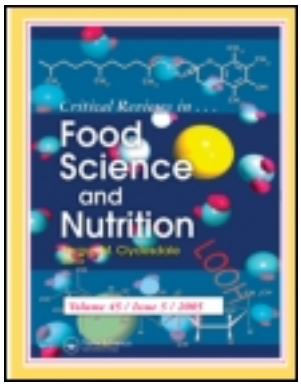

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REVIEW ARTICLE

Plant Essential Oils as Active Antimicrobial Agents

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Essential oils derived from plants have been recognized for decades to exhibit biological activities, including antioxidant, anticancer, and antimicrobial attributes. Antimicrobial activities of these natural plant materials have been intensively explored in recent years, mainly in response to the overwhelming concern of consumers over the safety of synthetic food additives. Gram-negative organisms are believed to be slightly less sensitive to essential oils than Gram-positive bacteria. Generally, a higher concentration is required to obtain the same efficacy in foods than in synthetic media. The combinations of different types of essential oils or with other food additives have been found to potentially exhibit synergistic if not additive effects. This suggests a cost-efficient and wholesome alternative to both food industry and consumers, at the same time adhering to the hurdle technology in inhibiting proliferation of foodborne pathogens. This review aims to examine the conventional methods commonly used for assessment of antimicrobial activities of essential oils and phytochemicals, the use of these substances as antimicrobials in food products, factors that affect their efficacy, synergism between components or with available food preservatives as well as the challenges and future directions of using essential oils and phytochemicals as natural food preservatives.

Keywords Essential oils, phytochemicals, antimicrobial activity, preservative, foodborne pathogens

INTRODUCTION

Foodborne illnesses are still a major problem in the world. Despite new improvements in slaughter hygiene, food production techniques, and control programs, food safety remains as a widespread public health issue. According to a recent report, the Centers for Disease Control and Prevention (CDC) (2011) estimates that 48 million Americans fall ill, 128,000 get hospitalized, and 3,000 die from foodborne diseases annually. Common foodborne causative agents of outbreaks include *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus cereus, Clostridium perfringens, Clostridium botulinum, Campylobacter jejuni, Escherichia coli* O157:H7, and *Listeria monocytogenes*. The problem is further aggravated as many of these bacterial strains develop antibiotic resistance from the wide usage of antibiotics, combined with over-prescription and indiscriminate patient compliance (de Souza et al., 2005; Oroojalian et al., 2010).

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Mild preservation techniques in modern food industries play an important role in producing food with better organoleptic quality, but which does not favor food safety grounds. Hence, coupled with the social and economic implications of foodborne outbreaks, there is a growing interest in exploring new methods of reducing and eliminating foodborne pathogens, which, following the hurdle principle, can be used with other existing methods to balance sensory acceptability and antimicrobial effectiveness (Burt, 2004; de Oliveira et al., 2010).

Concern over the negative perception of consumers on chemical preservatives has motivated food industries to pursue and develop natural preservatives that may offer as safer alternatives. In addition, natural products are preferred due to its wide availability and better biodegradability. Consequently, research on plant essential oils and phytochemicals, which show potential as natural antimicrobials in food systems, has gained much attention in recent years. If developed successfully, such natural antimicrobial agents will be useful in preventing proliferation of foodborne pathogens and thus increasing the shelf life of food products.

Essential oils are volatile natural mixtures extracted from different plant parts (flowers, buds, seeds, leaves, twigs, bark,

herbs, wood, fruits, and roots), and are composed of terpenoid structures with broad activities. They are usually obtained by hydro or steam distillation and by expression (in the case of citrus peel oils). Compositional analysis of essential oils is done by gas chromatography, mass spectrometry, or headspace analysis. It is well established that essential oils contain volatile secondary plant metabolites that exhibit insecticidal, antioxidant, anti-inflammatory, anti-allergic, and anticancer properties, in addition to their antimicrobial characteristics (Svoboda et al., 2006; Lee et al., 2011). Thus, wide applications of essential oils are seen in medical and clinical microbiology, pharmaceutical botany, fragrance industries as well as food flavoring and preservation (Svoboda et al., 2006; Oroojalian et al., 2010). Some essential oils are even used in livestock feeds for animals such as weaned piglets (Burt, 2004). Plant extracts from herbs and spices are rich in phenolics, of which some were proven to show antimicrobial activity (Burt, 2004; Ho et al., 2010). For instance, curcumin extracted from turmeric (*Curcuma longa*) was shown to suppress the growth of *S. aureus*, *S. albus*, *B. cereus*, *B. subtilis*, *E. coli*, and *Pseudomonas aeruginosa* (Jayaprakasha et al., 2005). At certain concentrations, essential oils may be selective against pathogens without affecting beneficial commensal gut bacteria. For example, thyme oil is able to eradicate growth of *Clostridium perfringens* without affecting *Lactobacillus* (Svoboda et al., 2006). Other plant extracts or essential oils possessing recognized antimicrobial spectrum include oregano (*Origanum vulgare*) (Seaberg et al., 2003; Lin et al., 2005), cinnamon (*Cinnamomum zeylanicum*) (Burt, 2004), onions (*Allium cepa*), and garlic (*Allium sativum*) (Benkeblia, 2004).

Phytochemicals, which mostly originate from essential oils, are non-nutritive chemicals with relatively complex structures and have mechanisms different from traditional antimicrobials. They can be categorized generally into alkaloids, flavanoids, isoflavones, monoterpenes, phenolic acids, carotenoids, etc. In plants, phytochemicals help to protect the plant against UV light, herbivores, and pathogens, and also act as signals to beneficial microbes and pollinators (Svoboda et al., 2006). The most active phytochemicals isolated from essential oils were shown to be thymol, carvacrol, *p*-cymene, γ-terpinene, 1,8-cineole, *cis*-ocimene, camphor, linalool, terpinene-4-ol, thujone, limonene, α-bisabolol, and chamazulen (Svoboda et al., 2006). Other phytochemicals that have been identified as effective antimicrobials include eugenol, perillaldehyde, cinnamaldehyde, and cinnamic acid (Burt, 2004).

In many cases, essential oils exert their beneficial effects better than a chemically synthesized pure compound. This may be due to the synergistic action of the complex mixture of components that can interact with multiple molecular sites. Thus, simultaneous resistance of target microorganisms at two different sites is needed to take effect (Svoboda et al., 2006). Some constituents of essential oils, however, may affect the absorption rates or bioavailability of other components.

The purpose of this paper is to review recent studies on the antimicrobial activity of essential oils and phytochemicals appropriate for food application. Suggested mechanisms of action on microorganisms, factors affecting antimicrobial activity of essential oils as well as their efficacy with respect to foodborne microorganisms will be described. Lastly, possible synergism with different components/oils and food additives will be discussed. Although some data provided in this review are for spoilage microorganisms, the paper focuses mostly on the antimicrobial effect of essential oils and phytochemicals on foodborne pathogens. For easier understanding the names of the plant essential oils mentioned in this review paper, the species and genus as well as their common names are listed in Table 1.

METHODS FOR ANTIMICROBIAL TESTING

There are various techniques to test the antimicrobial activity of essential oils. The two most universally accepted and commonly used methods are agar disk diffusion and agar dilution. This is mainly because compatible data have been obtained when these two methods were performed on the same phytochemical or essential oil. However, other methods, such as turbidimetry, bioimpedimetry, bioautography, microatmospheric method, and time killing assay are described and compared in brief with the two most common methods.

Agar Diffusion Method

Agar diffusion is the most common and effective method to test the antimicrobial activity of plant extracts, phytochemicals, and essential oils. It is deemed as a simple and quick method requiring very little amount of essential oil. In most cases, promising results are assured. Agar diffusion is usually used as a prescreening step for the antimicrobial activity of a large number of essential oils. The basic principle behind this method is the diffusion of essential oil through agar. An effective antimicrobial compound can inhibit the growth of microbes, resulting in the zone of inhibition. The measurement of the zone indicates the extent of antimicrobial property of the compound.

Petridishes are first filled with agar broth before inoculation with microorganisms. The essential oil is taken in different concentrations and applied onto filter paper discs or into wells impregnated on agar. Typically, plates are left at room temperature for approximately 30 minutes to allow proper diffusion of essential oil through the agar, prior to incubation at 37°C for 24 hours (Lee et al., 2011). The essential oil diffuses into the agar and prevents microbial growth in the area of its action. The essential oil concentration is maximum at the center of the disc or well, and decreases in the agar further from the disc or well. Clear areas around the disk or well, which mark the zone of inhibition, are measured and a greater clear area portrays higher antimicrobial efficacy of essential oil. Hence, by this method, the degree of antimicrobial activity of essential oil can be measured.

Table 1 Scientific and common names of essential oils

Scientific name		Common name(s)
Genus	Species/cultivars	
Allium	*A. cepa*	Onion
Allium	*A. sativum*	Garlic
Anethum	*A. graveolens* L.	Dill
Anthemis	*A. nobilis* L.	Camomile
Backhousia	*B. citriodora*	Lemon myrtle
Bidens	*B. pilosa* Linn var. *Radiata*	Hairy beggar ticks, sticks tights, Spanish needles
Bunium	*B. persicum*	Black cumin
Cestrum	*C. nocturnum*	Night cestrum, lady of the night, night-blooming jessamine, night-blooming jasmine
Chrysanthemum	*C. indicum*	Chrysanthemum
Cinnamomum	*C. zeylanicum, C. zeylanicum* Blume, *C. verum*	Cinnamon
Cinnamomum	*C. osmophloeum*	Indigenous cinnamon
Citrus	*C. bergamia* Risso	Bergamot
Citrus	*C. limon*	Lemon
Citrus	*C. senensis*, *C. sinensis* L. *Osbeck*	Valencia orange
Coriandrum	*C. sativum* L.	Coriander (seeds), Cilantro (immature leaves)
Cryptomeria	*C. japonica*	Japanese cedar
Cuminum	*C. cyminum*	Cumin
Cunila	*C. galioides, C. incisa, C. spicata, C. menthoides, C. angustifolia, C. microcephala*	Dittany
Curcuma	*C. longa*	Turmeric
Cymbopogon	*C. citrates*	Lemongrass
Eucalyptus	*E. dives, E. globules*	Eucalyptus
Hyssopus	*H. officinalis* L.	Hyssop
Juniperus	*J. phoenicea*	Phoenician juniper
Kadsura	*K. longipedunculata*	Chinese kadsura vine
Laurus	*L. nobilis* L.	Laurel
Melaleuca	*M. alternifolia*	Tea tree
Mentha	*M. spicata* L., *M. piperita* L.	Mint
Myristica	*M. fragrans* Houtt	Mace (aril)
Myrtus	*M. communis*	Myrtle
Nigella	*N. sativa* Linn	Black seed
Ocimum	*O. sanctum* L.	Holy basil
Ocimum	*O. gratissimum* L.	African basil
Ocimum	*O. basilicum* L.	Sweet basil
Origanum	*O. vulgare* L., *O. compactum*	Oregano
Pelargonium	*P. graveolens, P.* "Sweet Mimosa," *P.* "Mabel Grey," *P.* "Atomic Snowflake," *P.* "Royal Oak." *P.* "Attar of Roses," *P.* "Chocolate Peppermint," *P.* "Clorinda"	Geranium
Picea	*P. excelsa*	Norway spruce
Rosmarinus	*R. officinalis*	Rosemary
Salvia	*S. officinalis* L.	Sage
Salvia	*S. tomentosa*	Balsamic sage
Schinus	*S. molle* L.	Peruvian peppertree
Silene	*S. armeria* L.	Caryophyllaceae
Syzygium	*S. aromaticum*	Clove
Tachyspermum	*T. copticum*	Ajwain
Thymbra capitata	*T. capitata*	Thyme
Thymus	*T. vulgaris L., T. daenensis*, *T. tosevii* L., *T. pulgeioides, T. pallescens, T. algeriensis, T. dreatensis, T. kotschyanus, T. persicus*	Thyme
Zingiber	*Z. officinale* Roscue	Ginger
Zizyphus	*Z. jujuba*	Red date, Chinese date

However, certain disadvantages limit the use of the agar disk diffusion method for essential oils. First, agar disc diffusion may not be very well suited for volatile essential oils or volatile secondary plant metabolites as they evaporate very quickly and thus fail to produce a satisfactory zone of inhibition. Second, the method cannot be used for poorly soluble compounds, as they do not diffuse properly in the agar to produce effective results. Thus, dispersing and emulsifying agents are often used in an attempt to solve this problem (Kim et al., 1995a; Sari et al., 2006).

Agar Dilution Method

Direct Contact Assay

The agar or the broth dilution method can be applied to both bacteria and fungi for antimicrobial activity testing of plant extracts, secondary metabolites, and essential oils. In this method, bacterial cells are first pre-cultured at 37°C for 24 hours in a suitable growth broth. A fixed amount of this bacterial suspension is inoculated into nutrient broth, followed by the addition and mixing of relevant essential oils of different concentrations. The mixtures are then incubated at 37°C for 24 hours prior to measurement of optical density values. The minimum concentration of the compound, which shows no growth of microorganisms on determination of absorbance (no absorbance change from before), is deemed as the minimum inhibitory concentration (MIC) (Cristani et al., 2007). Thus, the MIC is said to exert a bacteriostatic effect which decreases inoculum viability, and helps in determining the antimicrobial activity of essential oil. To confirm the bacterial survival and verify if the inhibition is reversible or permanent, samples showing no increase in absorbance are further subcultured onto nutrient agar plates and incubated at 37°C for 48 hours. The lowest concentration of essential oil is required to kill 99.9% or more of an original inoculum of bacteria. This concentration is referred to as minimum bactericidal concentration (MBC).

Vapor Phase Assay

While most antimicrobial tests have been performed such that essential oils are in direct contact with the antimicrobial agent, there is interest in the antimicrobial effectiveness of essential oils in the vapor phase, such as in the case of active packaging when essential oils are added to packaging instead of directly into food. Moreover, due to high hydrophobicity and volatility of essential oils, low water solubility has to be overcome with addition of emulsifiers or solvents to the dilution assay, which may alter activity of essential oils (Nedorostova et al., 2009).

The vapor phase assay for essential oils was first described by López et al. (2005) to determine the antimicrobial activity of essential oils in the vapor phase. Agar medium is prepared in the same way as the direct contact assay described above. Essential oils are prepared by diluting in solvent before aliquots of each dilution are added to sterile blank filter disks and placed on the medium-free cover of each petridish, instead of media. Alternatively, the filter disks with essential oil dilutions may also be added to microscope slides before attaching to the cover of the petridish to prevent interaction between the oil and plastic dish (Becerril et al., 2007).

After the incubation period, the MIC is determined as the lowest essential oil concentration which made visible inhibition zone, and is expressed as microliters or milliliters of essential oil per volume unit of atmosphere above the organism growing on the agar surface.

Description and Comparison of Various Methods

Five different methods are described in brief, followed by a comparison of a few prominent methods with regard to their significance and limitations (Table 2).

Turbidimetry has been used to find out antimicrobial activity by optical density evaluation. This method involves the use of a 96-well plate containing the microbial culture and essential oils to determine absorbance. The plates are first incubated and then shaken vigorously and finally scanned by a multiscan photometer to get optical density values. Thus, by endpoint determination, the reduction in microbial cell growth is noted (Ponce et al., 2003).

Bioimpedimetry has also been used to detect antimicrobial activity. The impedance method is based on changes in conductance caused by metabolism of bacteria in the growth media. The technique measures antibacterial activity by detection time (DT) – defined as the time required by the microbial population to reach the concentration, also known as the threshold, causing rapid deviation in the curve of the percentage electrical variation. Detection time is a function of the initial microbial concentration, the microbial generation time, and the length of the lag phase (Marino et al., 2001). A delay in the detection time is an indication of antibacterial activity exhibited by the test sample.

Another method used is Bioautography. It involves the separation of phytochemical and essential oil components by paper or thin layer chromatography and application of microbe

Table 2 Comparison of significance and limitations among antimicrobial tests

Method	Significance	Limitations
Agar disk diffusion	Screening for presence or absence of antimicrobial activity.	Not suitable for volatile compounds.
Agar dilution	Determination of extent or degree of antimicrobial activity.	Discrepancy in data due to different MIC standards limits set.
Time killing assay	Determination of rapidity and duration of activity.	Does not help in detecting the components of antimicrobials and does not provide information about interaction between different antimicrobial agents.
Turbidimetry	Determination of extent or degree of antimicrobial activity.	Insensitive to small changes in concentration and is susceptible to air entrapment error (presence of air bubbles interfere with absorbance).
Bioautography	Determination of antimicrobial activity of all components of essential oils.	Not suitable for highly volatile compounds.

containing broth (Tellez et al., 2000; Rossi et al., 2011). Clear zones after elution indicate good antimicrobial activity. However, this technique is limited to a few phytochemicals and essential oils which are not very volatile (Kalemba and Kunicka, 2003).

Time killing assay is another method wherein the microbes are enriched in nutrient broth medium and incubated at 37°C for 24 hours. Essential oils of interest are added into media during the maximum growth period. Decrease in microbial cell count is observed either by colony counts (Moon et al., 2011), or turbidity via the UV spectrophotometer (Lee et al., 2011).

FACTORS AFFECTING ESSENTIAL OILS' ANTIMICROBIAL ACTIVITY

Experimental outcomes of antimicrobial tests of essential oils often depend on a host of factors such as composition of active agents, varietal differences, physical and chemical characteristics of the essential oil components, presence of starch, protein, or lipid which interfere with the essential oil antimicrobial activity, species and strain of microorganism, the test method used as well as culture conditions (Holley and Patel, 2005). For instance, the type and volume of broth, temperature, and time of incubation, as well as concentration and age of inoculums, all play a part in influencing the essential oil potency. It is imperative for such factors to be precisely stated in reports to allow valid comparisons of antimicrobial activity among different systems.

Concentration and Solubility

Similar to almost all types of antimicrobials, it is generally known that greater concentration of essential oils exerts greater antimicrobial activity. It was shown that essential oil concentration was the most significant factor influencing the bioactivity of essential oil from indigenous cinnamon (*Cinnamomum osmophloeum*) and Japanese cedar (*Cryptomeria japonica*) tissues against pathogenic *Legionella pneumophila* (Chang et al., 2008). However, the solubility of essential oil in the medium it is placed in may play a bigger role in affecting its activity. When studies were carried out on the antimicrobial effect of carvacrol and cinnamaldehyde in preventing *E. coli* O157:H7 and *Salmonella enterica* in apple juice, it was found that lower concentrations, which were miscible in the apple juice, showed significant decrease in the levels of these bacteria (Friedman et al., 2004). It was observed that in cloudy or turbid solutions, distribution of essential oil was non-uniform and the adsorption of some essential oils on the surface of apple pulp reduced its effectiveness in the whole juice system. Thus, miscibility of essential oil in the substrate at optimum concentration is the key criteria for effective antimicrobial activity.

Time

As with concentration, it is expected that greater exposure time would result in greater antimicrobial activity. A marked increase in fungicidal activity of Caryophyllaceae (*Silene armeria* L.) on *B. cinerea* spores was observed after 30-minute exposure at higher concentrations of 62.5 and 125 g/mL (Bajpai et al., 2008). However, there may reach a maximum limit, whereby antimicrobial activity no longer increases with time. Unlike *P. aeruginosa*, the membrane potential and permeability continued to be adversely affected by oregano (*Origanum compactum*) essential oil after 60-minute exposure (Bouhdid et al., 2009). According to Friedman et al. (2004), essential oils can be divided into the following two types based on the time they take to produce significant action: slow acting compounds and fast acting compounds. Some antimicrobials, such as carvacrol, cinnamaldehyde, and geraniol, are considered as fast acting compounds since they have shown to inactivate organisms like *E. coli* O157:H7 and *Salmonella* in a short span of five minutes. For the slow acting compounds, it was observed that a time span of 30–60 minutes was required to show efficient antimicrobial activity.

pH

In general, the susceptibility of bacteria to essential oils increases as the pH of food decreases (Burt, 2004; Basti et al., 2007; Gutierrez et al., 2009). High pH does not aid in antimicrobial activity whereas low pH coupled with a suitable essential oil may have an additional effect in reducing the number of microbes. Low pH values of about 5 appeared to have the greatest impact on the increase of antimicrobial effect of essential oils on *L. monocytogenes*, while pH values higher than that increased the growth rate of bacteria regardless of essential oil (Gutierrez et al., 2008). It is probably due to the fact that at low pH values, essential oil components tend to remain undissociated and are more hydrophobic, thus assisting their binding to hydrophobic parts of proteins and allowing them to dissolve more easily in the bacteria cell membrane lipids (Juven et al., 1994; Rivas et al., 2010). Studies have also proposed increase in carvacrol antimicrobial activity in oregano essential oil with addition of acetate moiety (in the form of acetic acid) (Angienda and Hill, 2011). However, acidic conditions may not always be effective for all microorganisms, especially if they are relatively acid-tolerant species. Such was the case for *E. coli* O157:H7, since this species is well known for its ability to grow well in acidic pasteurized and unpasteurized apple juice (Friedman et al., 2004).

Oxygen Availability

Growth conditions, such as availability of oxygen, also have an effect on antimicrobial activity of compounds. Thymol was found to be more effective in anaerobic conditions and expressed

a stronger antimicrobial activity compared with that of aerobic conditions (Kalemba and Kunicka, 2003). In addition, cinnamon and clove oils proved effective when used in combination with low oxygen levels (<0.05%) and high CO_2 concentrations (40%), since growth of bacteria could be delayed under such conditions. Such proportions were deemed suitable for active packaging systems for intermediate moisture foods (Matan et al., 2006). Anaerobic conditions could chemically alter tea tree oil components to more active antimicrobial compounds, or alter the metabolism of microorganisms to increase the uptake of the oil (Hammer et al., 1999).

Temperature

The effect of temperature on the antimicrobial activity of essential oils is controversial. While some studies reported an increase in activity with increasing temperature (Friedman et al., 2004; Kotzekidou et al., 2008), others showed the opposite trend (Tassou et al., 1995; Canillac and Mourey, 2004; Rivas et al., 2010). Low concentrations of orange, lemon, and mandarin essential oils had synergistic lethal effects with mild heat treatment on bacterial cells (Espina et al., 2011). It was found that 500 g/mL cinnamaldehyde could completely inhibit bacterial growth for more than 30 days at 30°C, but the effect was less at 20°C (Moleyar and Narasimham, 1992). Inhibitory activity of thyme essential oil against *E. coli* O157:H7 in minced meat was observed at 10°C storage condition but not at 4°C (Solomakos et al., 2008a). On the other hand, decreasing storage temperature appeared to have better antimicrobial effect of carvacrol on *E. coli* O157:H7 (Rivas et al., 2010). Ultimately, the optimum temperature would depend on the target matrix, essential oil used, and target organism to be inhibited, since different microbes also have different optimum temperature for growth. This may be especially so for psychotropic species (Valero and Salmerón, 2003).

Types of Microorganisms

In general, phytochemicals and essential oils have a better antimicrobial effect on Gram-positive microorganisms (Burt, 2004; Dung et al., 2008; Kotzekidou et al., 2008; Klančnik et al., 2011). Gram-negative microorganisms possess an outer membrane rich in lipopolysaccharide that is almost impermeable to lipophilic compounds, thus preventing various antimicrobial components of essential oil from penetrating through (Al-Reza et al., 2009). The hydrolytic enzymes present in its periplasmic space may also help in hydrolyzing antimicrobial substances. On the other hand, Gram-positive bacteria do not have this barrier, thus the hydrophobic components of essential oils can contact directly with the phospholipid bilayer of the cell membrane and cause increase in ion permeability, intracellular constituent leakages as well as impedance of enzyme systems (Gao et al., 2010). Nonetheless, essential oils can still act on Gram-negative bacteria due to the presence of porin proteins in the outer membrane which create sufficiently large channels to allow passage of small molecular mass compounds (Holley and Patel, 2005; Kotzekidou et al., 2008).

For instance, the essential oil of Chinese Kadsura Vine (*Kadsura longipedunculata*) was tested to exert good antimicrobial activity against all Gram-positive bacteria tested, moderate fungicidal activity against yeast, but missing activity against Gram-negative bacteria (Mulyaningsih et al., 2010). On the other hand, a study of essential oil of cinnamon (*Cinnamomum zeylanicum* Blume (Lauraceae)) was reported to show strong antimicrobial activity against all Gram-positive bacteria (including *Staphylococcus, Streptococcus*, and *Enterococcus*), Gram-negative bacteria (including *Pseudomonas* and *Clostridium*), and yeast (including *Candida* strains) tested (Unlu et al., 2010). The high amount of cinnamaldehyde may be due to high antimicrobial activity of cinnamon oil. Future investigation is warranted to elucidate the exact mechanism. Even though Gram-negative bacteria is often less susceptible, there are still certain essential oils that work against them in relation to the very specific structure–activity relationship.

Bacterial Cell Number

The extent of antimicrobial activity by essential oil components is dependent on cell numbers, although no correlation has been established yet (Kalemba and Kunicka, 2003). Many of the studies do not take into account the effects of inoculum's size and use relatively large number of cells (Lambert et al., 2001). Generally, as inoculum size increases, the MIC values increase accordingly (Lambert et al., 2001; Klančnik et al., 2011). However, some components, such as pulegone, tend to be more effective against *Erwinia amylovora* when the microbial cell concentration is low (3 log cfu/mL) but other components, such as pinene, could be effective even at high cell concentrations of 7 log cfu/mL (Scortichini and Rossi, 1991). Different essential oils can also respond differently to low or high inoculum numbers of the same bacterial species. The essential oil of *Satureja bachtiarica* was least efficient in decreasing *L. monocytogenes* at both low and high inoculum numbers as compared with the essential oil of thyme (*Thymus daenensis*) (Pirbalouti et al., 2010a).

Dispersing and Emulsifying Agents

Essential oils contain many water-insoluble hydrophobic components which make dispersion in the growth medium difficult. Only the more water-soluble components, such as carvone or 1,8-cineole, are able to diffuse into agar, while hydrophobic components may be left on the medium surface or evaporate (Sokovic et al., 2009). Thus, to improve the solubility of essential oils in the medium, many surface active agents (emulsifiers) and dispersing agents (solvents) have been used to ensure

maximum contact with microorganisms. Emulsifying agents and solvents, such as dimethyl sulfoxide (DMSO), Tween-20 or Tween-80, and ethanol (Remmal et al., 1993; Sokovic et al., 2009), as well as stabilizers such as agar and lecithin (Mann and Markham, 1998; Burt and Reinders, 2003), have been used previously. Stabilizers act by slowing down the separation of essential oils from water phase, thus allowing more effective inactivation of bacterial cells. However, such combination may alter the physical and chemical characteristics of essential oil components, and affect their functionality undesirably (Chalova et al., 2010). For example, some form of stabilizers such as lecithin may reduce the antimicrobial activity of phenols since lecithin can add another protection level to the bacterial cell membrane (Burt and Reinders, 2003).

Reports on the effect of Tween on the antimicrobial activity of essential oils are controversial. In positive cases, high antimicrobial activities of orange oil components of linalool, citral, and geraniol dispersed in Tween-20 were observed in a study by Kim et al. (1995b) and two to four-fold increase in the MIC of tea tree (*Melaleuca alternifolia*) oil against various bacteria was found to be attributed to Tween-80 in the study reported by Hammer et al. (1999). However, many studies have also shown the antagonistic effect of Tween on the antimicrobial activity of certain essential oils. This was the case for oregano and clove essential oils' activity (Remmal et al., 1993), Valencia orange oil (Chalova et al., 2010) as well as thyme (*Thymus* sp.) and mint (*Mentha* sp.) essential oils (Sokovic et al., 2009) by Tween-80. It has been proposed that beyond a certain concentration, the non-ionic Tween surfactant is capable of forming micelles to trap lipophilic molecules and prevent the exposure of bioactive compounds to microorganisms. On the other hand, DMSO and ethanol, both being polar solvents, do not form micelles and could better enhance the contact of materials with different polarity, thus increasing antimicrobial activity of Valencia orange oil (Chalova et al., 2010). Higher antimicrobial activity of ginger, fingerroot, and turmeric extracts against *Salmonella* and *Listeria* species was observed when prepared with isopropanol (polar solvent) than with hexane (non-polar solvent) (Thongson et al., 2004).

Food Components

Food components can affect antimicrobial activity of essential oils substantially; thus, apart from performing tests in common laboratory media, there is also a need to validate antimicrobial activity in food matrices. For example, fat in full cream milk can protect bacterial cells against antimicrobials, and damaged cells can also repair faster in food matrix than in laboratory media due to the presence of more nutrients (Klančnik et al., 2011). Antimicrobials are harder to disperse in food models and exposed to target microorganisms than in synthetic media (Gutierrez et al., 2008). In a recent research by de Oliveira et al. (2010), the combined application of thymol and carvacrol with lactic and acetic acids against *S. aureus* in meat was shown to be less effective than in meat broth. The lower efficacy of phenolic compounds in food models can be explained in terms of solubility and phase distribution parameters. Thymol and carvacrol, being lipophilic, dissolve better in other constituents of foods than in their aqueous phase where the bacterial growth occurs (Svoboda et al., 2006). In addition, the presence of nitrogenous compounds and fat in meat products could react with phenolic compounds and hinder their performance significantly. The phenolic groups and peptide links of proteins interact via hydrogen bonds and hydrophobic interactions to form complexes. This complication depends on protein characteristics, pH, as well as the molecular size, conformational flexibility, and water solubility of phenolic compound (de Oliveira et al., 2010). High concentration of sunflower oil was also found to reduce the antimicrobial activity of oregano and thyme essential oils (Gutierrez et al., 2008). A lower efficacy in vivo indicates a drawback of these natural substances as antimicrobial agents in food systems because high concentrations can adversely affect the organoleptic attributes of food (Naveena et al., 2006).

Sodium Chloride

Sodium chloride (NaCl) has shown both synergistic and antagonistic activities with essential oils and their components. At low concentrations, NaCl, in combination with clove oil, was effective against various *E. coli* strains (Angienda and Hill, 2011). Addition of NaCl to mint oil had additive antimicrobial effect against *S.* Enteritidis and *L. monocytogenes* (Tassou et al., 1995). However, antagonistic effect of 0.125% NaCl was found for carvacrol and p-cymene against *B. cereus* in rice (Ultee et al., 2000a), and inclusion of salt also did not improve the activity of cinnamaldehyde against Gram-positive or Gram-negative bacteria (Moleyar and Narasimham, 1992). The exact reasons for the discrepancy are not well understood.

Cations

Cations, such as Ca^{2+} and Mg^{2+}, can disrupt antimicrobial activity of essential oils by forming chelates with other ions and the essential oil component, hindering their entry through bacterial cell membranes (Hammer et al., 1999). Improvement of essential oil antimicrobial action was achieved by addition of ethylene diamine tetra acetic acid (EDTA) (Walsh et al., 2003).

SUGGESTED MECHANISMS OF ANTIMICROBIAL ACTIVITY

Antimicrobials' mechanisms can first be explored by looking at their respective structure–activity relationship. With a highly diversified groups of phytochemicals found in essential oils, it is suggested that the antimicrobial actions involve multiple targets within the cell rather than relying on one particular mechanism

(Lambert et al., 2001; Skandamis and Nychas, 2001; Carson et al., 2002). Due to the diversity of plant phytochemicals that may coexist in essential oils, the importance of detailed analysis of phytochemicals originating from different plant organs must therefore be stressed for accurate elucidation of the mechanism of action. By far, the most active phytochemicals are believed to be phenols followed by aldehydes and ketones, alcohols, and hydrocarbons (Knobloch et al., 1986).

Essential oils that exhibit most potent antimicrobial activities against pathogens possess high amount of phenolic compounds, such as thymol and carvacrol from oregano, and eugenol from clove. Therefore, the mechanism of action of essential oils is known to resemble those of phenolic compounds. One unique feature of essential oils is their hydrophobicity, rendering the abilities to react with lipids on the bacterial cell membrane. This increases the membrane permeability, disturbs the original cell structures, and breaks homeostasis (Knobloch et al., 1986; Sikkema et al., 1994). When there is substantial amount of leakage of cell content from bacteria, they become susceptible to cell death. Cell membrane has been thought to be the main site of action (Cristani et al., 2007). The interaction of essential oil and microbial cell envelope has been studied via scanning electron microscope to evaluate structural alterations (Di Pasqua et al., 2007). Results showed strong decrease of unsaturated fatty acids in the treated cells, resulting in changes in lipid profiles and structural alterations of cell envelopes.

Compound interaction with lipid membranes was investigated by Cristani et al. (2007) by monitoring the release, following exposure to essential oils, of the water-soluble fluorescent marker carboxyfluorescein from unilamellar vesicles with different lipid composition. Consistent with previous findings, it was evident that essential oils are able to interact with phospholipidic membrane by exerting a fluidifying effect to introduce lipophilic molecules into the ordered structure of lipid bilayer.

It is important to note that both hydrophilic and hydrophobic parts of phenolic compounds contributed to antimicrobial activity. As such, the hydrophilic part interacts with the polar part of the membrane, while hydrophobic part buries in the hydrophobic inner part of bacterial membrane. Furthermore, transmembrane transfer kinetic experiments, which investigate whether lipophilic medium better supports the absorption of bioactive compounds by biological membranes as compared to aqueous ones, showed that carvacol, which is more hydrophilic, has the fastest kinetic of interaction compared with thymol, *p*-cymene, and γ-terpinene, for it is more able to migrate through the aqueous membrane to reach the multilamellar liposomes surface and interact with lipid bilayers on the membrane (Cristani et al., 2007).

As the membrane integrity is being damaged, it further affects pH homeostasis, equilibrium of inorganic ions, nucleic acid synthesis, and balance of ATP pools. A study that utilized a mixture of thymol and carvacrol observed an increased permeability of *P. aeruginosa* and *S. aureus* cells to the fluorescence nuclear stain ethidium bromide, dissipated pH gradients irrespective of glucose availability, and leakage of inorganic ions (Lambert et al., 2001). However, it does not happen the same way in all microorganisms. For instance, when citrus oil blend was used against *Enterococcus faecium* and *Enterococcus faecalis*, it was found that as the membrane permeability increased, there was a loss of membrane potential, and decrease in both ATP synthesis and intracellular pH values (Fisher and Phillips, 2009). Nevertheless, existing findings have not been able to fully comprehend the exact effects of essential oils on specific enzymes, proteins, metabolic processes, and the mechanisms by which cells have adapted.

An antifungal study reported that clove essential oil resulted in an extensive lesion of cell membrane (Pinto et al., 2009). A considerable reduction in the quantity of ergosterol was also observed. Ergosterol is a major sterol component of fungal cell membrane, specific to fungi for the maintenance of cell function and integrity. Disruption of normal sterol biosynthetic pathways that leads to a reduction in ergosterol biosynthesis is known to be a primary mechanism of action by which antifungal drugs inhibit fungal cell growth (Kelly et al., 1995). In addition, germ tube formation, a change in morphogenesis that plays a critical role in pathogenicity, was almost completely inhibited by clove essential oil below the MIC values. A similar mechanism was found in the antifungal activities of essential oils of thyme (*Thymus pulgeioides*) (Pina-Vaz et al., 2004; Pinto et al., 2006), lemon grass (*Cymbopogon citrates*) (Tyagi and Malik, 2010), and thyme (*Thymbra capitata*) (Salgueiro et al., 2004).

By using flow cytometric approach, propidium iodide was found to have penetrated yeast cells rapidly within a short incubation period. This indicates that the mechanism of action involves an inherent killing of cells, implying a primary lesion of cell membrane rather than from metabolic impairment leading to secondary membrane damage (Pina-Vaz et al., 2004). With the aid of electron microscope, rapid leakage of potassium, rapid lysis of yeast spheroplast, severe cell membrane alterations with fracturing, and solubilization of membranes were observed (Pina-Vaz et al., 2004).

Hammer et al. (2004) investigated the mechanism of action of tea tree oil and its components against *Candida albicans*, *Candida albrata*, and *Saccharomyces cerevisiae*. Terpenes within the tea tree oil seem to have modified cell permeability, disrupted the lipid arrangement, resulting in altered membrane properties and functions. It is noteworthy that the position of terpene, in relation to its hydrophobicity strength, is closely connected to its efficacy within the membrane lipid bilayer. Other than the alteration in the membrane component, there was also an unexpected loss of inner material in the apical exocytosis of vesicles, which served to carry material for cell wall construction (Romagnoli et al., 2005). As a result, proper alignment of various parietal compounds, such as chitin, glucans, and glycoproteins, is then prevented.

As for virus, the mechanism of action of essential oils is thought to be due to the inhibition of viral protein synthesis at multiple stages of viral infection and proliferation. In a study, even though oil-treated virus continued to express viral mRNAs,

they have shown minimal expression of viral proteins, indicating suppression in viral protein translation (Wu et al., 2010).

On another note, to avoid false-positive or false-negative results, physical conditions capable of affecting essential oils' antimicrobial mechanism of action must be considered. As such, these will include fat content, complexity of foods, and temperature (Rattanachaikunsopon and Phumkhachorn, 2010). Other than that, low pH, low oxygen levels, high water activity, and salt level have been reported previously to be able to perturb the actions of essential oils. In conclusion, delicate design and careful investigation into the mechanisms of actions are needed to bring the efficacy of essential oils to the next level of understanding. Until then, use of essential oils as active antimicrobial agents shall remain restricted for now.

ACTIVITY OF ESSENTIAL OILS/COMPONENTS AGAINST MICROBES

Antibacterial Activity of Essential Oils and Components

Several studies have been performed to determine the various essential oils' and oil components' activities against foodborne bacterial pathogens. Common bacteria tested include Gram-positive *B. subtilis* and *S. aureus*, and Gram-negative *E. coli* and *P. aeruginosa*, as these are the most abundant surrogates of foodborne pathogens or representative toxin-producing microorganisms (Kalemba and Kunicka, 2003).

While most research groups focused on a specific plant, or group of plants, to investigate antimicrobial activities of these essential oils obtained from varying plant parts (Singh et al., 2007; Deba et al., 2008; Ennajar et al., 2010), species (Rasooli and Mirmostafa, 2003; Benkeblia, 2004; Hazzit et al., 2009), extraction techniques and solvents (Tepe et al., 2005; Celiktas et al., 2007; Al-Reza et al., 2009), environmental conditions (Canillac and Mourey, 2004), or geographical origins (Sari et al., 2006), some researches were focused on specific microbes, and demonstrated activities of up to 66 plant essential oils against these target microbes (Si et al., 2009). In addition, a fraction of papers proceeded to analyze the essential oil components to postulate which active compounds may be responsible for antimicrobial activity of the essential oil, and a number extended the experiment further by determining the active compounds' antimicrobial activities.

Table 3 lists common foodborne pathogens and various studied essential oils and their components that have demonstrated bacteriostatic and/or bactericidal activities toward these microorganisms. MIC and MBC values have also been included where determined in referenced papers.

Antifungal Activity of Essential Oils and Components

Apart from foodborne pathogens, several studies have also been performed to determine various essential oil and oil component activities against foodborne fungi such as *Aspergillus niger* and *Candida albicans*.

Table 4 lists common foodborne fungal pathogens and various studied essential oils and their components that have demonstrated antifungal activity toward these microbes. MIC values have also been included where determined in referenced papers.

Antimicrobial Activity of Essential Oils and Components in Food Matrices

Activities of antimicrobial essential oils have been investigated in a variety of natural and synthetic food matrices or packaging to reflect their ability to inactivate food spoilage pathogens when used in food manufacturing or as part of food packaging. Friedman et al. (2004) studied antimicrobial effects of a series of essential oils and components against *E. coli* O157:H7 and *S. enterica* in filtered and clear apple juices, at various levels of apple juice pH, and incubation time and temperature conditions. In general, their antimicrobial activities in apple juice were not significantly affected by juice pH, but they were increased with juice clarity, incubation temperature and time, and were dependent on the miscibility of antimicrobial components in the juice. Post-harvest strawberries have also been coated with essential oil components eugenol, menthol, and thymol to determine their antimicrobial activities (Wang et al., 2007). Eugenol, menthol, and thymol were successful in delaying the rate of berry decay and were able to promote decay resistance in the berries through increase in phenolic compounds, anthocyanins, flavonoids, and antioxidant capabilities (Wang et al., 2007).

Apart from fruits, essential oils' antimicrobial activities have also been evaluated in meat products. Tunisian sage (*Salvia officinalis* L.) and Peruvian peppertree (*Schinus molle* L.) essential oils were added into minced beef meat inoculated with *S. Anatum* and *S. Enteritidis* and stored the beef meat for up to 15 days. As a result, bacteriostatic effects were observed throughout the duration of the study for concentrations of less than 0.1% (w/v) (Hayouni et al., 2008). However, at concentrations of up to 3.0%, bactericidal effects were apparent, and more pronounced with increasing concentration (Hayouni et al., 2008).

In addition, synthetic food systems have been utilized in several studies to minimize confounding factors of natural food products and as preliminary work before optimizing essential oils as antimicrobial agents for application into real food. Seven cultivars of geranium (*Pelargonium* sp.) essential oils mixed into a model food system – soup made of broccoli and white flour inoculated with *S. aureus* – were observed to be able to completely inhibit the pathogen (Lis-Balchin et al., 2003). Gutierrez et al. (2009) utilized food model systems of lettuce, meat, and milk inoculated with *Listeria* spp. to optimize antimicrobial efficacy and elucidate the sensory impact of lemon balm, marjoram, oregano, and thyme essential oils. Results of the experiments showed that antimicrobial activities of essential oils were encouraged by high protein concentrations, low pH, and

Table 3 Antimicrobial activity of essential oils and components

Microbe	Gram	Essential oil/activity	Essential oil component/activity
Aeromonas hydrophila	–	*Chrysanthemum indicum* 1.25 μg/mL[a] 5 μg/mL[b] (Lee et al., 2011), *Juniperus phoenicea* (Ennajar et al., 2010), *Cunila* sp. 2.50 mg/mL[a] (Sandri et al., 2007), *Satureja subspicata* Vis. 1.56 μL/mL[a] 3.12 μL/mL[b] (Skocibusić et al., 2006).	Thujone 1.25 μg/mL,[a] 5 μg/mL,[b] chrysanthemyl alcohol 5 μg/mL,[a] 20 μg/mL,[b] camphor 5 μg/mL,[a] 20 μg/mL,[b] γ-terpinene 1.25 μg/mL[a] (Lee et al., 2011).
Bacillus cereus	+	*Bunium persicum* 0.18 mg/mL,[a,b] *Cuminum cyminum* 0.37 mg/mL,[a,b] *Tachyspermum copticum* 0.03 mg/mL[a,b] (Oroojalian et al., 2010), *Chrysanthemum indicum* 1.25 μg/mL[a] 10 μg/mL[b] (Lee et al., 2011), *Juniperus phoenicea* (Ennajar et al., 2010), *Cinnamomum zeylanicum* Blume bark, leaf (Singh et al., 2007), *Bidens pilosa* Linn var. *Radiata* (Deba et al., 2008), *Thymus pallescens*, *T. algeriensis*, *T. dreatensis* (Hazzit et al., 2009), *Salvia tomentosa* 9.0 mg/mL[a] (Tepe et al., 2005), *Cunila* sp. $\geq$ 0.04 mg/mL[a] (Sandri et al., 2007), *Satureja subspicata* Vis. 3.12 μL/mL[a] 6.25 μL/mL[b] (Skocibusić et al., 2006), *Anisochilus carnosus* (Senatore et al., 2003), *Laurus nobilis* L. 0.20% v/v[a] (Erkmen and Özcan, 2008), *Phlomis ferruginea* Ten. (Formisano et al., 2006), *Nigella sativa* Linn (Singh et al., 2005b), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c).	Thujone 1.25 μg/mL,[a] 5 μg/mL,[b] chrysanthemyl alcohol 20 μg/mL,[a] camphor 5 μg/mL,[a] 20 μg/mL,[b] γ-terpinene 1.25 μg/mL,[a] 20 μg/mL[b] (Lee et al., 2011), eugenol, cinnamaldehyde (Singh et al., 2007), carvacrol (Ultee et al., 2000a).
Bacillus subtilis	+	*Chrysanthemum indicum* 10 μg/mL[a] 10 μg/mL[b] (Lee et al., 2011), *Juniperus phoenicea* (Ennajar et al., 2010), *Cinnamomum zeylanicum* Blume bark, leaf (Singh et al., 2007), *Bidens pilosa* Linn var. *Radiata* (Deba et al., 2008), *Thymus kotschyanus* 0.625 ppm,[a,b] *T. persicus* 0.625 ppm[a,b] (Rasooli and Mirmostafa, 2003), *Rosmarinus officinalis* (Celiktas et al., 2007), *Cestrum nocturnum* 25.0 μg/mL[a] (Al-Reza et al., 2009), *Cunila* sp. $\geq$ 0.31 mg/mL[a] (Sandri et al., 2007), *Satureja subspicata* Vis. 3.12 μL/mL[a] 3.12 μL/mL[b] (Skocibusić et al., 2006), *Anisochilus carnosus* (Senatore et al., 2003), *Laurus nobilis* L. 0.20% v/v[a] (Erkmen and Özcan, 2008), *Phlomis ferruginea* Ten. (Formisano et al., 2006), *Nigella sativa* Linn (Singh et al., 2005b), *Plectranthus cylindraceus* 31.3 μg/mL[a] (Marwah et al., 2007), *Citrus bergamia* Risso 1.00 mg/mL[a] (Mandalari et al., 2007), *Herba Moslae* 0.236 mg/mL[a] 0.472 mg/mL[b] (Li et al., 2010), *Myristica fragrans* Houtt (aril) (Singh et al., 2005a), *Zataria multiflora* (Mahboubi and Bidgoli, 2010).	Thujone 1.25 μg/mL,[a] 5 μg/mL,[b] chrysanthemyl alcohol 2.5 μg/mL,[a] camphor 5 μg/mL,[a] 20 μg/mL,[b] γ-terpinene 1.25 μg/mL[a] (Lee et al., 2011), eugenol, cinnamaldehyde (Singh et al., 2007), eriodictyol 0.25 mg/mL[a] (Mandalari et al., 2007).
Escherichia coli O157:H7	–	*Bunium persicum* 1.50 mg/mL,[a,b] *Cuminum cyminum* 3.0 mg/mL,[a,b] *Tachyspermum copticum* 0.25 mg/mL[a] 0.50 mg/mL[b] (Oroojalian et al., 2010), *Anthemis nobilis* L., *Hyssopus officinalis* L., *Mentha piperita* L. (Marino et al., 2001), *Origanum vulgare* L. (Marino et al., 2001; Friedman et al., 2004), cinnamon leaf, lemongrass, lemon (Friedman et al., 2004), cinnamon 0.133 mg/mL[b], clove 0.283 mg/mL[b] (Friedman et al., 2004; Si et al., 2006), *Satureja subspicata* Vis. 1.56 μL/mL[a] 3.12 μL/mL[b] (Skocibusić et al., 2006), *Zataria multiflora* (Mahboubi and Bidgoli, 2010), *Coriandrum sativum* L. (immature leaves) 0.40% v/v[a], *Anethum graveolens* L. 0.47% v/v[a], *Eucalyptus dives* 0.27% v/v[a], *C. sativum* L. (seeds) 0.23% v/v[a] (Delaquis et al., 2002).	Carvacrol 0.283 mg/mL,[b] geraniol 0.283 mg/mL,[b] eugenol 0.466 mg/mL[b] (Friedman et al., 2004; Si et al., 2006), citral (Friedman et al., 2004), *m*-anisaldehyde 0.933 mg/mL,[b] thymol 0.166 mg/mL,[b] 2-*tert*-butyl-4-methylphenol 0.433 mg/mL,[b] mandelonitrile 0.766 mg/mL[b] (Si et al., 2006).
Listeria monocytogenes	+	*Bunium persicum* 0.75 mg/mL,[a,b] *Cuminum cyminum* 1.50 mg/mL,[a,b] *Tachyspermum copticum* 0.25 mg/mL[a,b] (Oroojalian et al., 2010), *Chrysanthemum indicum* 1.25 μg/mL[a] (Lee et al., 2011), *Juniperus phoenicea* (Ennajar et al., 2010), *Thymus pallescens*, *T. Algeriensis*, *T. dreatensis* (Hazzit et al., 2009), *Cunila* sp. $\geq$ 1.25 mg/mL[a] (Sandri et al., 2007), *Satureja subspicata* Vis. 1.56 μL/mL[a] 6.25 μL/mL[b] (Skocibusić et al., 2006), *Laurus nobilis* L. 0.02% v/v[a] (Erkmen and Özcan, 2008), *Cestrum nocturnum* 12.5 μg/mL[a] (Al-Reza et al., 2009), *Coriandrum sativum* L. (immature leaves) 0.02% v/v[a], *Eucalyptus dives* 0.37% v/v[a], *C. sativum* L. (seeds) 0.47% v/v[a] (Delaquis et al., 2002), *Mosla chinensis* Maxim 62.5 μg/mL[a] (Cao et al., 2009), *Zizyphus jujuba* 31.25 μg/mL[a] (Ozturk and Ercisli, 2007), *Picea excelsa* (Canillac and Mourey, 2004), *Cymbopogon citratus*, *Ocimum basilicum* L., *O. gratissimum* L., *Thymus vulgaris* L., *Zingiber officinale* Roscue (Nguefack et al., 2004).	Thujone 1.25 μg/mL,[a] 20 μg/mL,[b] chrysanthemyl alcohol 5 μg/mL,[a] camphor 5 μg/mL,[a] γ-terpinene 1.25 μg/mL[a] (Lee et al., 2011).

Table 3 Antimicrobial activity of essential oils and components *(Continued)*

Microbe	Gram	Essential oil/activity	Essential oil component/activity
Proteus mirabilis	–	*Anthemis nobilis* L., *Hyssopus officinalis* L., *Mentha piperita* L., *Origanum vulgare* L. (Marino et al., 2001), *Cunila* sp. ≥ 5.00 mg/mL[a] (Sandri et al., 2007), *Satureja subspicata* Vis. 1.56 μL/mL[a] 3.12 μL/mL[b] (Skocibusić et al., 2006), *Anisochilus carnosus* (Senatore et al., 2003), *Phlomis ferruginea* Ten. (Formisano et al., 2006).	
Pseudomonas aeruginosa	–	*Juniperus phoenicea* (Ennajar et al., 2010), *Origanum compactum* 2.19% v/v[a], *Tachyspermum copticum* 7.4% v/v[a] (Mayaud et al., 2008), *Cinnamomum verum* bark 0.24% v/v[a] (Mayaud et al., 2008; Bouhdid et al., 2010), *C. zeylanicum* Blume (leaf, bark) (Singh et al., 2007), *Cestrum nocturnum* 12.5 μg/mL[a] (Al-Reza et al., 2009), *Origanum glandulosum* spp. 62.50 μg/mL[a] (Sari et al., 2006), *Satureja subspicata* Vis. 12.5 μL/mL[a] 25.00 μL/mL[b] (Skocibusić et al., 2006), *Anisochilus carnosus* (Senatore et al., 2003), *Phlomis ferruginea* Ten. (Formisano et al., 2006), *Nigella sativa* Linn (Singh et al., 2005b), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c), *Plectranthus cylindraceus* 0.125 mg/mL[a] (Marwah et al., 2007), *Zataria multiflora* (Mahboubi and Bidgoli, 2010), *Mosla chinensis* Maxim 0.125 mg/mL[a] (Cao et al., 2009), oregano 1.648 mg/mL[a] (Lambert et al., 2001).	Eugenol, cinnamaldehyde (Singh et al., 2007), thymol 0.385 mg/mL,[a] carvacrol 0.450 mg/mL[a] (Lambert et al., 2001).
Salmomella enterica	–	*Tachyspermum copticum* 0.50 mg/mL[a] 1.0 mg/mL[b] (Oroojalian et al., 2010), *Chrysanthemum indicum* 1.25 μg/mL[a] (Lee et al., 2011), *Allium cepta*, *A. sativum* (Benkeblia, 2004), Melissa oil, oregano, lemon, lemongrass, cinnamon leaf (Friedman et al., 2004), *Thymus pallescens*, *T. algeriensis*, *T. dreatensis* (Hazzit et al., 2009), *Bunium persicum* 3.0 mg/mL,[a,b] *Cuminum cyminum* 3.0 mg/mL,[a,b] *Plectranthus cylindraceus* 0.125 mg/mL[a] (Marwah et al., 2007), *Backhousia citriodora* (Wilkinson et al., 2003).	Thujone 1.25 μg/mL,[a] chrysanthemyl alcohol 2.5 μg/mL,[a] camphor 5 μg/mL,[a] γ-terpinene 1.25 μg/mL[a] (Lee et al., 2011), carvacrol, terpeineol, geraniol, citral, linalool (Friedman et al., 2004), eriodictyol 0.80 mg/mL,[a] hesperitin 1.0 mg/mL,[a] naringen 1.0 mg/mL[a] (Mandalari et al., 2007).
Salmonella typhimurium	–	*Cinnamomum zeylanicum* Blume (bark, leaf) (Singh et al., 2007), *Thymus pallescens*, *T. algeriensis*, *T. dreatensis* (Hazzit et al., 2009), *Cestrum nocturnum* 25.0 μg/mL[a] (Al-Reza et al., 2009), *Cunila* sp. ≥ 5.00 mg/mL[a] (Sandri et al., 2007), *Satureja subspicata* Vis. 6.25 μL/mL[a] 12.50 μL/mL[b] (Skocibusić et al., 2006), *Anisochilus carnosus* (Senatore et al., 2003), *Citrus bergamia* Risso 0.40 mg/mL (Mandalari et al., 2007), *Myristica fragrans* Houtt (aril) (Singh et al., 2005a), clove 0.283 mg/mL[b], cinnamon 0.133 mg/mL[b] (Si et al., 2006), *Eucalyptus dives* 0.30% v/v[a] (Delaquis et al., 2002), *Mosla chinensis* Maxim 0.250 mg/mL[a] (Cao et al., 2009), *Backhousia citriodora* (Wilkinson et al., 2003).	Eugenol, cinnamaldehyde (Singh et al., 2007), Carvacrol 0.167 mg/mL,[b] geraniol 0.367 mg/mL,[b] eugenol 0.400 mg/mL,[b] *m*-anisaldehyde 0.933 mg/mL,[b] thymol 0.233 mg/mL,[b] 2-*tert*-butyl-4-methylphenol 0.433 mg/mL,[b] mandelonitrile 0.333 mg/mL[b] (Si et al., 2006).
Shigella sonnei	–	*Chrysanthemum indicum* 1.25 μg/mL[a] 20 μg/mL[b] (Lee et al., 2011), *Backhousia citriodora* (Wilkinson et al., 2003).	Thujone 5 μg/mL,[a] 10 μg/mL,[b] chrysanthemyl alcohol 1.25 μg/mL,[a] 20 μg/mL,[b] camphor 1.25 μg/mL[a] (Lee et al., 2011).
Staphylococcus aureus	+	*Bunium persicum* 0.75 mg/mL,[a,b] *Cuminum cyminum* 0.75 mg/mL[a] 1.50 mg/mLb[c], *Tachyspermum copticum* 0.60 mg/mL[a,b] (Oroojalian et al., 2010), *Chrysanthemum indicum* 1.25 μg/mL[a] 20 μg/mL[b] (Lee et al., 2011), garlic, onion (Benkeblia, 2004), *Anthemis nobilis* L., *Hyssopus officinalis* L., *Mentha piperita* L. (Marino et al., 2001), *Origanum vulgare* L. 0.575 mg/mL[a] (Marino et al., 2001; Lambert et al., 2001), *Cinnamomum verum* leaf 1.25% v/v[a] (Mayaud et al., 2008), *C. verum* bark 0.125% v/v[a] (Mayaud et al., 2008; Bouhdid et al., 2010), *C. zeylanicum* Blume (leaf, bark) (Singh et al., 2007), *Thymus pallescens*, *T. algeriensis*, *T. dreatensis* (Hazzit et al., 2009), *T. kotschyanus* 1.25 ppm,[a,b] *T. persicus* 1.25 ppm[a,b] (Rasooli and Mirmostafa, 2003; Mayaud et al., 2008), *Salvia tomentosa* 18.0 mg/mL[a] (Tepe et al., 2005), *Cunila* sp. ≥ 0.62 mg/mL[a] (Sandri et al., 2007), *Juniperus phoenicea* (Ennajar et al., 2010), *Rosmarinus officinalis* (Celiktas et al., 2007), *Cestrum nocturnum* 25.0 μg/mL[a] (Al-Reza et al., 2009), *Satureja subspicata* Vis. 0.09 μL/mL[a] 0.19 μL/mL[b] (Skocibusić et al., 2006), *Anisochilus carnosus* (Senatore et al., 2003), *Laurus nobilis* L. 0.02% v/v[a] (Erkmen and Özcan, 2008), *Phlomis ferruginea* Ten. (Formisano et al., 2006), *Nigella sativa* Linn (Singh et al., 2005b),	Thujone 1.25 μg/mL,[a] 5 μg/mL,[b] chrysanthemyl alcohol 2.5 μg/mL,[a] camphor 5 μg/mL,[a] γ-terpinene 1.25 μg/mL[a] (Lee et al., 2011), eugenol, cinnamaldehyde (Singh et al., 2007), eriodictyol 0.80 mg/mL[a] (Mandalari et al., 2007), thymol 0.14 mg/mL,[a] carvacrol 0.175 mg/mL[a] (Lambert et al., 2001).

(Continued on next page)

Table 3 Antimicrobial activity of essential oils and components *(Continued)*

Microbe	Gram	Essential oil/activity	Essential oil component/activity
		Anethum graveolens L. (Umbelliferae) (Singh et al., 2005c), *Plectranthus cylindraceus* 31.3 μg/mL[a] (Marwah et al., 2007), *Herba Moslae* 0.118 mg/mL[a] 0.236 mg/mL[b] (Li et al., 2010), *Coriandrum sativum* L. (immature leaves) 0.02% v/v[a], *C. sativum* L. (seeds) 0.40% v/v[a], *Anethum graveolens* L. 0.37% v/v[a], *Eucalyptus dives* 0.23% v/v[a] (Delaquis et al., 2002), *Mosla chinensis* Maxim 62.5 μg/mL[a] (Cao et al., 2009), *Melaleuca alternifolia* 10% v/v[a] (Mayaud et al., 2008), *Cymbopogon citratus*, *Ocimum basilicum* L., *O. gratissimum* L., *Thymus vulgaris* L., *Zingiber officinale* Roscue (Nguefack et al., 2004), *Hymenocrater longiflorus* 0.12 mg/mL[a] (Ahmadi et al., 2010), *Pelargonium graveolens* (Lis-Balchin et al., 2003).	
Vibrio parahaemolyticus	–	*Chrysanthemum indicum* 1.25 μg/mL[a] 10 μg/mL[b] (Lee et al., 2011).	Thujone 1.25 μg/mL,[a] 5 μg/mL,[b] chrysanthemyl alcohol 5 μg/mL,[a] 20 μg/mL,[b] camphor 1.25 μg/mL,[a] 20 μg/mL,[b] γ-terpinene 1.25 μg/mL[a] (Lee et al., 2011).
Vibrio vulnificus	–	*Chrysanthemum indicum* 1.25 μg/mL[a] 20 μg/mL[b] (Lee et al., 2011).	Thujone 1.25 μg/mL,[a] 5 μg/mL,[b] chrysanthemyl alcohol 5 μg/mL,[a] camphor 5 μg/mL,[a] γ-terpinene 1.25 μg/mL[a] (Lee et al., 2011).
Yersinia enterocolitica	–	*Laurus nobilis* L. 1.0% v/v[a] (Erkmen and Özcan, 2008), *Anthemis nobilis* L., *Hyssopus officinalis* L., *Mentha piperita* L., *Origanum vulgare* L. (Marino et al., 2001), *Heracleum lasiopetalum* 39 μg/mL[a], *Achillea kellalensis* 39 μg/mL[a], *Mentha longifolia* 156.25 μg/mL[a], *Dracocephalam multicaule* 156.25 μg/mL[a], *Kelussia odoretascima* 156.25 μg/mL[a], *Echiophora platyloba* 39 μg/mL[a], *Thymus daenensisv* 39 μg/mL[a] (Pirbalouti et al., 2010b).	

Note: [a]MIC: Minimum inhibitory concentration is the lowest concentration of test compound resulting in the lack of visible microorganism growth change.
[b]MBC: Minimum bactericidal concentration is the concentration where 99.9% or more of the initial inoculums are killed.

presence of simple sugars, and are thus a function of ingredient manipulation. Some combinations of essential oils were also observed to have synergistic effects, and usage of these combinations may possess the potential for reduction of possible undesirable effects caused by high single essential oil concentrations to organoleptic properties (Gutierrez et al., 2009). A review of some of these essential oil combinations' synergistic antimicrobial abilities, and other possible interactions with food preservatives and antibiotics will be presented in the next section.

Antimicrobial Activity in Active Food Packaging

Apart from addition of antimicrobial essential oils into food, recent development of natural food preservatives has led to increasing interest in application of essential oils into food packaging. Active packaging involves the support, coat, or absorption of active components on a solid matrix from which they can be released to the atmosphere and act as food preservation agents. Antimicrobial active packaging aims to extend shelf life and improve consumer safety of products by reducing, inhibiting, and/or retarding the growth of pathogenic microbes in packed foods and packaging materials (Seydim and Sarikus, 2006).

The highly volatile and antimicrobial nature of natural plant essential oils make them attractive candidates for this purpose, as the oils do not require direct contact with food, thereby reducing possible sensory changes, and provide an alternative to less desirable synthetic additives (Becerril et al., 2007). The design and production of active food packaging using essential oils require extensive studies on the efficacy of essential oils in vapor phase, rate of release of essential oils from packaging matrix, microbial kill time, identification of active oil components, understanding of bacteriostatic and/or bactericidal mechanisms of the components, and the concentrations required with various food matrices, thereby limiting available literature on active food packaging using essential oils.

Becerril et al. (2007) studied the use of oregano and cinnamon essential oils in active packaging material developed by an industrial partner, and found fast antimicrobial activity by the active packaging incorporated with low essential oil concentrations. In fact, death time curves of *E. coli* and *S. aureus* by active packaging with oregano and cinnamon essential oils showed shorter optimum time of exposure against the bacteria than that of pure essential oils in vapor phase, likely to be due to different volatile compositions generated when incorporated into the matrix.

Table 4 Antifungal activity of essential oils and components

Microbe	Essential oil/activity	Essential oil component/activity
Aspergillus niger	*Satureja subspicata* Vis. 0.78 μL/mL,[a] 0.78 μL/mL[b] (Skocibusić et al., 2006), *Cinnamomum zeylanicum* Blume (bark, leaf) (Singh et al., 2007), *Laurus nobilis* L. 0.02% v/v[a] (Erkmen and Özcan, 2008), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c), *Myristica fragrans* Houtt (aril) (Singh et al., 2005a), *Backhousia citriodora* (Wilkinson et al., 2003), *Mosla chinensis* Maxim 62.5 μg/mL[a] (Cao et al., 2009), *Allium cepa, A. sativum* (Benkeblia, 2004), *Hymenocrater longiflorus* 0.48 mg/mL[a] (Ahmadi et al., 2010), *Citrus limon*, *Eucalyptus globules*, *Lippia alba*, *L. microphylla*, *Peumus boldus* (de Souza et al., 2005), *Citrus sinensis* L. Osbeck (Sharma and Tripathi, 2008).	Citral 0.5% v/v[a] (de Souza et al., 2005), eugenol, cinnamaldehyde (Singh et al., 2007).
Aspergillus flavus	*Satureja subspicata* Vis. 0.19 μL/mL[a] 3.12 μL/mL[b] (Skocibusić et al., 2006), *Cinnamomum zeylanicum* Blume leaf (Singh et al., 2007), *Myristica fragrans* Houtt (aril) (Singh et al., 2005a), *Mosla chinensis* Maxim 62.5 μg/mL[a] (Cao et al., 2009), *Cimbopogom citrates*, *Citrus limon*, *Peumus boldus* (de Souza et al., 2005), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c).	Citral 0.5% v/v,[a] eugenol 2.0% v/v[a] (de Souza et al., 2005; Singh et al., 2007), cinnamaldehyde (Singh et al., 2007).
Aspergillus terreus	*Cinnamomum zeylanicum* Blume leaf (Singh et al., 2007), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c), *Mosla chinensis* Maxim 62.5 μg/mL[a] (Cao et al., 2009).	Eugenol, cinnamaldehyde (Singh et al., 2007).
Candida albicans	*Juniperus phoenicea* (Ennajar et al., 2010), *Satureja subspicata* Vis. 0.09 μL/mL[a] 0.19 μL/mL[b] (Skocibusić et al., 2006), *Salvia tomentosa* 18.0 mg/mL[a] (Tepe et al., 2005), *Thymus pallescens*, *T. algeriensis*, *T. dreatensis* (Hazzit et al., 2009), *Zataria multiflora* (Mahboubi and Bidgoli, 2010), *Origanum glandulosum* spp. 31.25 μg/mL[a] (Sari et al., 2006), *Mosla chinensis* Maxim 62.5 μg/mL[a] (Cao et al., 2009), *Hymenocrater longiflorus* 0.24 mg/mL[a] (Ahmadi et al., 2010).	
Candida krusei	*Salvia tomentosa* 18.0 mg/mL[a] (Tepe et al., 2005).	
Fursarium spp.	*Cimbopogom citratus*, *Lippia alba*, *L. microphylla*, *Peumus boldus* (de Souza et al., 2005).	Citral 0.5% v/v,[a] eugenol 4.0% v/v,[a] micrene (de Souza et al., 2005).
Fusarium graminearum	*Cinnamomum zeylanicum* Blume bark, leaf (Singh et al., 2007), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c), *Myristica fragrans* Houtt (aril) (Singh et al., 2005a), *Backhousia citriodora* (Wilkinson et al., 2003).	Eugenol, cinnamaldehyde (Singh et al., 2007).
Fusarium oxysporum	*Allium cepa*, *A. sativum* (Benkeblia, 2004)	
Penicillium spp.	*Lippia alba*, *Peumus boldus* (de Souza et al., 2005).	Citral 2.0% v/v,[a] eugenol 4.0% v/v[a] (de Souza et al., 2005).
Penicillium citrinum	*Cinnamomum zeylanicum* Blume bark, leaf (Singh et al., 2007), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c), *Myristica fragrans* Houtt (aril) (Singh et al., 2005a).	Eugenol, cinnamaldehyde (Singh et al., 2007).
Penicillium cyclopium	*Allium cepa*, *A. sativum* (Benkeblia, 2004).	
Penicillium viridicatum	*Cinnamomum zeylanicum* Blume bark, leaf (Singh et al., 2007), *Anethum graveolens* L. (Umbelliferae) (Singh et al., 2005c), *Myristica fragrans* Houtt (aril) (Singh et al., 2005a).	Eugenol, cinnamaldehyde (Singh et al., 2007).
Rhizopus spp.	*Citrus limon*, *Cimbopogom citratus*, *Eucalyptus globules*, *Lippia alba*, *L. microphylla* (de Souza et al., 2005)	Citral 8.0% v/v[a], eugenol 4.0% v/v,[a] micrene (de Souza et al., 2005).
Rhizopus oryzae	*Laurus nobilis* L. 0.02% v/v[a] (Erkmen and Özcan, 2008).	

Note: [a]MIC: Minimum inhibitory concentration is the lowest concentration of test compound resulting in the lack of visible microorganism growth change.

Another potential use of active packaging is to limit the growth of microbes on food surfaces by direct contact of edible films of milk proteins, chitosan, or alginates incorporated with various essential oils. Possible applications of such edible active packaging include meats, fish, sausages, and cheeses.

In a recent study, Seydim and Sarikus (2006) developed whey protein isolate (WPI)-based edible film with essential oils of oregano, cinnamon, and rosemary. WPI film incorporated with 2% oregano oil was determined to be most effective against *E. coli* 0157:H7, *S. aureus*, *S.* Enteritidis, and *L. monocytogenes*, while 3 to 4% of garlic oil in WPI film was required for inhibitory activity, whereas rosemary essential oil in WPI film had no antimicrobial effect on the studied microbes.

Successful preservation of beef slices and fish using edible active packaging was illustrated using milk protein-based and gelatin–chitosan films incorporated with essential oils (Oussalah et al., 2004; Gómez-Estaca et al., 2010). Milk protein-based film with 1.0% (w/v) oregano, 1.0% (w/v) pimento, or

1.0% oreganopimento (1:1) essential oils mix applied on whole beef muscle was successful in reducing *E. coli* O157:H7 and *Pseudomonas* spp. microbial load during seven days of storage at 4°C. Diffusion of essential oil from the film to the meat surface took place by progressive release of essential oil phenolic compounds throughout storage, and milk protein-based film with oregano was found to have the highest antimicrobial activity, with up to 1.12 log reduction of *E. coli* O157:H7 after seven days, while pimento had the lowest antimicrobial activity with no effect on *E. coli* O157:H7 throughout storage (Oussalah et al., 2004; Gómez-Estaca et al., 2010). In a more recent study, gelatin–chitosan-based edible film incorporated with clove essential oil was applied to fish under chilled storage conditions (Oussalah et al., 2004; Gómez-Estaca et al., 2010). Likewise, clove film was able to reduce microbial load on the surface of fish, thereby successfully extending the shelf life of fish under storage.

Incorporation of essential oils in active food packaging provides the food industry with a new natural and effective form of preservation. However, more research is needed in order to establish effective and safe concentrations of essential oils, and better understand and control release rates of active components to the food.

SYNERGISM WITH OTHER ESSENTIAL OILS, FOOD PRESERVATIVES, AND ANTIBIOTICS

By definition, synergism occurs when two or more agents work together to produce an effect greater than the sum of their individual effects. Antagonism, which is opposite of synergism, is defined when the combined effect of two agents is smaller than the effect of any one of them. The third possible outcome, termed additive effect, is when the combined effect of two or more agents is equal to the sum of their individual effects (Popovich et al., 2010).

Synergistic studies involving essential oils and their phytochemical components to exhibit antimicrobial activity have been remarkably challenging. To date, synergism has been demonstrated in several ways, these include the studies of effects between two different essential oils, essential oils and food additives, and also between essential oils and antibiotics. Synergistic effects of essential oils in inhibition–resistance microorganisms have also been of interest for many. However, effectiveness thus far has been dubious; this may be reasoned by the structural differences of phytochemicals, differences in the nature of various pathogenic bacterial strains as well as differences in food matrices. All these factors could very well affect their unique action mechanisms in cell. On another note, when it comes to using natural plant essential oils as antimicrobial agents, flavors may be introduced into food products unintentionally. Moreover, when two or more essential oils are added together, undesirable organoleptic effects may prevail. Therefore, it is mandatory to optimize concentrations between substances for both optimization of antimicrobial activities and organoleptic qualities.

Essential Oils

The approach to study synergism in antimicrobial effects was first performed in various essential oil combinations. For instance, the greatest inactivation of *L. monocytogenes* in salads was achieved in highest activity when combination of essential oils was used. The essential oils used included thyme verbena, thyme red, Spanish oregano, ajowan, tea tree, clove, and sage oils tested at 1% as well as with 2% rosemary oil (Antonio et al., 2009). Often, additive, rather than synergistic, effect was observed when a mixture of carvacrol and thymol was utilized in *P. aeruginosa* and *S. aureus* (Lambert et al., 2001). Parallel to the findings, when two monosubstances derived from thyme oil, which includes linalool, thymol, or carvacrol, were used in combination against six different strains, additive antimicrobial activity was observed (Iten et al., 2009). It was then concluded that mixing carvacrol and thymol in proper amounts may exert total inhibition, and the activity is partly based on additive effects, which especially increase the kill-rate (Lambert et al., 2001; Iten et al., 2009).

Food Preservatives

Similar to the idea of hurdle technology, food preservation using several preservatives in small amounts is preferred over the use of large amount of single preservative. This shall enhance metabolic exhaustion and increase stress reactions across a variety of microorganisms that may be specifically addressed by different essential oils and/or food additives. Also, this helps maintain sensory, nutritive, and economic properties of foods. Significant inhibition of Staphylococcal enterotoxin was observed when the essential oil of *Zataria multiflora* Boiss. was used in combination with nisin. Results showed that 42.59–43.70% hemolysis due to α-toxin activity of *S. aureus* was obtained with the used drug combination (Parsaeimehr et al., 2010). In vitro studies showed synergy of essential oils of eucalyptus and mint when combined with methylparaben against *P. aeruginosa*, while essential oils of mint, oregano, and sage showed synergy when combined with propylparaben and imidazolidinyl urea against *S. aureus* (Patrone et al., 2010).

Related to application in food industry, when thyme oil was used in combination with chitosan on a ready-to-cook chicken pepper kebab skewer under modified atmosphere packaging, the shelf life was found to have been improved by a significant decrease in lactic acid bacteria, *Pseudomonas* spp., *Brochothrix thermosphacta*, *Enterobacteriaceae*, and yeasts and molds (Giatrakou et al., 2010). It has also retained the desirable sensory characteristics throughout the 14-day period. Similarly, an anti-listerial activities study was conducted in Russian-type salad. The anti-listerial activity of Enterocin AS-48 at a concentration of 30 μg/g was intensified when used in combination with a wide range of essential oils, including thyme verbena, Spanish oregano, ajowan, tea tree, clove, sage oils, etc (Antonio et al., 2009). Another study targeting *L. monocytogenes* in minced beef

during refrigerated storage showed that when thyme essential oil was used with 0.6% nisin at 500 or 1000 IU/g, synergistic activity against the pathogen was reported. This is significant for the reason that this treatment successfully kept the population of *L. monocytogenes* below the acceptable limit of 2 log cfu/g at 4°C (Solomakos et al., 2008b). The use of electrolyzed NaCl solution in combination with 0.5% carvacol and 0.5% thymol on carp fillets during convectional air-drying has also shown much stronger antimicrobial and antioxidant effects (Mahmoud et al., 2006). All these have implied limitless possibilities in combining essential oils with various food additives, suggesting good alternative to artificial preservatives in food industry.

Antibiotics

Synergistic effects of antibiotics and phytochemicals in essential oils have also been evaluated in antibiotic-resistant pathogens. This is one strategy employed to counter the resistance mechanisms developed in multidrug-resistant pathogens across the years. Many studies have shown positive correlations and these phytochemicals were active against antibiotic-resistant bacteria under minimal concentration, hence minimizing potential toxic effects.

In fungi, a study that utilized essentials oils of Moroccan endemic thymes against *Candida albicans* showed synergistic effect of essential oils when used together with fluconazol, suggesting possible reduction of dose of drugs, and minimizing toxic side effects and treatment cost (Saad et al., 2010). When the essential oil of *Ligusticum chuanxiong* was used with ketoconazole and itraconazole, this was found to have increased *Trichophyton* species susceptibility both synergistically or additively, depending on the concentration used (Sim and Shin, 2008). Essential oil derived from myrtle (*Myrtus communis*) leaves worked synergistically with amphotericin B against *C. albicans* and *Aspergillus* species and exhibited good fungal activity (Mahboubi and Ghazian, 2010).

Synergistic activity against antibiotic-resistant bacteria has also been demonstrated in several studies. When oxacillin was used in combination with Korean herb *Artemisia iwayomogi* oil fraction or vulgarone B, the MIC of *A. iwayomogi* oil against *S. aureus* CCARM3511 (oxacillin-resistant strain) is significantly lowered by eight or four times (Chung et al., 2009). As the susceptibility criterion specified by the Clinical and Laboratory Standards Institute was ≥ 2 μg/mL, *A. iwayomogi* oil fraction or vulgarone B can potentially be used as a safe anti-*S. aureus* agent alternative in food (Chung et al., 2009). Similarly, antimicrobial activity of *Zataria multiflora* Boiss. essential oil was studied in combination with vancomycin against methicillin-resistant and methicillin-sensitive strains of *S. aureus*. Synergistic activity was confirmed by microtiter assay, displaying the potential for developing combination antibiotic against MRSA infections (Mahboubi and Bidgoli, 2010). Antifungal activity of Holy basil (*Ocimum sanctum* L.) essential oil and azole antimycotics-fluconazole and ketoconazole assessed in both fluconazole-resistant *Candida* isolates has shown selective, potent, and augmented in vitro antifungal effects (Amber et al., 2010). Chung et al. (2009) confirmed the inhibitory activities of *A. iwayomogi* oils, especially by vulgarone B, against antibiotic-susceptible and antibiotic-resistant bacteria of important human pathogens *S. aureus*, *S. Enteritidis*, and *S. Typhimurium*.

Similar to pure essential oils mixture, rather than synergism, additive effects are observed at times. When the interactions of peppermint oil and menthol with four different antibiotics (ampicillin, oxytetracycline, erythromycin, and gentamycin) were studied, only the combinations of peppermint oil and oxytetracycline and that of menthol and oxytetracycline were found to have additive effects. The rest expressed indifferent interactions with essential oils (Schelz et al., 2006). Association of Nystatin, an antifungal compound, with tea tree (*Melaleuca alternifolia*) essential oil only had additive effect on the *Candida* strains evaluated (Mahboubi and Ghazian, 2010). Combinations of essential oils and methanol extracts obtained from aerial parts of *Thymus vulgaris* and *Pimpinella anisum* seeds too displayed additive action against nine Gram-positive and Gram-negative pathogenic bacteria, especially *P. aeruginosa* (Al-Bayati, 2008). Optimal synergistic combination is difficult to achieve or to be stabilized in different food matrices.

Recent research appears to have focused on the synergistic effects in inhibition resistance microorganisms. The synergistic activity attained may therefore be of benefit to the public, especially the immunocompromised, the young, and the elderly. However, while many research results advocate the potential use of essential oils as antimicrobial agents in food, there is still relatively limited information in relation to the safety and/or upper limit of use. This needs to be carefully addressed in future synergistic studies involving essential oils.

CHALLENGES AND FUTURE DIRECTIONS

Although extensive research has been done on the antimicrobial action of essential oils, reports tend to contradict each other in terms of antimicrobial efficacy and response to various environmental factors such as storage temperature and presence of salt. This is probably due to difference in essential oils, presence of impurities in essential oils, target microorganisms, and test conditions used. Moreover, different authors denote slightly different definitions for MIC and MBC. Discrepancies in results cause difficulties in making a firm conclusion of antimicrobial mechanism of essential oils, and most of the current research rely on postulations and past reports to explain their results. However, it is unlikely to standardize a single method for all research, since studies often have different aims and objectives that require different experimental designs (Holley and Patel, 2005).

While essential oils appear promising as antimicrobial alternatives, information about the toxic levels is almost nonexistent. To date, there is limited information about the possibility of essential oils affecting the stress tolerance of pathogens. As

discussed by Owen and Palombo (2007), natural plant materials do not have "generally regarded as safe" status when used as antimicrobials. Hence, the challenge of future analysis is to set the optimum range of each essential oil and phytochemical more clearly, assess the possibility of any residues from their usage as well as their potential toxicity or allergenicity (Svoboda et al., 2006).

The stability of essential oils also imposes a problem during food processing. While the relative biochemical stability of some phytochemicals, such as allicin and thiosulfonates, is not exactly known, some chemicals are heat-labile and decompose at high temperatures. For example, cinnamaldehyde was found to decompose to benzaldehyde at around 60°C when heated alone, but was stable for 30 minutes at 200°C when heated with eugenol or cinnamon leaf oil. Encapsulation techniques are recently studied to stabilize essential oils against oxidation, volatilization losses, low solubility, interaction with other components, and changes in sensory properties (Liolios et al., 2009; Oroojalian et al., 2010; Donsì et al., 2011). However, further research on the action of encapsulated oils as antimicrobial agents has to be conducted to confirm their efficacy.

Despite the availability of a wide range of essential oils, relatively few are reported to be appropriate for practical use in specific food systems. As mentioned, the efficacy of natural antimicrobials may be reduced by certain food components. To allow more extensive use of essential oils as natural food preservatives, there is a need to evaluate the efficacy of essential oils within food model systems that closely resemble the target food composition to affirm the potential interactions between essential oils and food components so as to allow optimized application on real food.

In addition, although studies have shown synergism between certain oils/components and food additives, there is scarce information regarding the use of specific combinations in food, and interactions may vary with different foods. Thus, the antimicrobial mechanisms of key combinations have to be further evaluated and confirmed so that an optimized profile can be applied reliably in food preservation. It was also reported that individual phytochemicals do not work as effectively as heterogeneous extracts (Shetty and Labbe, 1998; Seaberg et al., 2003), suggesting that certain phytochemicals can only exhibit antimicrobial action when they are combined with other constituents of extracts or essential oils. Thus, researchers have to be careful when concluding about the antimicrobial activity of these individual essential oil components.

Essential oils could be introduced readily into foods that are commonly prepared with herbs and spices, such as savory meat, fish, cheese, soups, and sauces. However, if used alone in mild flavored food products, the organoleptic quality may become unacceptable to consumers. Thus, future areas of research may focus on the levels of synergists to be added with essential oils and phytochemicals so that effective antimicrobial responses can be maximized while preventing undesirable changes to the food sensory properties. Besides flavor, essential oils may interact with food components and result in unwanted appearance characteristics. For example, clove and oregano oils can react with iron and form dark pigmentations, which impair their use in food with light colors. However, such effects vary with different compounds and food constituents, and must be evaluated beforehand so that they can be avoided in actual commercial applications (Burt, 2004).

More research could be done to assess the degree of adaptation of bacteria to essential oils. In a study by Ultee et al. (2000b), *Bacillus cereus* was shown to become less susceptible to carvacrol after growth in mild concentrations of the compound. An increase in sensitivity was obtained upon fluidity and passive permeability reduction of cell membrane by altering the fatty acid and phospholipid head compositions. Moreover, the mechanism of action of essential oil components on cell membrane proteins and phospholipids is not exactly understood and could be further explored. Rees et al. (1995) recommended the study of essential oil antimicrobial activity against bacterial cells in the stationary growth phase. Further investigation and clarification of these mechanisms is relevant and would help in deciding the approach to the development of new technological applications (Burt, 2004).

Nevertheless, with the growing awareness of "green consumerism," more and more essential oils and phytochemicals are expected to be exploited in the future (Tuley and Stevens, 1997). This also implies an increasing popularity of commercial plant-derived products in cosmetics, medicinal, and food sectors. Bioengineering of phytochemical synthesis in plants may be considered for producing greater yields, should large amounts be required (McCaskill and Croteau, 1999).

CONCLUSION

A significant number of essential oils and phytochemicals are bioactive against foodborne pathogens in vitro, and to a smaller degree, in foods. Gram-positive organisms are by and large more susceptible to essential oils and phytochemicals than Gram-negative organisms. Further investigation into the efficacy of such natural substances in different food matrices is likely to be a productive area of research. To prevent off flavor or other undesirable sensory qualities, essential oils have to be carefully selected according to the specific food type. Synergism between different phytochemicals or other chemical compounds has to be investigated further before they can be applied commercially. A compound should be checked for its safety limits if its antimicrobial activity depends on a concentration greater than the usual for flavorings. Despite having disadvantages, essential oils prove beneficial with their broad antimicrobial activities and lack of apparent resistance development. On the whole, investigating and collecting relevant information for antimicrobial activity of essential oils and phytochemicals in food can aid in optimizing activity, predicting resistance, and finally deciding the most appropriate natural preservatives to be employed in food conservation systems.

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