the market. These oils have less valuable quality but they are more stable and less sensitive to oxidation. The oils from leaves, twigs or flowers of different citrus trees were also produced. Three citrus peel oils have monographs in EP 5, lemon, mandarin and sweet orange oil.

Lemon (*Citrus limon* L.; Rutaceae). The essential oil is isolated by cold pressing of peels or peel pulp, yield of 0.4–4%. Lemon oil is a clear, pale yellow to greenish-yellow liquid that becomes cloudy at low temperatures. It has characteristic odor and taste of outer lemon peel part. Lemon oil is widely used in lemon and other natural flavors: pineapple, butterscotch and banana flavors, and can be mixed with other citrus oils like lime, orange and grapefruit.

Major components are: limonene (56.0–78.0%), β-pinene (7.0–17.0%), γ-terpinene (6.0–12.0%), sabinene (1.0–3.0%), geranial (0.5–2.3%), neral (0.3–1.5%), neryl acetate (0.2–0.9%), geranyl acetate (0.1–0.8%), α-terpineol (max. 0.6%), β-caryophyllene (0.5%) (EP 5).

Mandarin (*Citrus reticulata* Blanco; Rutaceae). The essential oil is isolated from rind of almost ripe fruits, yield of 0.5%. The oil is greenish or yellow to reddish orange liquid showing blue fluorescence. It is widely used alone or in conjunctions with orange oil in beverages, confectionery and in many natural flavors (mango, peach, apricot).

Major components are: limonene (65.0–75.0%), γ-terpinene (16.0–22.0%), α-pinene (1.6–3.0%), myrcene (1.5–2.0%), β-pinene (1.2–2.0%), p-cymene (max. 1.0%), methyl N-methylanthranilate (0.3–0.6%), sabinene (max. 0.3%) (EP 5).

Orange sweet (*Citrus sinensis* (L.) Osbeck, syn. *C. aurantium* var. *dulcis* L.; Rutaceae). The oil is pale yellow to orange, clear liquid that may become cloudy when chilled. It has a mild bitter, astringent flavor, yield of 0.3–0.5%. Orange oil is generally used in orange flavors and many other natural flavors.

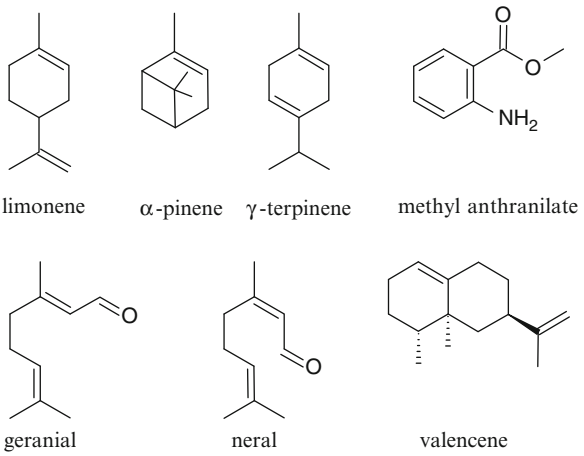


Fig. 5.5 Main components of citrus oils

Major components are: limonene (92.0–97.0%), myrcene (1.7–2.5%), sabinene (0.2–1.1%), linalol (0.2–0.7%), α-pinene (0.4–0.6%), valencene (0.02–0.5%), decanal (0.1–0.4%), octanal (0.1–0.4%), β-pinene (0.02–0.3%), geranial (0.03–0.2%), neral (0.02–0.1%) (EP 5) (Fig. 5.5).

5.9.1 Antibacterial Activity

The main constituent of all citrus peel oils is limonene – a monoterpene hydrocarbon. Antimicrobial activity of hydrocarbons is lower than that of oxygenated essential oil components. Despite of this fact, entirely good antibacterial and antifungal properties were observed for citrus oils mainly due to oxygenated monoterpenes. It is worth to mention that pressed citrus peel oils contain 1–15% non-volatile components.

The potential antimicrobial uses of citrus oils in food have been recently reviewed by Fisher and Philips (2008). In their previous work Fisher and Philips (2006) assessed antibacterial effect of lemon and sweet orange oil on the survival of five bacteria species. Both oils showed good activity against *L. monocytogenes* (MIC 0.25%) and weak activity against *C. jejuni*, *E. coli*, *S. aureus* and *B. cereus* (MIC 1–>4%). Lemon, mandarin and sweet orange oils exhibited high activity against *C. jejuni* (BA_{50} 0.009–0.044%), less against two *L. monocytogenes* strains (BA_{50} 0.0056–0.665%), and were merely active against *E. coli* and *S. aureus* (BA_{50} 0.41–0.67%) (Friedman et al. 2002). When tested against nine bacteria species (e.g. *E. faecalis*, *E. coli*, *K. pneumoniae*, *Serratia marcescens*) these three oils exhibited only weak antibacterial activity (MIC 2–>2%) with the exception of orange and lemon oil toward *Aeromonas sobria* (Hammer et al. 1999). Recently pronounced activity of mandarin oil was assessed against three Gram-positive bacterial strains (MIC 1–2 μl/ml) and lower one against Gram-negative bacteria such as

E. coli O157:H7, *S.* Enteritidis and *P. eruginosa* (MIC 5 µl/ml). Lemon oil and orange oil showed poor activity against Gram-negative bacteria while lemon oil revealed the highest activity against *Enterococcus faecium* and orange oil against *L. monocytogenes* (both MIC 0.5 µl/ml) (Espina et al. 2011).

Similar MIC 2.5 ml/100 ml for lemon oil against *E. coli* was reported by Moreira et al. (2005) and Ezzat (2001). According to Rossi et al. (2007) essential oils of *C. sinensis* and *Citrus reticulata* exhibited high activity against *C. jejuni* (MIC 0.125% and 0.25%, respectively), they were effective against *S. aureus* and showed weak activity against *E. coli*, *E. aerogenes* and *P. aeruginosa*. Orange oil at 300 ppm was partially sporicidal against *B. cereus* and *C. botulinum*, which was a little more resistant (Chaibi et al. 1997). It was only slightly active against 9 bacteria and 3 fungi (Chao et al. 2000). Lemon oil and sweet orange oil were among the most effective in the set of 21 essential oils against *P. vulgaris* (MIC 6.4 mg/ml) and 5 other bacteria (MIC 6.4–12.8 mg/ml) (Prabuseenivasan et al. 2006).

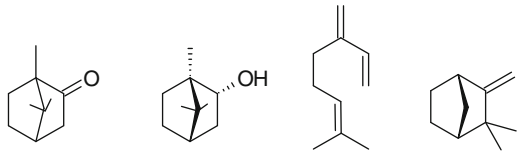
5.9.2 Antifungal Activity

The hydrodistilled fruit essential oils of lemon picked at three different period as well as 6 cultivars of sweet orange showed antifungal action on *Penicillium digitatum* and *Penicillium italicum*. Effective dose ED_{50} toward these two strains was 600–1,050 ppm and 1,400–2,500 ppm, respectively for lemon oil and 1,000–2,400 ppm and 3,200–5,400 ppm, respectively for orange oil (Caccioni et al. 1998). Orange oil was only slightly active against three fungi (Chao et al. 2000). Orange oil showed better activity than lemon and mandarin oils toward *C. albicans* (MIC 1%) (Hammer et al. 1999) and demonstrated fungistatic activity against *A. niger* at 1.5 µg/ml with 79% growth inhibition, at 750 ppm completely inhibited *A. parasiticus* and at 500 ppm aflatoxin B_1 production (Singh et al. 2010). However, orange oil had limited effect the growth of rye bread spoilage fungi, e.g. *Penicillium roqueforti*, *A. flavus* (Suhr and Nielsen 2003). Citrus oils at 1.6% reduce the level of *A. parasiticus* (Fisher and Philips 2008).

5.10 Rosemary Oil

Rosemary oil is obtained from flowering tops and leaves of *Rosmarinus officinalis* L. (evergreen shrub, Lamiaceae) with the yield of 0.5–2.5%. It is clear, mobile, colorless to pale yellow liquid with a characteristic odor. The main use of the oil is in seasoning blends. Two types of rosemary oil are available on the market, that differ mainly in percentages of main constituents, although several other chemotypes are known.

The main components of rosemary oil, Spanish type, are: α-pinene (18.0–26.0%), 1,8-cineole (16.0–25.0%), camphor (13.0–21.0%), camphene (8.0–12.0%), β-pinene



1,8-cineole camphor borneol myrcene camphene

Fig. 5.6 Main components of rosemary oil

(2.0–6.0%), limonene (2.5–5.0%), β-myrcene (1.5–5.0%), borneol (2.0–4.5%), α-terpineol (1.0–3.5%), verbenone (0.7–2.5%), bornyl acetate (0.5–2.5%), p-cymene (1.0–2.2%) (EP 5).

For rosemary oil, Moroccan and Tunisian type they are: 1,8-cineole (38.0–55.0%), camphor (5.0–15.0%), α-pinene (9.0–14.0%), β-pinene (4.0–9.0%), camphene (2.5–6.0%), borneol (1.5–5.0%), limonene (1.5–4.0%), α-terpineol (1.0–2.6%), p-cymene (0.8–2.5%), myrcene (1.0–2.0%), bornyl acetate (0.1–1.5%), verbenone (max. 0.4%) (EP 5).

It is very rarely indicated what type of rosemary oil was investigated for its antimicrobial activity and only sometimes the composition of the oil is given (Fig. 5.6).

5.10.1 Antibacterial Activity

Rosemary oil was among the most effective in the set of 21 essential oils against 6 bacteria, e.g. *B. subtilis*, *P. vulgaris*, *P. aeruginosa* and *K. pneumoniae* (MIC 6.4–12.8 mg/ml) (Prabuseenivasan et al. 2006) and in a set of other 21 oils against *S. aureus*, *L. monocytogenes* (MIC 0.04% and 0.02%, respectively) and *C. jejuni* (MIC 0.5%) (Smith-Palmer et al. 1998). According to Friedman et al. (2002) the oil showed higher activity toward *C. jejuni* (BA50 0.06%) and lower toward two *L. monocytogenes* strains (>0.6%).

The activity of rosemary oil toward four *E. coli* strains was quite good when compared with 10 oils tested, MIC 0.6 ml/100 ml (Moreira et al. 2005). According to other reports the oil was less effective against *E. coli* and *S. epidermidis* revealing MIC 11.3 mg/ml (Schelz et al. 2006) or 10–20 mg/ml (Celiktas et al. 2007) as well as against *P. putida* (MIC >0.8%) (Oussalah et al. 2007) and four other bacteria species (Celiktas et al. 2007). Rosemary oil had inhibitory effect on bacteria species used in food industry *Lactobacillus survatus*, *L. sakei*, *Staphylococcus xylosus*, *Staphylococcus carnosus*, and food spoilage bacteria *Enterobacter gergoviae* and *Enterobacter amnigenus* (Viuda-Martos et al. 2008).

Rosemary oil showed medium activity in the set of 13 oils against different strains of respiratory tract pathogens, e.g. *S. aureus*, *S. agalactiae*, *H. influenzae* (Fabio et al. 2007). However, its activity was lower (MID 50–100 mg/l air) than that of other 13 oils tested in vapor phase against respiratory tract pathogens (Inouye et al. 2001).

High activity of rosemary oil, Spanish type was established in diffusion test against 25 bacteria species (Baratta et al. 1998) and 29 bacteria strains (Mangena and Muyima 1999). Only tiny differences were found in activity of three oil samples of rosemary oil with different composition against five bacteria species (e.g. *E. coli*, *S. aureus*, *P. aeruginosa*) (Tommasi et al. 2009) and in activity of six oil samples (four with high 1,8-cineole content ca. 50% and two with similar 1,8-cineole and camphor content ca. 25%) toward three Gram-negative and five Gram-positive bacteria strains (Zaouali et al. 2010). In the latter research *P. aeruginosa* (MIC >10 µl/ml), *S. epidermidis* and *S. faecalis* (MIC 10 µl/ml) appeared to be resistant.

5.10.2 Antifungal Activity

Rosemary oil exhibited higher activity against yeast and moulds than bacteria (Luqman et al. 2007; Celiktas et al. 2007). It was in the group of 13 oils out of 51 tested demonstrating activity against four yeast species, e.g. *C. albicans*, *Saccharomyces cerevisiae* (Hili et al. 1997). MIC against two *S. cerevisiae* strains was 2.8–5.7 mg/ml (Schelz et al. 2006). Luqman et al. (2007) established MIC 2.75–5.5 mg/ml and MFC 5.5–11 mg/ml against ten drug-resistant mutants of *C. albicans* as well as MIC <2.75 mg/ml and MBC <5.5 mg/ml against dermatophytes. Hammer et al. (1999) reported similar MIC 1% while Angioni et al. (2004) considered rosemary oil as low active with MIC over 900 µg/ml against *C. albicans*.

From three rosemary oils the sample with composition corresponding to Moroccan and Tunisian type revealed significantly higher activity against *C. albicans* and *C. glabrata* then two others (Tommasi et al. 2009). High activity of rosemary oil, Spanish type was established in diffusion test against 12 yeast species (Mangena and Muyima 1999).

Rosemary oil was much more effective against rye bread spoilage fungi in the vapor than in agar medium. At 270 µl/l in air the oil totally inhibited the growth of *Endomyces fibuliger* and in 80% the growth of *Eurotium repens* being less active against other three fungi (Suhr and Nielsen 2003).

5.11 Conclusions

Essential oils are important natural products used for their flavor and fragrance in food, pharmaceutical and perfumery industries. The spectrum of biological and pharmacological activities of essential oils is exceptionally broad and has been extensively researched and reviewed. Their antimicrobial properties have been exploited intensively in recent years, mainly in respect to the extensive ban on antibiotics in the animal industries and antibiotic overuse in human medicine. These properties assessed by different *in vitro* methods are well documented and have been reported in this chapter for some essential oils selected for their importance as food additives.

However, it should be taken into account that essential oils are much more active in the *in vitro* conditions than in *in situ* (e.g. in food and cosmetic) or *in vivo* (in patients) model systems. The effective content of individual essential oil is usually too high to be acceptable for the application to food products because of the intensity of aroma. In the last decade, the assessments of antibacterial and antifungal activity of essential oils in product model systems have been more and more numerous. Such research revealed synergistic or at least additive effects in the mixtures of essential oils or essential oil with other food additives (Bassole et al. 2010; Tajkarimi et al. 2010). This suggests that such mixtures could be used in order to diminish the odor of each individual component and improve the preservative properties. Essential oils therefore will continue to be indispensable natural ingredients and they may provide alternatives to conventional antimicrobial additives in food.

References

Abdolahi A, Hassani A, Ghosta Y et al (2010) Essential oils as control agents of postharvest *Alternaria* and *Penicillium* rots on tomato fruits. J Food Safety 30:341–352

Aggarwal KK, Khanuja SPS, Ateeque A et al (2002) Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. Flavour Fragr J 17:59–63

Al-Bayati FA (2008) Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J Ethnopharmacol 116:403–406

Amvam Zollo PH, Biyiti L, Tchoumbougnang F et al (1998) Aromatic plants of tropical central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flavour Fragr J 13:107–114

Angioni A, Barra A, Cereti E et al (2004) Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. J Agric Food Chem 52:3530–3535

Baratta MT, Dorman HJD, Deans SG et al (1998) Antimicrobial and antioxidant properties of some commercial essential oils. Flavour Fragr J 13:235–244

Basilico MZ, Basilico JC (1999) Inhibitory effects of some spice essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxin A production. Lett Appl Microbiol 29:238–241

Bassole IHN, Lamien-Meda A, Bayala B et al (2010) Composition and antimicrobial activities of *Lippia multiflora* Meldenke. Mentha x piperita L. and Ocimum basilicum L. essential oils and their major monoterpene alcohols alone and in combination. Molecules 15:7825–7839

Beg AZ, Ahmad I (2002) *In vitro* fungitoxicity of the essential oil of Syzygium aromaticum. World J Microbiol Biotechnol 18:313–315

Biavati B, Franzoni S, Ghazvinizadeh H et al (1997) Antimicrobial and antioxidant properties of plant essential oils. In: Franz C, Mathe A, Buchbauer G (eds) Proceedings 27th international symposium essential oils, Vienna, Austria, Allured Publishing Corporation, Carol Stream, IL, USA, pp 326–331

Bourrel C, Vilarem G, Michel G et al (1995) Analyse chimique et propriétés fongistatiques de quelques huiles essentielles en milieu liquid. Effects sur la morphogénes hyphale. Riv Ital EPPOS 17:31–41

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 94:223–253

Burt SA, Reinders RD (2003) Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett Appl Microbiol 36:162–167

Caccioni DRL, Guizzardi M, Biondi DM et al (1998) Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. Int J Food Microbiol 49:73–79

Carmo ES, de Oliviera LE, de Souza EL et al (2008) Effect of *Cinnamomum zeylanicum* Blume essential oil on the growth and morphogenesis of some potentially pathogenic *Aspergillus* species. Braz J Microbiol 39:91–97

Celiktas OY, Hames Kocabas EE, Bedir E et al (2007) Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis* L., depending on location and seasonal variations. Food Chem 100:553–559

Ceylan E, Fung DYC (2003) Comparison and determination of minimum inhibitory concentration of essential oils against *Escherichia coli* O157: H. ARI Bull Istanbul Tech Univ 53:93–97

Chaibi A, Ababouch LH, Belasri K et al (1997) Inhibition of germination and vegetative growth of *Bacillus cereus* T and *Clostridium botulinum* 62A spores by essential oils. Food Microbiol 14:161–174

Chao SC, Young DG, Oberg CJ (2000) Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. J Essent Oil Res 12:639–649

Combrinck S, Regnier T, Kamatou GPP (2011) *In vitro* activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. Ind Crop Prod 33:344–349

Crociani F, Biavati B, Alessandrini A et al (1997) Growth inhibition of essential oils and other antimicrobial agents towards Bifidobacteria from dental caries. In: Franz C, Mathe A, Buchbauer G (eds) Proceedings of 27th international symposium on essential oils, Vienna, Austria, Allured Publishing Corporation, Carol Stream, IL, USA, pp 40–44

Deans SG, Noble RC, Hiltunen R et al (1995) Antimicrobial and antioxidant properties of *Syzygium aromaticum* (L.) Merr. & Perry: impact upon bacteria, fungi and fatty acid levels in ageing mice. Flavour Fragr J 10:323–328

Delespaul Q, Billerbeck VG, Roques Ch et al (2000) The antifungal activity of essential oils as determined by different screening methods. J Essent Oil Res 12:256–266

Donaldson JR, Warner SL, Cates RG et al (2005) Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods. Pharm Biol 43:687–695

Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88:308–316

Edris AE (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual constituents: a review. Phytother Res 21:308–323

El-Baroty GS, El-Baky HH, Farag RS et al (2010) Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. African J Biochem Res 4:167–174

Espina L, Somolinos M, Loran S et al (2011) Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. Food Control 22:896–902

European Pharmacopoeia of the Council of Europe (2005) 5th edition, 5.0, 5.1–5.8., Strasbourg, France (EP 5)

Ezzat SM (2001) *In vitro* inhibition of *Candida albicans* growth by plant extracts and essential oils. World J Microbiol Biotechnol 17:757–759

Fabio A, Cermelli C, Fabio G et al (2007) Screening of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infections. Phytother Res 21:374–377

Ferhout H, Bohatier J, Guillot J et al (1999) Antifungal activity of selected essential oils, cinnamaldehyde and carvacrol against *Malassezia furfur* and *Candida* albicans. J Essent Oil Res 11:119–129

Fisher K, Philips C (2008) Potential antimicrobial uses of essential oils of food: is citrus the answer? Food Sci Technol 19:156–164

Fisher K, Phillips CA (2006) The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus in vitro* and in food systems. J Appl Microbiol 101:1232–1240

Franz Ch, Novak J (2010) Sources of essential oils. In: Baser KHC, Buchbauer G (eds) Handbook of essential oils. Science, technology, and applications. CRC Press Taylor & Francis Group, Boca Raton

Friedman M (2006) Antimicrobial activities of plant compounds against antibiotic-resistant *Micrococcus luteus*. Int J Antimicrob Agents 28:156–158

Friedman M, Henika PR, Mandrell RE (2002) Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes* and *Salmonella enteric*a. J Food Prot 65:1545–1560

Gayoso CW, Lima EO, Oliveira VT et al (2005) Sensitivity of fungi isolated from onychomycosis to *Eugenia caryophyllata* essential oil and eugenol. Fitoterapia 76:247–249

Giamperi L, Fraternale D, Ricci D (2002) The *in vitro* action of essential oils on different organisms. J Essent Oil Res 14:312–318

Goni P, Lopez P, Sanchez C et al (2009) Antimicrobial activity in the vapor phase of a combination of cinnamon and clove essential oils. Food Chem 116:982–989

Hajlaoui H, Snoussi M, Noumi E et al (2010) Chemical composition, antioxidant and antibacterial activities of the essential oils of five Tunisian aromatic plants. Ital J Food Sci 3:320–329

Hammer KA, Carson CF, Riley TV (1999) Antimicrobial activity of essential oils and another plant extracts. J Appl Microbiol 86:985–990

Hili P, Evans CS, Veness RG (1997) Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Lett Appl Microbiol 24:269–275

Horne D, Holm M, Oberg C et al (2001) Antimicrobial effects of essential oils on *Streptococcus pneumoniae*. J Essent Oil Res 13:387–392

Hussain AI, Anwar F, Nigam PS et al (2010) Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. J Sci Food Agric 90:1827–1836

Imai H, Osawa K, Yasuda H et al (2001) Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. Microbios 106:31–39

Inouye S (2003) Laboratory evaluation of gaseous essential oils (Part 1). Int J Aromather 13:95–107

Inouye S, Yamaguchi H, Takizawa T (2001) Screening of antibacterial effect of variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infect Chemother 7:251–254

Inouye S, Abe S, Yamaguchi H et al (2003) Comparative study of antimicrobial and cytotoxic effects of selected essential oils by gaseous and solution contacts. Int J Aromather 13:33–41

Iscan G, Kirimer N, Kurkcuoglu M et al (2002) Antimicrobial screening of *Mentha piperita* essential oils. J Agric Food Chem 50:3943–3946

Jantan B, Moharam BAK, Santhanam J et al (2008) Correlation between chemical composition and antifungal activity of the essential oils of eight *Cinnamomum* species. Pharm Biol 46:406–412

Jirovetz L, Buchbauer G, Stoyanova A et al (2004) Antimicrobial testing and chiral phase gas chromatographic analysis of dill oils and related key compounds. Nutrition 28:257–260

Jirovetz L, Buchbauer G, Bali S et al (2009) Antimicrobial activities of essential oils of mint and peppermint as well as some of their main compounds. J Essent Oil Res 21:363–366

Kalemba D, Kunicka A (2003) Antibacterial and antifungal properties of essential oils. Curr Med Chem 10:813–829

Kouidhi B, Zmantar T, Bakhrout A (2010) Anticariogenic and cytotoxic activity of clove essential oil (*Eugenia caryophyllata*) against a large number of oral pathogens. Ann Microbiol 60:599–604

Lis-Balchin M, Deans SG, Eaglesham E (1998) Relationship between bioactivity and chemical composition of commercial essential oils. Flavour Fragr J 13:98–104

Lis-Balchin M, Ochocka RJ, Deans SG et al (1999) Differences in bioactivity between the enantiomers of α-pinene. J Essent Oil Res 11:393–397

Lopez P, Sanchez C, Batlle R et al (2005) Solid vapor phase antimicrobial activity of six essential oils: susceptibility of selected foodborne bacteria and fungal strains. J Agric Food Chem 53:6939–6946

Luqman S, Dwivedi GR, Darokar MP et al (2007) Potential of rosemary oil to be used in drug-resistant infections. Altern Ther Health Med 13:54–59

Mangena T, Muyima NYO (1999) Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. Lett Appl Microbiol 28:291–296

Marino M, Bersani C, Comi G et al (1999) Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. J Food Prot 62:1017–1023

Marino M, Bersani C, Comi G et al (2001) Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. Int J Food Microbiol 67:187–195

Mastura M, Nor Azah MA, Khozirah S et al (1999) Anticandidal and antidermatophytic activity of *Cinnamomum* species essential oils. Cytobios 98:7–23

Matan N, Rimkeeree H, Mawson AJ et al (2006) Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. Int J Food Microbiol 107:180–185

Mimica-Dukic N, Bozin B, Sokovic M et al (2003) Antimicrobial and antioxidant activities of three *Mentha* species essential oils. Planta Med 69:413–419

Misra N, Khare U, Shukla D (2000) Antifungal activity of essential oil of *Cinnamomum zeylanicum* Nees against *Stachybotrys chartarum*. J Essent Oil Bear Pl 3:97–110

Moreira MR, Ponce AG, del Valle CE et al (2005) Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT Food Sci Technol 38:565–570

Nevas M, Korhonen A-R, Lindstroem M et al (2004) Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. J Food Prot 67:199–202

Neves J, Pinto E, Amaral MH et al (2009) Antifungal activity of a gel containing *Thymus vulgaris* essential oil against *Candida* species commonly involved in vulvovaginal candidosis. Pharm Biol 47:151–153

Ooi LSM, Li Y, Kam S-L et al (2006) Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume. Am J Chin Med 34:511–522

Ouattara B, Simard RE, Holley RA et al (1997) Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int J Food Microbiol 37:155–162

Oussalah M, Caillet S, Saucier L et al (2006) Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. Meat Sci 73:236–244

Oussalah M, Caillet S, Saucier L et al (2007) Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control 18:414–420

Özek T, Tabanca N, Demirci F et al (2010) Enantiomeric distribution of some linalool containing essential oils and their biological activities. Rec Nat Prod 4:180–192

Panday AK (2003) Composition and *in vitro* antifungal activity of the essential oil of menthol mint (*Mentha arvensis* L.) growing in central India. Indian Drugs 40:126–128

Paparella A, Taccogna L, Aguzzi I et al (2008) Flow cytometric assessment of the antimicrobial activity of essential oils against *Listeria monocytogenes*. Food Control 19:1174–1182

Paster N, Menasherov M, Ravid U et al (1995) Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J Food Prot 58:81–85

Pattnaik S, Subramanyam VR, Bapaji M et al (1996) Antibacterial and antifungal activity of ten essential oils *in vitro*. Microbios 86:237–246

Pattnaik S, Padhan DK, Jana GK (2010) Evaluation of cinnamon oil, peppermint oil, cardamom oil & orange oil as antimicrobial agents. J Pharm Res 3:414–416

Penalver P, Huerta B, Borge C et al (2005) Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS 113:1–6

Pozzatti P, Scheid LA, Spader TB et al (2008) *In vitro* activity of essential oils extracted from plants used as spices against fluconazole-resistant and fluconazole-susceptible *Candida* spp. Can J Microbiol 54:950–956

Prabuseenivasan S, Jayakumar M, Ignacimuthu S (2006) *In vitro* antibacterial activity of some plant essential oils. BMC Complement Altern Med 6:1–8

Ranasinghe L, Jayawardena B, Abeywickrama K (2002) Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M. Perry against crown rot and anthracnose pathogens isolated from banana. Lett Appl Microbiol 35:208–211

Rath CC, Dash SK, Mishra RK (2001) *In vitro* susceptibility of Japanese mint (*Mentha arvensis* L.) essential oil against five human pathogens. Indian Perfum 45:57–61

Rattanachaikunsopon P, Phumkhachorn P (2010) Antimicrobial activity of basil (*Ocimum basilicum*) oil against *Salmonella enteritidis in vitro* and in food. Biosci Biotechnol Biochem 74:1200–1204

Reddy MVB, Angers P, Gosselin A et al (1998) Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochem 47:1515–1520

Reichling J, Schnitzler P, Suschke U et al (2009) Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties – an overview. Forsch Komplementmed 16:79–90

Roengsumran S, Petsom A, Thaniyavarn S et al (1997) Antibacterial activity of some essential oils. J Sci Res Chula Univ 22:13–19

Rossi P-G, Berti L, Panighi J et al (2007) Antibacterial action of essentials oils from Corsica. J Essent Oil Res 19:176–182

Rota C, Carrami ana JJ, Burillo J et al (2004) *In vitro* antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. J Food Prot 67:1252–1256

Schelz Z, Molnar J, Hohmann J (2006) Antimicrobial and antiplasmid activities of essential oils. Fitoterapia 77:279–285

Singh G, Maurya S, de Lampasona MP et al (2007) A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem Toxicol 45:1650–1661

Singh P, Shukla R, Prakash B et al (2010) Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of *Citrus maxima* Burm. and *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. Food Chem Toxicol 48:1734–1740

Sivamani P, Sahul Hameed AS (2010) *In vitro* antibacterial activity of essential oils of selected herbals against isolates from HIV/AIDS patients. J Pharm Res 3:672–678

Smith-Palmer A, Stewart J, Fyfe L (1998) Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Appl Microbiol 26:118–122

Sokovic M, Glamoclija J, Marin PD et al (2010) Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. Molecules 15:7532–7546

Soliman KM, Badeaa R (2002) Effect of oil extracted from some medical plants on different mycotoxigenic fungi. Food Chem Toxicol 40:1669–1675

Suhr KI, Nielsen PV (2003) Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. J Appl Microbiol 94:665–674

Tajkarimi MM, Ibrahim SA, Cliver DO (2010) Antimicrobial herb and spice compounds in food. Food Control 21:1199–1218

Tassou C, Koutsoumanis K, Nychas G-JE (2000) Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. Food Res Int 33:273–280

Thoppil J, Tajo A, Deena MJ et al (2001) Microbicidal properties of mint essential oils. Indian Perfum 45:143–145

Tommasi L, Negro C, Miceli A et al (2009) Antimicrobial activity of essential oils from aromatic plants grown in the Mediterranean area. J Essent Oil Res 21:185–189

Tullio V, Nostro A, Mandras N et al (2007) Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. J Appl Microbiol 102:1544–1550

Tzortzakis NG (2009) Impact of cinnamon oil-enrichment on microbial spoilage of fresh produce. Innov Food Sci Emerg 10:97–102

Unlu M, Ergene E, Unlu GV et al (2010) Composition, antimicrobial activity and *in vitro* cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (*Lauraceae*). Food Chem Toxicol 48:3274–3280

Varma J, Dubey NK (2001) Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. Int J Food Microbiol 68:207–210

Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J et al (2007) Antifungal activity of thyme, clove and oregano essential oils. J Food Safety 27:91–101

Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J et al (2008) Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. Int Food Sci Technol 43:526–531

Wilson CL, Solar JM, El Ghaouth A et al (1997) Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis 81:204–210

Wright J (2004) Flavor creation. Allured Publishing Corporation, Carol Stream

Xing Y, Li X, Xu Q et al (2010) Antifungal activities of cinnamon oil against *Rhizopus nigricans, Aspergillus flavus* and *Penicillium expansum in vitro* and *in vivo* fruit test. Int J Food Sci Tech 45:1837–1842

Zabka M, Pavela R, Slezakova L (2009) Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi. Ind Crops Prod 30:250–253

Zaki HM, Mohamed SR, Salman AM et al (2001) Exotoxigenicity of *Aeromonas* species isolated from different sources: inhibitory effect of essential oils on their growth. Vet Med J Giza 49:111–121

Zambonelli A, D'Aulerio AZ, Bianchi A et al (1996) Effects of essential oils on phytopathogenic fungi *in vitro*. J Phytopathol 144:491–494

Zaouali Y, Bouzaine T, Boussaid M (2010) Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem Toxicol 48:3144–3152

Zu Y, Yu H, Liang L et al (2010) Activities of ten essential oils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7 cancer cells. Molecules 15:3200–3210