Antimicrobial herb and spice compounds in food

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Abstract

Herbs and spices containing essential oils (EOs) in the range of 0.05–0.1% have demonstrated activity against pathogens, such as Salmonella typhimurium, Escherichia coli O157:H7, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus, in food systems. Application of herbs, spices and EOs with antimicrobial effects comparable to synthetic additives is still remote for three major reasons: limited data about their effects in food, strong odor, and high cost. Combinations of techniques have been successfully applied in several in-food and in vitro experiments. This paper aims to review recent in-food applications of EOs and plant-origin natural antimicrobials and recent techniques for screening such compounds.

Keywords: Herbs and spices
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1. Introduction

Strong consumer demand for safe and high-quality foods can be attributed in part to the widespread availability and accessibility of quality health data and information. There are also new concerns about food safety due to increasing occurrence of new food-borne disease outbreaks caused by pathogenic micro-organisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic micro-organisms (Arques, Rodriguez, Nunez, & Medina, 2008; Aslim & Yucel, 2007; Brandi, Amagliani, Schiavano, De Santi, & Sisti, 2006; Cusnieh & Lamb, 2005; Demirci, Guven, Demirci, Dadandi, & Basar, 2008; Friedman, Henika, Levin, & Mandrell, 2006; Ionnouy, Poiata, Hancianu, & Tzakou, 2007; Li, Tajkarimi, & Osburn, 2008; Lopez-Malo vigil, Palou, & Alzamora, 2005; Murdak, Cleveland, Matthews, & Chikindas, 2007; Nguelfack et al., 2007; Petitclerc et al., 2007; Turner, Thompson, & Auldist, 2007). As a consequence, natural antimicrobials are receiving a good deal of attention for a number of micro-organism-control issues. Reducing the need for antibiotics, controlling microbial contamination in food, improving shelf-life extension technologies to eliminate undesirable pathogens and/or delay microbial spoilage, decreasing the development of antibiotic resistance by pathogenic micro-organisms or strengthening immune cells in humans are some of the benefits (Abou-taleb & Kawai, 2008; Fisher & Phillips, 2008; Gaysinsky & Weiss, 2007; Gutierrez, Barry-Ryan, & Bourke, 2008a; Lopez-Malo vigil et al., 2005; Nazef, Belgesueima, Tani, Prevost, & Drider, 2008; Patrignani et al., 2008; Periago, Conesa, Delgado, Fernandez, & Palop, 2006; Ponce, Roura, Del Valle, & Moreira, 2008; Raybaudi, Mosqueda-Melgar, & Martin-Belloslo, 2008; Yamamoto, Matsunaga, & Friedman, 2004).

Antimicrobials are used in food for two main reasons: (1) to control natural spoilage processes (food preservation), and (2) to prevent/control growth of micro-organisms, including pathogenic micro-organisms (food safety). Natural antimicrobials are derived from animal, plant and microbial sources. There is considerable potential for utilization of natural antimicrobials in food, especially application to fresh fruits and vegetables. However, methods and mechanisms of action, as well as the toxicological and sensory effects of natural antimicrobials, are not completely understood (Burt, 2004; Davidson, 2006; Gaysinsky & Weiss, 2007; Gutierrez, Rodriguez, Barry-Ryan, & Bourke, 2008b; Lopez-Malo vigil et al., 2005; Moriera, Ponce, Del Valle, & Roura, 2007; Patrignani et al., 2008; Periago et al., 2006; Ponce et al., 2008; Raybaudi, Mosqueda-Melgar et al., 2008; Zaika, 1988). Even without a more comprehensive understanding of how natural antimicrobial substances work, there is a growing effort to develop new effective methods that rely primarily on their use to enhance food safety (Ayala-Zavala et al., 2008; Brandi et al., 2006; Chen et al., 2008; FAO/WHO Codex Alimentarius Commission, 2007; Liu, O’Connor, Cotter, Hill, & Ross, 2008; Lopez-Malo vigil et al., 2005; Martinez, Obeso, Rodriguez, & Garcia, 2008; Murdak et al., 2007; Nazef et al., 2008; Oussalah, Caillet, Salmiea, Saucier, & Lacroix, 2004; Pellegrini, 2003; Vagahasiya & Chanda, 2007).

In addition to their flavoring effects, some spices and herbs have antimicrobial effects on plant and human pathogens (Brandi et al., 2006). Food processing technologies such as chemical preservatives cannot eliminate food pathogens such as Listeria monocytogenes or delay microbial spoilage totally (Gutierrez, Barry-Ryan, & Bourke, 2009). Cold distribution of perishable food can help, but it cannot guarantee the overall safety and quality of the product. Moreover, changes in dietary habits and food processing practices and increasing demand for ready-to-eat products. Fruits and vegetables have been followed by increasing reports of food-borne pathogenic micro-organisms because of the presence of pathogens in raw materials (Lanciotti et al., 2004; Li, Tajkarimi, & Osburn, 2008; Li, Zhu et al., 2008).

There are new techniques such as pulsed light, high pressure pulsed electric and magnetic fields for food preservation and controlling pathogens and spoilage micro-organisms in food. However, technologies such as mild heat processing, modified-atmosphere packaging, vacuum packaging and refrigeration are not sufficiently effective, neither for eliminating undesirable pathogens nor delaying microbial spoilage. Moreover, some of these methods, such as vacuum cooling, can increase the probability of food contamination. Incorporation of natural antimicrobials into packaging materials, to protect the food surface rather than the food, has also been developed recently (Abou-taleb & Kawai, 2008; Fisher & Phillips, 2008; Gaysinsky & Weiss, 2007; Gutierrez et al., 2008a; Holley & Patel, 2005; Lanciotti et al., 2004; Li, Tajkarimi et al., 2008; Li, Zhu et al., 2008; Lopez-Malo vigil et al., 2005; Nazef et al., 2008; Patrignani et al., 2008; Periago et al., 2006; Ponce et al., 2008; Raybaudi, Mosqueda-Melgar et al., 2008; Raybaudi, Rojas-Grau, Mosqueda-Melgar, & Martin-Belloslo, 2008). A growing body of data indicates that there is considerable potential for utilization of natural antimicrobials in food, especially application to fresh fruits and vegetables, for their oxidative degradation of lipids and improvement of the quality and nutritional value of food, in addition to their strong antifungal effects. EOs derived from spices and plants have antimicrobial activity against L. monocytogenes, Salmonella typhimurium, Escherichia coli O157:H7, Shigella dysenteriae, Bacillus cereus and Staphylococcus aureus at levels between 0.2 and 10 μl mL⁻¹ (Burt, 2004).

For example, combined mild heat treatment with addition of cinnamon and clove EOs to apple cider significantly reduced the D-value and time to 5-log reduction of Escherichia coli O157:H7 (Knight & McKellar, 2007). Application of concentrations of 2.0% of citric acid or up to 0.1% of cinnamon bark oil in tomato juice, followed by treatment with high-intensity pulsed electric fields, successfully achieved the pasteurization level (reduction of at least 5.0 log_{10} units) (Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloslo, 2008a, 2008b).

The purpose of this paper is to provide an overview of the data published mostly in the past 10 years on plant-derived compounds that have been reported to be effective against spoilage or pathogenic bacteria, and practical methods for screening these compounds.
2. Historical overview of plant antimicrobials

Natural antimicrobial agents derived from sources such as plant oils have been recognized and used for centuries in food preservation. EOs and spices were used by the early Egyptians and have been used for centuries in Asian countries such as China and India. Some of the spices, such as clove, cinnamon, mustard, garlic, ginger and mint are still applied as alternative health remedies in India. EO production can be traced back over 2000 years to the Far East, with the beginnings of more modern technologies occurring in Arabia in the 9th century. However, it was also during this time period that the medical applications of EOs became secondary to their use for flavor and aroma. Plant extracts and spices, in addition to contributing to taste and flavor, can act against Gram-positive pathogens such as L. monocytogenes. They can also enhance storage stability by means of active components including phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons, especially in such spices as cinnamon, clove, garlic, mustard, and onion. The first scientific studies of the preservation potential of spices, describing antimicrobial activity of cinnamon oil against spores of anthrax bacilli, were reported in the 1880s. Moreover, clove was used as a preservative to disguise spoilage in meat, syrups, sauces and sweetmeats. In the 1910s, cinnamon and mustard were shown to be effective in preserving applesauce. Since then other spices, such as allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage and thyme, have been reported to have significant bacteriostatic properties (Burt, 2004; Ceylan & Fung, 2004; Gutierrez et al., 2008a; Jayaprakash, Negi, Jena, & Jagan Mohan Rao, 2007; Kwon, Kwon, Kwon, & Lee, 2008; Sofia, Prasad, Vijay, & Srisavastava, 2007; Zaika, 1988).

Most spices have eastern origins; however, some of them have been introduced after discovery of the New World – spices such as chili peppers, sweet peppers, allspice, annatto, chocolate, epazote, sassafras, and vanilla, which have been used for food flavoring and medicinal purposes (Ceylan & Fung, 2004).

3. Major spice and herb antimicrobials

Spices and EOs are used by the food industry as natural agents for extending the shelf life of foods. A variety of plant- and spice-based antimicrobials is used for reducing or eliminating pathogenic bacteria, and increasing the overall quality of food products. Plant-origin antimicrobials are obtained by various methods from aromatic and volatile oily liquids from flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots of plants. EOs in plants generally are mixtures of several components. Some of these presence exert antimicrobial effects such as components in oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage and vanillin (Angioni et al., 2004; Arques et al., 2008; Daferera, Zigos, & Polissiou, 2000; Davidson & Naidu, 2000; Gutierrez et al., 2008a; Holley & Patel, 2005; Kim, Kubec, & Musah, 2006; Ponce et al., 2008; Raybaudi, Rojas-Grau et al., 2008; Zazzagh-Abyaneh et al., 2008; Zaika, 1988). Commercially based plant-origin antimicrobials are most commonly produced by SD (steam distillation) and HD (hydro distillation) methods, and alternative methods such as SFE (supercritical fluid extraction) provide higher solubility and improved mass transfer rates. Moreover, the manipulation of parameters such as temperature and pressure leads to the extraction of different components when a particular component is required. Bioengineering of the EO components also provides more available commercial products (Burt, 2004). Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savory, and fennel, have been successfully used alone or in combination with other preservation methods. They exert direct or indirect effects to extend food-stuff shelf life or as antimicrobial agent against a variety of Gram-positive and Gram-negative bacteria. However, their efficacy depends on the pH, the storage temperature, the amount of oxygen, the EO concentration and active components (Burt et al., 2007; Du & Li, 2005; Gutierrez et al., 2008a; Holley & Patel, 2005; Koutsoumanis, Lambropoulou, & Nychas, 1999; Sandasi, Leonard, & Viljoen, 2008; Svoboda, Brooker, & Zrustova, 2006; Viuda-Martos, Ruiz-Navajas, Fernandez-Lopez, & Angel Perez-Alvarez, 2008).

3.1. Chemical components present in EOs

EOs are a group of terpenoids, sesquiterpenes and possibly diterpenes with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones (Fish & Phillips, 2006). EOs and other plant extracts are principally responsible for antimicrobial activities in plants, herbs and spices. These extracts can be obtained from plants and spices by various methods, such as steam, cold, dry and vacuum distillation. These plant compounds, including glucosides, saponins, tannins, alkaloids, EOs, organic acids and others, are present as parts of the original plant defense system against microbial infection (Bajpai, Rahman, & Kang, 2008; Ceylan & Fung, 2004). Major components of the EOs can constitute up to 85%, and other components are usually at trace levels (Burt, 2004; Grosso et al., 2008).

There are various chemical components present in plant-origin antimicrobials including, saponin and flavonoids, thiosulfonates, glucosinolates and saponins.

Saponin and flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. They commonly form a soapy lather after shaking in water. The antimicrobial activity of saponins and flavonoids in plants like oats (Avena sativa) and anthraquinones, carbohydrates and alkaloids derived from plants like Bersema engleriana (Melanthaceae) have been proven when extracted from roots, stem bark, leaves and wood (Bahraminejad, Asenstorfer, Riley, & Schultz, 2008; Cunshie & Lamb, 2005; Kuete et al., 2008; Musyimi, Muema, & Muema, 2008; Naidu, 2000a). Thiosulfonates are prepared from garlic by mild procedures. They have strong antimicrobial activities against Gram-negative bacteria (Kim et al., 2008; Naidu, 2000a; Yoshida et al., 1999). Glucosinolates are present in broccoli, Brussels sprouts, cabbage, and mustard powder and cause the pungent flavor of mustard and horseradish. They demonstrate a wide range of antibacterial and antifungal properties with direct or synergistic effect in combination with other compounds (Almajo, Carbo, Lopez Jimenez, & Gordon, 2008; Graumann & Holley, 2008; Gutierrez et al., 2008a; Naidu, 2000a; Tolonen et al., 2004). Generally, phenolic compounds of EOs such as citrus oils extracted from lemon, olive oil (oleuropein) and tea-tree oil (terpenoids), orange and bergamot have broader antimicrobial effects and are not cate-
4. Uses of plant-origin antimicrobials

Food spoilage can occur from raw food materials to the processing and distribution. Spoilage sources might be chemical, physical and microbiological. Preservation techniques for microbiological spoilage have been dramatically improved in recent years to minimize any growth of micro-organisms including pathogenic micro-organisms (Gould, 1996). Research concerning plant-origin food-preservative EOs has increased since the 1990s, with more utilization of spices and their EOs as natural bio-preservatives, to increase shelf life and overall quality of food products and reduce or eliminate pathogenic micro-organisms (Burt, 2004; Moriera et al., 2007; Simitzis et al., 2008). Application of Thymus eugii showed stronger antimicrobial activity compared to vancomycin (30 mcg) and erythromycin (15 mcg) (Toroglu, 2007). Ginger (Zingiber officinale), galangal (Alpinia galanga), turmeric (Curcuma longa), and fingerroot (Boesenbergia pandurata) extracts against Gram-positive and Gram-negative pathogenic bacteria at 0.2–0.4% (v/v) for fingerroot and 8–10% (v/v) for all of the spices (Pattaratanawadee, Thongson, Mahakarnchanakul, & Wanchatanaanawong, 2006). Cinnamon, cloves, and cumin showed the strongest antimicrobial effects against Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Mycobacterium smegmatis, Micrococcus luteus, and Candida albicans as test strains, with inhibition zones between <10 and >30 mm by the disc-diffusion method (Aguacong, Dostbil, & Alemdar, 2007).

α-Pinene, cineole, limonene, linalool and geranyl acetate are five common antimicrobial compounds that have been effective against developing antimicrobial resistance (Ceylan & Fung, 2004). There are a variety of effective antimicrobial components in EOs derived from herbs such as oregano and thyme: carvacrol and p-cymene are two of them (Burt et al., 2007). Application of yarrow (Achillea millefolium) as an ancient spice showed antimicrobial effect at 30% concentration against Staphylococcus aureus and Escherichia coli (Tajkarimi et al., 2009). Oregano and thyme, oregano with marjoram, and thyme with sage had the most effective EOs against Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli O157:H7 and L. monocytogenes (Almajano et al., 2008; Graumann & Holley, 2008; Gutierrez et al., 2008a; Naidu, 2000a; Tolonen et al., 2004). Phenolic compounds of spices, which contain a high percentage of eugenol, carvacrol and/or thymol, are primarily responsible for bacteriocidal/bacteriostatic properties (Gutierrez, Rodriguez, Barry-Ryan, & Bourke, 2008b; Gutierrez et al., 2008a; Mandalari et al., 2007; Naidu, 2000b; Olonisakin, Oladimeji, & Lagide, 2007; Rodriguez et al., 2009).

Table 1 presents some antimicrobial activities and components of spices and herbs.

Table 1

<table>
<thead>
<tr>
<th>Category</th>
<th>Species</th>
<th>Plant part</th>
<th>Major flavor component</th>
<th>Bacterial inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Herbs</strong></td>
<td>Basil, sweet (Ocimum basilicum)</td>
<td>Leaves</td>
<td>Linalool/methyl chavicol</td>
<td>&lt;50</td>
</tr>
<tr>
<td></td>
<td>Oregano (Oregum vulgare)</td>
<td>Leaves/flowers</td>
<td>Carvacrol/thymol</td>
<td>75–100</td>
</tr>
<tr>
<td></td>
<td>Rosemary (Rosmarinus officinalis)</td>
<td>Leaves</td>
<td>Camphor/1,8-cineole/borneol/camphor</td>
<td>75–100</td>
</tr>
<tr>
<td></td>
<td>Sage (Salvia officinalis)</td>
<td>Leaves</td>
<td>Thujone, 1,8-cineole/borneol/camphor</td>
<td>50–75</td>
</tr>
<tr>
<td></td>
<td>Thyme (Thymbus vulgares)</td>
<td>Leaves</td>
<td>Thymol/carvacrol</td>
<td>75–100</td>
</tr>
<tr>
<td><strong>Spices</strong></td>
<td>Allspice, pimento (Pimenta dioica)</td>
<td>Berry/leaves</td>
<td>Eugenol/β-caryophyllene</td>
<td>75–100</td>
</tr>
<tr>
<td></td>
<td>Cinnamon (Cinnamomum zeylanicum)</td>
<td>Bark</td>
<td>Cinnamic aldehyde/eugenol</td>
<td>75–100</td>
</tr>
<tr>
<td></td>
<td>Clove (Syzygium aromaticum)</td>
<td>Flower bud</td>
<td>Eugenol</td>
<td>75–100</td>
</tr>
<tr>
<td></td>
<td>Mustard (Brassica)</td>
<td>Seed</td>
<td>Allyl isothiocyanate</td>
<td>50–75</td>
</tr>
<tr>
<td></td>
<td>Nutmeg (Myristica fragrans)</td>
<td>Seed</td>
<td>Myristicin/α-piene/Sabinene</td>
<td>50–75</td>
</tr>
<tr>
<td></td>
<td>Vanilla (Vanilla planifolia, V. pompona, V. tahitensis)</td>
<td>Fruit/seed</td>
<td>Vanillin (4-hydroxymethoxybenzaldehyde)/p-OH-benzyl methyl ether</td>
<td>–</td>
</tr>
<tr>
<td><strong>Oils</strong></td>
<td>Olive oil</td>
<td>Fruit</td>
<td>Oleuropein</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Tea-tree oil (Melaleuca alternifolia)</td>
<td>Leaves</td>
<td>Terpenoids</td>
<td>–</td>
</tr>
</tbody>
</table>
4.1. Mechanism of action

It has been demonstrated that the antimicrobial effects of the EOs acts by causing structural and functional damages to the bacterial cell membrane. It is also indicated that the optimum range of hydrophobicity is involved in the toxicity of the EOs (Goni et al., 2009).

Spices and herbs are mostly used in the range of 0.05–0.1% (500–1000 ppm) in food systems. Some spices have stronger antimicrobial activity than others and can be effective at 1000 ppm. However, some spices require higher concentrations (Ceylan & Fung, 2004). Application of antimicrobials by different exposure methods, such as vapor phase compared to direct contact method, of mustard and clove EOs showed noteworthy differences (Goni et al., 2009). The stereochemistry, lipophilicity and other factors affected the biological activity of these compounds which might be altered positively or negatively by slight modifications (Veluri, Weir, Bais, Stermitz, & Vivanco, 2004). It has been shown that plant substances affect microbial cells by various antimicrobial mechanisms, including attacking the phospholipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material of bacteria, and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids (Argues et al., 2008; Burt et al., 2007; Lanciotti et al., 2004; Proestos et al., 2008; Silva et al., 2007; Skocibusic et al., 2006). Allyl isothiocyanate derived from mustard seems to have multi-targeted mechanisms of action in metabolic pathways, membrane integrity, cellular structure and statistically significant higher release of the cell compounds of Escherichia coli O157:H7 (Turgis et al., 2009).

Carvacrol increases the heat shock protein 60 HSP 60 (GroEL) protein and inhibited the synthesis of flagellin highly significantly in E. coli O157:H7 (Burt et al., 2007). There are concerns regarding the enhanced aroma and taste of oregano at the higher levels of application in food items, especially at 1% (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007). The influence of seasonal harvest of plants, geographical location and altitude, storage and extraction procedures still has to be investigated in detail in order to be able to draw the utmost benefit for nutritional and even more for industrial use (Proestos et al., 2008; Silva et al., 2007). Seasonal variations might have some effects on EOs of the cerrado species (Silva et al., 2007); however, they did not have a significant effect on the EOs derived from Myrcia myrtifolia (De Cerqueira et al., 2007). The crude extract of Sorghum bicolor Moench has useful antimicrobial properties and plant extract and fractions showed variable antimicrobial properties (Kil et al., 2009). Table 2 lists some of these compounds, their reported plant origins and antimicrobial effects. Generally, higher concentrations of EOs are necessary in food, compared to in vitro trials: twofold differences in semi-skim milk, 10-fold in pork liver sausage, 50-fold in soup and 25–100-fold in soft cheese. However, for Aeromonas hydrophila there was no difference between in vitro and in-food experiments on cooked pork and lettuce (Burt, 2004; Naidu, 2000a). The apparent antimicrobial efficacy of plant-origin antimicrobials depends on factors such as the method of extracting EOs from plant material, the volume of inoculum, growth phase, culture medium used, and intrinsic or extrinsic properties of the food such as pH, fat, protein, water content, antioxidants, preservatives, incubation time/temperature, packaging procedure, and physical structure of food (Brandi et al., 2006; Burt, 2004; Lis-Balchin, Steyril, & Krenn, 2003; Lopez-Malo vigil et al., 2005). Another important parameter regarding effects of food preservatives is ability to reduce the pH level inside the bacterial cell (pHin). It has been shown that pHin of both E. coli and Salmonella has been reduced by the effect of mustard’s EOs (Turgis et al., 2009).

Generally, Gram-negative bacteria are less sensitive to the antimicrobials because of the lipopolysaccharide outer membrane of this group, which restricts diffusion of hydrophobic compounds. However, this does not mean that Gram-positive bacteria are always more susceptible (Burt, 2004). Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even when used to prevent possible off flavor caused by clove and tea tree when used to protect against Escherichia coli O157:H7 and minimize ways more susceptible (Burt, 2004). Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria (Ramesh Kumar, George, & Shiburaj, 2007; Stefanello et al., 2008).

4.2. Synergistic and antagonistic effects of components

When the combined effect of substances is higher than the sum of the individual effects, this is synergy; antagonism happens when a combination shows less effect compared to the individual applications (Burt, 2004). Synergistic effects of some compounds, in addition to the major components in the EOs, have been shown in some studies (Abdalla, Darwish, Ayad, & El-Hamahmy, 2007; Becerril, Gomez-Lus, Goni, Lopez, & Nerin, 2007; Rota, Herrera, Martinez, Sotomayor, & Jordan, 2008). Application of a certain combination of carvacrol-thymol can improve the efficacy of EOs against pathogenic micro-organisms (Lambert, Skandamis, Coote, & Nychas, 2001).

Synergism between carvacrol and p-cymene, a very weak antimicrobial, might facilitate carvacrol’s transportation into the cell by better swelling the B. cereus cell wall (Burt, 2004). Antimicrobial activity of combination of cinnamon and clove EOs in vapor phase showed better antimicrobial with less active concentration in the vapor phase compared to liquid phase (Goni et al., 2009). Thymol and carvacrol showed synergistic and antagonistic effects, in different combinations of cilantro, coriander, dill and eucalyptus EOs (each containing several components) and mixtures of cinnamaldehyde and eugenol, against Staphylococcus sp., Micrococcus sp., Bacillus sp. and Enterobacter sp. (Burt, 2004). An antagonistic effect on Bacillus cereus was seen in rice when carvacrol and p-cymene were used with salt; high-hydrostatic pressure showed a synergistic effect in combination with thymol and carvacrol against L. monocytogenes (Burt, 2004). Vacuum packing in combination with oregano EOs showed a synergistic effect against L. monocytogenes with 2–3 log10 reduction. Similar results have been recorded when clove and coriander EOs have been used against Aeromonas hydrophila on vacuum-packed pork. Application of oregano EO has a synergistic effect in modified-atmosphere packaging (MAP) including 40% CO2, 30% N2 and 30% O2 (Burt, 2004). The available oxygen is another factor antagonistic on EO activities; by decreasing the oxygen level, the sensitivity of micro-organisms to the EOs has been increased (Burt, 2004). Residual hydrosols after distillation of EOs from plant materials can be used as economical sources of antimicrobial components (Lis-Balchin et al., 2003). Table 3 summarizes the results of various experiments regarding application of plant-origin antimicrobials in food. Table 4 demonstrates some aspects and studies in the area of plant-derived antimicrobials; due to methodological differences, such as choice of plant extract(s), test micro-organism(s) and antimicrobial test method, these findings are not directly comparable.

Application of nisin with carvacrol or thymol has been positively effective against Bacillus cereus with temperatures increasing from 8°C to 30°C (Burt, 2004). Application of nisin with rosemary extract enhanced the bacteriostatic and bactericidal activity of the nisin (Thomas & Isak, 2006). Oregano EOs, in combination with modified-atmosphere packaging, have effectively increased the shelf life of fresh chicken (Chouliara et al., 2007). Antimicrobial resistance did not develop in Yersinia enterocolitica and Salmonella choleraesuis after sub-inhibitory passes with cinnamon, by direct contact or vapor phase (Goni et al., 2009). Combination of linalool and 1, 8-cineole (1:1) created more resistance in E. coli, compared to application of pure linalool. Either synergism or antagonism of 1, 8-cineole and linalool derived from Cinnamomum fragrans could happen against Gram-negative bacteria and Fusarium oxysporum (Randrianarivo et al., 2008). The synergistic effect of different components could offer a way to prevent possible off flavor caused by clove and tea tree when used to protect against Escherichia coli O157:H7 and minimize
off flavor effects in meat products (Moriera et al., 2007). Combinations of EOs of oregano and thyme, oregano with marjoram and thyme with sage had the most effects against Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli O157:H7 and L. monocytogenes (Gutierrez et al., 2008a).

5. Review of some findings of in vitro experiments

In vitro experiments on plant-origin antimicrobials are well described in the literature. However, the antimicrobial activity of herbs and spices might vary based on the tested organism (Bagamboula, Uyttendaele, & De Bevere, 2003). Below are some examples of reported findings organized by the target microbial species.

5.1. A. hydrophila

This is the most sensitive to antimicrobials among Gram-negative species (Burt, 2004). Linalool and methyl chavicol vanillin extracted from sweet basil vanilla showed an inhibitory effect on A. hydrophila at 0.125% v/v methyl chavicol, 1% v/v linalool (Davidson & Naidu, 2000).

5.2. Bacillus sp.

Bacillus sp. were inhibited by: guarana extract (Majhenic, Skerget, & Knez, 2007), EOs of Syzygium gardneri leaves (Raj, George, Pradeep, & Sethuraman, 2008), supercritical fluid extract of the shiitake mushroom Lentinula edodes (Kitzbberger, Smania, Pedrosa, & Ferreira, 2007), hydro-distilled fresh leaves of Pittosporum nepalense Wight et Arn (John, George, Pradeep, & Sethuraman, 2008), leaves, bark and fruits of Neolitsea fischeri (John et al., 2008), EOs of the flower heads and leaves of Santolina rosmarinifolia L. (Compositeae) (Jonnouy et al., 2007), different extracts of thyme, hydro distillation of Syzygium gardneri leaves (Raj et al., 2008), aerial parts of fresh Plectranthus ciliatus leaves (Mohsenzadeh, 2007), EOs of Actinidia macrospora (Lu, Zhao, and Romeo et al., 2008).

### Table 2
Some reported sources of natural antimicrobials in plant within the last 10 years.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Effective against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coriander (Coriandum sativum), Oregano (Origanum vulgare), Rosemary (Rosmarinus officinalis), Parsley (Petroselinum crispum)</td>
<td>Gram-positive and Gram-negative bacteria, including Listeria monocytogenes</td>
<td>Angioni et al. (2004), Ceylan and Fung (2004), Daferera et al. (2000), El-Zemity, Radwan, El-Monam, and Sherby (2008), Gutierrez et al. (2008a, 2008b), Kim, Wei et al. (1995), Lopes-Lutz et al. (2008), and Santos and Rao (2001)</td>
</tr>
<tr>
<td>Allipse (Pimenta dioica), Basil (Ocimum basilicum), Blue mallee (Eucalyptus polybractea), Bay (Laurus nobilis), Caraway seed (Carum carvi), Lemongrass (Cymbopogon citratus), Lemon balm (Melissa officinalis), Marjoram (Origanum majorana), Rosemary (Rosmarinus officinalis), and Sage (Salvia officinalis)</td>
<td>Bacillus subtilis, Clostridium botulinum, Escherichia coli, Listeria monocytogenes, Salmonella typhimurium and Staphylococcus aureus</td>
<td>Angioni et al. (2004), Burt (2004), Ceylan and Fung (2004), Daferera et al. (2000), El-Zemity et al. (2008), Greule and Mosandl (2008), Gutierrez et al. (2008a), Gutierrez et al. (2008b), Kim, Wei et al. (1995), Lopes-Lutz et al. (2008), Lopez-Malo vigil et al. (2005), and Santos and Rao (2001)</td>
</tr>
<tr>
<td>Clove (Syzygium aromaticum) Sage (Salvia officinalis), Cinnamon (Cinnamomum zeylanicum) and Marijoram (Origanum majorana), Allipse (Pimenta dioica)</td>
<td>Pathogens such as Bacillus subtilis, Clostridium botulinum, Escherichia coli, Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus</td>
<td>Amiri et al. (2008), Burt (2004), Ceylan and Fung (2004), Greule and Mosandl (2008), Gutierrez et al. (2008a, 2008b), Lopez-Malo vigil et al. (2005), and Romeo et al. (2008)</td>
</tr>
<tr>
<td>Basil (Ocimum basilicum), Bay (Laurus nobilis), and Lemongrass (Cymbopogon citratus)</td>
<td>Broad spectrum antibacterial effect against Gram-positive and Gram-negative pathogenic micro-organisms</td>
<td>Ceylan and Fung (2004) and Skobicin et al. (2006)</td>
</tr>
<tr>
<td>Blackberry (Rubus spp.), Bay (Laurus nobilis), Basil (Ocium basilicum), Cilantro (immature leaves of Coriandrum sativum), Coriander (Coriandum sativum seeds), Cinnamon (Cinnamomum zeylanicum), Coriander (Coriandum sativum), Marjoram (Origanum majorana) and Thyme (Thymus vulgaris)</td>
<td>Staphylococcus spp.</td>
<td>Burt (2004), Greule and Mosandl (2008), Gutierrez et al. (2008a, 2008b), Lopez-Malo vigil et al. (2005), and Romeo et al. (2008)</td>
</tr>
<tr>
<td>Oregano (Origanum vulgare), Sage (Salvia officinalis), Thyme (Thymus vulgaris) and Satureja hortensis L.</td>
<td>Staphylococcus aureus and Escherichia coli</td>
<td>Bousmaha-Marroki et al. (2007), Burt (2004), and Razzaghi-Abyaneh et al. (2008)</td>
</tr>
<tr>
<td>Marjoram (Origanum majorana)</td>
<td>Alternative for synthetic preservatives routinely used in the food industry</td>
<td>Mahmoud et al. (2007)</td>
</tr>
<tr>
<td>Dill (Anethum graveolens)</td>
<td>Clostridium botulinum, Pseudomonas aeruginosa, Staphylococcus aureus, Yersinia enterocolitica</td>
<td>Ceylan and Fung (2004)</td>
</tr>
<tr>
<td>Fennel (Foeniculum vulgare)</td>
<td>Bacillus cereus, Clostridium botulinum, Salmonella enteritidis, Staphylococcus aureus, Yersinia enterocolitica</td>
<td>Ceylan and Fung (2004)</td>
</tr>
</tbody>
</table>
### Table 3

Inhibitory activities of plant-origin antimicrobials against pathogenic bacteria, protein toxins and fungi, listed alphabetically – representative studies conducted within the last 10 years.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Adverse effects</th>
<th>Inhibitors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>Food poisoning</td>
<td>Teas, leaf volatile oil, leaf oleoresin, eugenol, bark volatile oil, bark oleoresin, E-cinnamaldehyde, oil-macerated garlic extract, tannins, polymers of flavanols, cassia bark-derived substances, crude extracts of bulbs (Lycoris chinensis), stems and leaves of (Nandina domestica), (Mahonia fortunei), (Mahonia bealei), stems of Berberis thunbergii and stems, leaves and fruits of Camptotheca acuminata, methanol and acetone extracts of 14 plants belonging to different families</td>
<td>Cong, Wang, and Xiong (2007), Li, Zhu et al. (2008), Singh et al. (2007), Vaghasiya and Chanda (2007), Yoshida et al. (1999), and Yilmaz (2006)</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>Food poisoning; diarrhea</td>
<td>Black and green tea, cinnamaldehyde and carvacrol</td>
<td>Friedman (2007), Arques et al. (2008), and Ravishankar, Zhu, Law, Joens, and Friedman (2008)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Food poisoning; diarrhea</td>
<td>Cinnamon, oregano oil (Oregano vulgare), pure essential oils, leaf volatile oil, eugenol, bark volatile oil, bark oleoresin, E-cinnamaldehyde, carvacrol, oregano oil, citra, lemongrass oil, cinnamaldehyde, cinnamono oil, (Oregano in whey protein isolate (WPI) films containing, at 23% level, garlic essential oil at 38-45), lemongrass, thyme, carvacrol, cinnamaldehyde, citral, and thymol, clove (Eugenia caryophylata), glucosinolates naturally present in mustard powder, mustard, Bersama engleri (Melianthaceae), chrysanthemum extracts, cabbages, vegetable, Petiveria alliacea L. roots, Aristolochia indica L., catechin, chlorogenic acid and chloridizin, ground yellow mustard, Brassica oleracea juice, dried garlic powder, commercial garlic products, and garlic oil, onion, marjoram and basil essential oils, Scutellaria, Forsythia suspensa (Thunb), and rosemary and clove oil with 75% ethanol, cassia bark-derived substances, thyme (Thymus vulgaris), bay (Pimenta racemosa), iron, extracts, lemongrass, crude extracts of Lycoris chinensis (bulbs), Nandina domestica/Mahonia fortunei/Mahonia bealei (stems and leaves), Berberis thunbergii (stems) and Camptotheca acuminata (stems, leaves and fruits), methanol and acetone extracts of 14 plants belonging to different families, caffeine, 1,3,7-trimethylxanthine.</td>
<td>Arques et al. (2008), Recerril et al. (2007), Ben Sassi, Harzallah-Skhir, Bourgougou, and Aouni (2008), Davidson and Naidoo (2000), Falcone et al. (2005), Ghosh, Kaur, and Gangu (2007), Graumann and Holley (2008), Gutierrez et al. (2008a, 2008b), Hammer et al. (1999), Ibrahim, Salameh, Phetsomphou, Yang, and Seo (2006), Ibrahim, Yang, and Seo (2008), Kim, Wei et al. (1995), Kim et al. (2008), Knight and McKellar (2007), Kong et al. (2007), Kuete et al. (2008), Kyung and Lee (2001), Li, Zhu et al. (2008), Majhenic et al. (2007), Rojas-Grau et al. (2007), Singh et al. (2007), Seydim and Sarikus (2006), Sivakami and Rupasinghe (2007), Tolonen et al. (2004), Vaghasiya and Chanda (2007), and Winward et al. (2008)</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Food poisoning; listeriosis</td>
<td>Tea, pure essential oils, (Oregano in whey protein isolate (WPI) films containing at 23% level, garlic essential oil at 38-45), cabbages, vegetable, cinnamon bark, cinnamon leaf, and clove, Brassica oleracea juice, lemon balm and sage essential oils, cassia bark-derived substances, citral, linalool and bergamot vapor</td>
<td>Brandi et al. (2006), Cava et al. (2004), Fisher and Phillips (2008), Friedman (2007), Gutierrez et al. (2008a, 2008b), Kong et al. (2007), Lopez et al. (2007), Seydim and Sarikus (2006), and Tolonen et al. (2004)</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Tuberculosis</td>
<td>Catechins, Bersama engleri (Melianthaceae)</td>
<td>Friedman (2007), Kuete et al. (2008)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Food spoilage</td>
<td>Tea extract, [Milk protein-based edible films containing oregano, 1.0% (w/v), pimento, or 1.0% oregano pimento (1:1)], tannins, polymers of flavanols, lemongrass, oregano and bay, crude extracts of Lycoris chinensis (bulbs), Nandina domestica/Mahonia fortunei/Mahonia bealei (stems and leaves), Berberis thunbergii (stems) and Camptotheca acuminata (stems, leaves and fruits), certain combinations of carvacrol-thymol, oregano essential oil: methanol and acetone extracts of 14 plants belonging to different families</td>
<td>Friedman (2007), Hammer et al. (1999), Lambert et al. (2001), Li et al. (2008), Oussalah et al. (2004), and Yilmaz (2006)</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Food spoilage</td>
<td>Teas, [Milk protein-based edible films containing oregano, 1.0% (w/v) pimento, or 1.0% oregano pimento (1:1)], tannins, polymers of flavanols</td>
<td>Friedman (2007), Oussalah et al. (2004), and Yilmaz (2008)</td>
</tr>
<tr>
<td>Shigella spp. Tea</td>
<td>Diarrhea</td>
<td>Tea, Bersam engleri (Melianthaceae), tannins, polymers of flavanols, crude extracts of Lycoris chinensis (bulbs), Nandina domestica/Mahonia fortunei/Mahonia bealei (stems and leaves), Berberis thunbergii (stems) and Camptotheca acuminata (stems, leaves and fruits)</td>
<td>Arques et al. (2008), Friedman (2007), Kuete et al. (2008), Li, Zhu et al. (2008), and Yilmaz (2006)</td>
</tr>
</tbody>
</table>
Table 3 (continued)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Adverse effects</th>
<th>Inhibitors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella spp.</strong></td>
<td>Food poisoning; Salmonellosis</td>
<td>Teas, leaf volatile oil; leaf oleoresin; eugenol; bark volatile oil; bark oleoresin, E-cinnamaldehyde, [oeregano in whey protein isolate (WPI) films containing at 2% level, garlic essential oil at 36.4%], oregano (Origanum vulgare), and cinnamon (Cinnamomum zeylanicum), lemongrass, thyme (Thymus vulgaris), carvacrol, cinnamaldehyde, citral, and thymol; methanol extract of Aspilia mussambicensis (Compositae), Bersama engleriiana (Melianthaceae), Aristolochia indica L., Brassica oleracea juice, dried garlic powder, commercial garlic products, and garlic oil, marjoram and basil essential oils, cassia bark-derived substances, lemongrass, bay, methanol and acetone extracts of 14 plants belonging to different families, thymol</td>
<td>Davidson and Naidu (2000), Friedman (2007), Gutierrez et al. (2008a), 2008b, Hammer et al. (1999), Kong et al. (2007), Kuette et al. (2008), Singh et al. (2007), Seydim and Sarkus (2006), Vagahasiya and Chanda (2007), and Yoshida et al. (1999)</td>
</tr>
<tr>
<td><strong>Spore forming bacteria</strong></td>
<td></td>
<td>Catechins</td>
<td>Friedman (2007)</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>Food poisoning; infection</td>
<td>Theasinsessin, tea, cinnamon, oregano (Origanum vulgare), pure essential oils, leaf volatile oil, leaf oleoresin, eugenol, bark volatile oil, bark oleoresin, E-cinnamaldehyde, [oeregano in whey protein isolate (WPI) films containing at 2% level, garlic essential oil at 3% and 4%], dried garlic powder, commercial garlic products, clove, mustard, rosemary (Rosmarinus officinalis), lemon balm (Melissa officinalis), sage (Salvia officinalis), chocolate mint (Mentha piperita), and oregano (Origanum vulgare), Bersama engleriiana (Melianthaceae), chrysanthemum extracts, oil-macerated garlic extract, Petiveria alliacea L. root extract, Aristolochia indica L., tannins, polymers of flavanols, lemongrass and bay; certain combinations of carvacrol-thymol, citral, linalool and vapor, methanol and acetone extracts of 14 plants belonging to different families</td>
<td>Arques et al. (2008), Ben Sassi et al. (2008), Fisher and Phillips (2006), Friedman (2007), Gutierrez et al. (2008a), Gutierrez et al. (2008b), Hammer et al. (1999), Kuette et al. (2008), Kwon et al. (2007), Lambert et al. (2001), Lopez et al. (2007), Musyimi et al. (2008), Singh et al. (2007), Seydim and Sarkus (2006), Vagahasiya and Chanda (2007), Winward et al. (2006), Yilmaz (2006), and Yoshida et al. (1999)</td>
</tr>
<tr>
<td><strong>V. cholerae</strong></td>
<td>Cholera</td>
<td>Tea extract</td>
<td>Arques et al. (2008)</td>
</tr>
<tr>
<td><strong>V. para-haemolyticus</strong></td>
<td>Mild gastroenteritis;</td>
<td>Basil, clove, garlic, horseradish, marjoram, oregano, rosemary, thyme, cassia bark-derived substances</td>
<td>Kong et al. (2007) and Yano et al. (2006)</td>
</tr>
<tr>
<td><strong>Yersinia enterocolitica</strong></td>
<td>Diarrhea</td>
<td>Teas, pure essential oils</td>
<td>Friedman (2007), Lopez et al. (2007)</td>
</tr>
<tr>
<td><strong>Toxins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botulinum</td>
<td>Neurotoxin botulism</td>
<td>Black tea</td>
<td>Friedman (2007)</td>
</tr>
<tr>
<td><strong>Molds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>Mycotoxins</td>
<td>Pure essential oils, leaf oleoresin, leaf volatile oil, eugenol, bark volatile oil, bark oleoresin, E-cinnamaldehyde, cassia bark-derived substances</td>
<td>Kong et al. (2007) and Lopez et al. (2007)</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Ochratoxins</td>
<td>Methanol extract of Aspilia mussambicensis (Compositae)</td>
<td>Musyimi et al. (2008)</td>
</tr>
<tr>
<td>Aspergillus parasiticus</td>
<td>Mycotoxins</td>
<td>Thymol</td>
<td>Davidson and Naidu (2000)</td>
</tr>
</tbody>
</table>

Wang, Chen, & Fu, 2007), specific derivative from the fruits of Eucalyptus globulus (Tan et al., 2008), EOs from thymol and carvacrol at 1: 2000 dilutions and cinnamic aldehyde and eugenol extracted from cinnamon and clove at 0.1–1.0 w/v, 0.06 v/v concentration(Davidson & Naidu, 2000), aerial parts of Ammoudes atlantica (Coss. et Dur.) Wolf. (Apiaceae) (Laouer et al., 2008), leaves, bark and fruits of Neolitsea fischeri (John et al., 2008), hydro-distilled dried and oil samples of Thymus carmuncum (Nejad Ebrahimi et al., 2008), and EOs from Anzer teas and wild-grown leaves of Acorus calamus (Radusine, Judzentiene, Pecuilyte, & Janulis, 2007; Sekeroglu, Deveci, Buruk, Gurbuz, & Ipek, 2007). Extracts of different spices, evaluated by disc diffusion assay, had the following relative inhibitory effects on Bacillus cereus: clove > mustard > cinnamon > garlic > ginger > mint (Sofia et al., 2007).

5.3. C. jejuni

It has been shown that Campylobacter jejuni is more sensitive to natural antimicrobials compared to other pathogenic microorganisms (Sudjana et al., 2009). Linalool vapor of bergamot and linalool oils (Fisher & Phillips, 2008); cinnamic aldehyde and eugenol extracted from cinnamon and clove at 0.05% concentration, linalool and methyl chavicol vanillin extracted from sweet basil vanilla at 0.25% concentration (Davidson & Naidu, 2000). EO of Origanum minitiflorum with a capacity as a food preservative showed a variety of antimicrobial effects against Campylobacter jejuni (Aslim & Vucel, 2007). The ciprofloxacin resistance of Campylobacter strains has been shown to be strongly diminished by application of EO of Origanum minitiflorum (Aslim & Vucel, 2007).
Table 4
Some studies regarding application of EOs or their components in food studies conducted in the past 10 years.

<table>
<thead>
<tr>
<th>Food group</th>
<th>EO or component</th>
<th>Bacterial species</th>
<th>Inhibitory effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat and poultry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fried meat</td>
<td>Oregano and thyme, oregano with marjoram and thyme with sage</td>
<td>B. cereus and P. aeruginosa, Escherichia coli O157:H7 and Listeria monocytogenes</td>
<td>Yes (+)</td>
<td>Du and Li (2008)</td>
</tr>
<tr>
<td>Ground beef</td>
<td>Chinese cinnamon and winter savory EOs</td>
<td>Pathogenic micro-organisms</td>
<td>Yes (increased radiosensitivity)</td>
<td>Turgis et al. (2008)</td>
</tr>
<tr>
<td>Meat</td>
<td>EOs and nisin</td>
<td>Listeria monocytogenes</td>
<td>Yes</td>
<td>Solomakos et al. (2008)</td>
</tr>
<tr>
<td>Meat surfaces</td>
<td>Oregano, pimento, or oregano:pimento</td>
<td>Escherichia coli O157:H7 or Pseudomonas spp.</td>
<td>Yes</td>
<td>Mosqueda-Melgar et al. (2008a, 2008b), and Oussalah et al. (2004)</td>
</tr>
<tr>
<td>Fresh sausage</td>
<td>Marjoram (Origanum majorana L.) EO</td>
<td>Several species of bacteria</td>
<td>Yes</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Sage oil</td>
<td>Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium</td>
<td>No</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Oregano</td>
<td>Increase shelf life</td>
<td>Yes (with modified-atmosphere packaging)</td>
<td>Chouliara et al. (2007)</td>
</tr>
<tr>
<td>Pate</td>
<td>Mint oil</td>
<td>Listeria monocytogenes, Salmonella enteritidis</td>
<td>No</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Minced pork</td>
<td>Thyme oil</td>
<td>Listeria monocytogenes</td>
<td>No</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Vacuum-packed ham</td>
<td>Cilantro oil</td>
<td>Listeria monocytogenes</td>
<td>No</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Vacuum-packed minced pork</td>
<td>Oregano oil</td>
<td>Clostridium botulinum spores</td>
<td>No</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Liver pork sausage</td>
<td>Rosemary</td>
<td>Listeria monocytogenes</td>
<td>Yes (encapsulated has more effect than standard)</td>
<td>Hayouni, Chraief et al. (2008)</td>
</tr>
<tr>
<td>Minced pork sausage</td>
<td>Winter savory (Satureja montana) EO</td>
<td>Food-borne bacteria and improve quality</td>
<td>Yes (in combination with other techniques)</td>
<td>Carraminana et al. (2008)</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cod fillets</td>
<td>Oregano oil</td>
<td>Photobacterium phosphoreum</td>
<td>Yes (+)</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Salmon fillets</td>
<td>Oregano oil</td>
<td>Photobacterium phosphoreum</td>
<td>Yes (+)</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Cod’s roe salad</td>
<td>Mint oil</td>
<td>Photobacterium phosphoreum</td>
<td>No</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Coated semi-fried tuna slices</td>
<td>Eugenol and linalool</td>
<td>Salmonella enteritidis</td>
<td>Yes (+++)</td>
<td>Burt (2004)</td>
</tr>
<tr>
<td>Fresh-water fish</td>
<td>Wild-thyme hydrosol</td>
<td>Increased shelf life, preservative effect</td>
<td>Yes</td>
<td>Oral et al. (2008)</td>
</tr>
<tr>
<td>Lightly salted cultured sea bream (Sparus aurata) fillets</td>
<td>Oregano</td>
<td>Increased shelf life, antioxidant activity</td>
<td>Yes</td>
<td>Goulas and Kontominas (2007)</td>
</tr>
<tr>
<td>Carp fillets</td>
<td>Carvacrol + Thymol</td>
<td>Four times increased shelf life</td>
<td>Yes</td>
<td>Mahmoud, Kawai, Yamazaki, Miyashita, and Suzuki (2006)</td>
</tr>
<tr>
<td>Carp</td>
<td>Thymol, carvacrol and cinnamaldehyde</td>
<td>Increased shelf life</td>
<td>Yes (+++)</td>
<td>Mahmoud et al. (2004)</td>
</tr>
<tr>
<td>Carp</td>
<td>Allyl isothiocyanate, carvacrol, cinnamaldehyde, citral, cuminaldehyde, eugenol, isoeugenol, linalool and thymol</td>
<td>Increased shelf life</td>
<td>Yes (+)</td>
<td>Mahmoud et al. (2004)</td>
</tr>
<tr>
<td>Soft cheese</td>
<td>DMC Base Natural preservative comprising 50% EOs of rosemary, sage and citrus</td>
<td>Listeria monocytogenes</td>
<td>From 1.0 µl g⁻¹ (+)</td>
<td>Burt (2004)</td>
</tr>
</tbody>
</table>

(continued on next page)
Yoghurt
Oil, clove, cinnamon, cardamom, peppermint oil

Streptococcus thermophilus
Yes [Mint oil +, Cardamom, clove: ++, Cinnamon:++]

Burt (2004)

Tzatziki (yogurt and cucumber salad)
Mint oil

Salmonella enteritidis
From 1.5%v/w ++

Burt (2004)

Butter
Satureja ciliaca essential oil

Antioxidant
Yes

Ozkan et al. (2007)

Dairy industries
Oregano, thyme, rosemary, sage, cumin and clove

Starter cultures and related food spoilage
Yes

Viuda-Martos et al. (2008)

Vegetables
Lettuce, romaine

Thyme oil

E. coli 0157:H7
Yes (+)

Burt (2004)

Carrots
Thyme oil

E. coli 0157:H7
Yes (+−++)

Burt (2004)

Lettuce, iceberg green
Basil methyl chavicol (BMC)

Natural flora
Yes (++)

Burt (2004)

Eggplant salad
Oregano oil

E. coli 0157:H7
Yes (++)

Burt (2004)

Alfalfa seeds
Cinnamaldehyde, thymol

Salmonella spp., six serotypes
Yes (50 °C: +, 70 °C: 0)

Burt (2004)

Rice
Boiled rice
Carvacrol

Natural flora
Yes (+++)

Burt (2004)

Boiled rice
Sage oil

Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium
No

Burt (2004)

Rice
Ocimum basilicum

Three stored-rice pests (Sitophilus oryzae, Rhyzopertha dominica and Cryptolestes pusillus)
Yes

Lopez et al. (2008)

Fruit
Kiwi fruit
Carvacrol, cinnamic acid

Natural flora
Yes (+++)

Burt (2004)

Honeydew melon
Carvacrol, cinnamic acid

Natural flora
Yes (0−+)

Burt (2004)

5.4. Clostridium perfringens

Clove EOs have an inhibitory effect against Clostridium perfringens at 0.4% (v/w) concentration (Davidson & Naidu, 2000).

5.5. Escherichia coli

A variety of antimicrobial effects on Escherichia coli has been shown by: guaraná extract (Majhenic et al., 2007), chloroform extract of the plant of Abrus precatorius L. roots at 84% concentration (Zore et al., 2007), EOs from leaves, stems and flowers of Salvia reuterana (Lamiaceae) (Esmaeili et al., 2008), linalool vapor of bergamot and linalool oils (Fisher & Phillips, 2008), water-distilled EO from leaves and flowers of Micromeria nubigena H.B.K. (Lamiaceae) (El-Seedi et al., 2008), hydro distillation leaf oil of Cinnamomum chemungianum (Raj et al., 2008), Rosmarinus officinalis oil (Gachkar et al., 2007), anzer tea EOs (Sekeroglu et al., 2007), EOs of Actinidia macrosperrma (Lu et al., 2007), oleroequin extracted from olive oil at 0.4 mg/ml concentration; clove EOs at 0.4% (v/w) concentration; thymol and carvacrol EOs at 5%, 10%, 15%, 20% concentration, and cinnamaldehyde and eugenol extracted from cinnamon and clove at 0.05% and 0.04% concentrations (Davidson & Naidu, 2000), linalool and methyl chavicol vanillin extracted from sweet basil vanilia at 0.25% concentration; terpenes extracted from tea-tree oil at 0.12−0.25% v/v concentration (Davidson & Naidu, 2000), aerial parts of Ammodites atlantica (Coss. et Dur.) Wolf. (Apiaceae) (Lacour et al., 2008), mango seed kernel (Abdalla et al., 2007), EOs of the flower heads and leaves of Santolima rosmarinifolia L. (Compositae) (Innonuy et al., 2007), sorghum extracts and fractions (Kil et al., 2009), and hydro-distilled fresh leaves of Pittosporum neelligherense Wight et Arn (John et al., 2008). Extracts and oil extracts of various spices had relative inhibitory effects on Escherichia coli as follows: mustard > clove > cinnamon > garlic > ginger > mint (Sofia et al., 2007).

5.6. L. monocytogenes

Rosmarinus officinalis oil showed a strong antimicrobial effect against L. monocytogenes (Gachkar et al., 2007). Thymol and carvacrol EOs showed inhibitory effects against L. monocytogenes at 24 °C in a microbiological medium at 0.5−0.7 w/v and were bacteriostatic at 0.5−1.0% concentration; and borneol extracted from sage and rosemary had inhibitory effect against L. monocytogenes at 0.02−0.05% concentration (Davidson & Naidu, 2000).

5.7. Molds and yeasts

Some EOs demonstrate a broad range of natural fungicidal effects against post-harvest pathogens, especially because of their bioactivity in the vapor phase for storage applications (Tripathi, Dubey, & Shukla, 2008). However, more time is needed for vapor-phase bioactivity effect, possible absorption into the food material needed to be considered (Fisher & Phillips, 2008). Anti-fungal activity might be affected by the targeted fungal physiological activity (Daferera et al., 2000).

The reported effective compounds against food-borne fungi, including Aspergillus niger; A. flavus; and A. parasiticus are: guaraná extract (Majhenic et al., 2007), EO and methanol extract of Satureja hortensis (Dikbas, Kotan, Dadosoglu, & Sahin, 2008), Satureja hortensis L. containing carvacrol and thymol (Razzaghi-Abyaneh et al., 2008), water-distilled EO from leaves and flowers of Microcerma nubigena H.B.K. (Lamiaceae) (El-Seedi et al., 2008), oleroequin extracted from olive oil at 0.2 mg/ml concentration, EOs from thymol at 500 μg/ml concentration and at 1.0% and 100 μg/ml concentrations; cinnamaldehyde and eugenol extracted from cinnamon and clove at 1.0% and 100 μg/ml concentrations (Davidson & Naidu, 2000), Aspergillus parasiticus growth and Aflatoxins production has been inhibited by Thymus vulgaris and Citrus aurantifolia, whereas Mentha spicata L., Foeniculum miliar, Azadirachta indica A.
According to a study on Mueller–Hinton (MH) Agar and MH broth, clove showed antimicrobial effect against Shigella sonnei, Shigella flexneri and E. coli at 1% weight/volume concentration (Bagamboula et al., 2003).

### 5.10. Staphylococcus aureus

A variety of antimicrobial activities against Staphylococcus aureus:

Chloroform and ethanol fractions of Abru s precatorius L. roots was 93% (percentage of inhibition) effective (Zore et al., 2007), hydro-distilled EOs from leaves, stems and flowers of Salvia reuterana (Lamiaceae) (Esmaeili et al., 2008), EOs from an alpine needle leaf of Abies koreana (Jeong, Lim, & Jeon, 2007), hydro-distilled extract of Syzygium gardneri leaves (Raj et al., 2008), hydro-distilled EOs of fresh leaves and mature fruits of Pittosporum viridulum (John et al., 2007), EOs from an alpine needle leaf of Abies koreana (Jeong et al., 2007), Rosmarinus officinalis oil (Gachkar et al., 2007); EOs of Zataria multiflora Boiss (Akhondzadeh Basti et al., 2007), EOs of Actinidia macroasperma (Lu et al., 2007), oleuropein extracted from olive at 0.1% w/v concentration of the extract delayed growth of the bacteria at 0.4–0.6% w/v concentration; clove EOs at 0.4% (v/w) concentration; EOs from thymol and carvacrol 5%, 10%, 15%, and 20% concentration, EOs from thymol and carvacrol 175–225 μg/ml concentration; cinnamic aldehyde and eugenol extracted from cinnamomum and clove at 0.03% and 0.04% concentrations, linalool and methyl chavicol vanillin extracted from sweet basil valinna at 0.1% concentration, terpenes extracted from sweet basil oil, cuminal, and EOs from Cinnamomum cassia oils at 0.12%–0.25% v/v concentration (Davidson & Naidu, 2000), aerial parts of Ammodauc atlantica (Coss. et Dur.) Wolf. (Apiaceae) (Laouer et al., 2008), leaves, bark and fruits of Neolitsea fischeri (John et al., 2008), water-distilled EOs from leaves and flowers of Micromeria nubigena H.B.K. (Lamiaceae) (El-Seedi et al., 2008), Curcuma domestica and Curcuma viridiflora at ratios of 20/15 (Chen et al., 2008), anzer tea's EOs (Sekeroglu et al., 2007), EOs of the flower heads and leaves of Santolina rosmarinifolia L. (Compositae) (Jonnouy et al., 2007), and hydro-distilled EOs of fresh leaves and mature fruits of Pittosporum viridulum (John et al., 2007).

### 5.11. Vibrio paraahaemolyticus

EOs from thymol and carvacrol at 0.5% concentration as whole spices and 100 μg/ml as essential oil (Davidson & Naidu, 2000), tannins and polymers of flavanols (Yilmaz, 2006), and Curcuma domestica and Curcuma viridiflora at ratios of 18/15 (Chen et al., 2008) showed antimicrobial activity against Vibrio paraahaemolyticus.

### 5.12. General Gram-positive and Gram-negative bacteria

Mint (Mentha piperita) EO was more effective against S. enteritidis compared to L. monocytogenes when in Greek appetizers taramosalata and tzatziki. In another study, using freshly distilled EOs showed more susceptibility to Gram–positive bacteria compared to Gram–negative (Burt, 2004). Lemon, orange and bergamot EOs and their components (Fisher & Phillips, 2006), Hydro-distilled EOs of stems, leaves (at vegetative and flowering stages) and flower of Eucalyptus chlorophylla O. Berg. (Myrtaceae) are effective against Gram-positive bacteria (Stefanello et al., 2008).

*Phomopsis* species from the Lamiaceae family, borneol extracted from sage and rosemary had an inhibitory effect against Gram-positive and Gram-negative bacteria at 2% concentration for two groups, 0.3% bacteriostatic and 0.5% bactericidal for Gram-positive bacteria (Bajpai et al., 2008). Linalool and methyl chavicol vanillin extracted from sweet basil vanilla showed inhibitory effect against 33–35 bacteria, yeasts and molds (Davidson & Naidu, 2000).
Bergamot peel (Mandalari et al., 2007) is effective against gram-negative bacteria. Origanum oil, extracted by steam distillation from *Thymus capitatus* L. (Labiatae) by Hoffmanns and Link (common name: Spanish oregano; synonyms: *Coridothymus capitatus* or *Thymus capitatis*), at 486 mg/L concentration in treated-grey-water reed-bed effluent, can prevent coliform re-growth for up to 14 days (Winward, Avery, Stephenson, & Jeffferson, 2008). Antimicrobial activity of EOs extracted from *Thymus vulgaris* (thymol chemotype), *Thymus zygis* subsp. *gracilis* (thymol and two linalool chemotypes) and *Thymus hyemalis* L. (thymol, thymol/linalool and carvacrol chemotypes) was active against 10 pathogenic micro-organisms (Sabulal, George, Pradeep, & Dan, 2008). Generally, Gram-positive bacteria are more sensitive to saponin, with MIC (minimum inhibitory concentration) between 0.3 and 1.25 mg/ml, compared to 1.25–5 mg/ml for Gram-negatives (Oleszek, 2000). MIC of oregano EOs were lower than thyme and cinnamon EOs, against Gram-negative pathogenic micro-organisms (Escherichia coli, Versinia enterocolitica, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis*) compared to Gram-positive ones (*Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus*, and Enterococcus faecalis) as well as molds (*Penicillium islandicum* and *Aspergillus flavus*) (Lopes-Lutz et al., 2008; Lopez, Sanchez, Battle, & Nerian, 2007).

6. Some in-food experiments with plant-origin antimicrobials

In-food studies depend on several additional factors, which have not been tested in similar in vitro studies (Stoico, Saffari, & Houghton, 2009). Spices and herbs can be used as an alternative preservative and pathogen-control method in food materials. Application of both extracts and EOs of plant-origin antimicrobials such as floral parts of *Nandina domestica* Thunb could be a potential alternative to synthetic preservatives (Bajpai et al., 2008). Generally, effective EOs in decreasing order of antimicrobial activities are: oregano > clove > coriander > cinnamon > thyme > mint > rosemary > mustard > cilantro/sage (Burt, 2004). However, in another study, mint showed less antimicrobial effect compared to mustard (Sofia et al., 2007). There are differences between *in vitro* and *in food* trials of plant-origin antimicrobials, mainly because only small percentages of EOs are tolerable in food materials. Finding the most inhibitory spices and herbs depends on a number of factors such as type, effects on organoleptic properties, composition and concentration and biological properties of the antimicrobial and the target micro-organism and processing and storage conditions of the targeted food product (Gutierrez et al., 2008a; Naidu, 2000a; Romeo, De Luca, Piscopo, & Poiana, 2008). In a study on blanched spinach and minced cooked beef, using clove and tea-tree EOs, three and four times the MIC in *in vitro* studies were needed to restrict *E. coli* O157:H7 populations in the food materials (Moriera et al., 2007). Despite some positive reports in regard to application of plant-origin natural antimicrobials, two major issues are faced regarding application of plant-origin antimicrobials in food: odors created mostly by the high concentrations, and the costs of these materials (Proestos et al., 2008; Silva et al., 2007).

6.1. Meat and poultry products

It has been shown that plant extracts are useful for reduction of pathogens associated with meat products. However, some authors have recorded low antimicrobial effects against pathogens in contaminated meat products (Grosso et al., 2008). High-fat-content of the food materials has effects on the application of EOs (Burt, 2004; Lis-Balchin et al., 2003). It may be because of the lipid solubility of EOs compared to aqueous parts of food (Lis-Balchin et al., 2003). Combination of 1% cloves and oregano in broth culture showed inhibitory effect against *L. monocytogenes*, however, the same concentration was not effective in meat slurry (Lis-Balchin et al., 2003). According to Table 4, recent studies regarding certain oils such as eugenol and coriander, clove, oregano and thyme oils showed high effect against *L. monocytogenes, Aeromonas hydrophila* and autochthonous spoilage flora in meat products. However, mustard, cilantro, mint and sage oils were less effective or ineffective (Burt, 2004). A 5–20 μl 1 g–1 level of eugenol and coriander, clove, oregano and thyme oil inhibits growth of *L. monocytogenes, Aeromonas hydrophila* and autochthonous spoilage flora in meat products (Burt, 2004).

Survival and growth of both susceptible and antibiotic-resistant *Campylobacter* strains have been inhibited effectively on agar plates and in contaminated ground beef by application of roselle (*Hibiscus sabdariffa* L.) (Yano, Satomi, & Oikawa, 2006; Yin & Chao, 2008).

EOs’ activity in meat products can be reduced by high levels of fat. Encapsulated rosemary EOs showed better antimicrobial effect compared to standard rosemary EOs against *L. monocytogenes* in pork liver sausage: neither the direct effect of the level used nor manner and effect of encapsulation are reported (Carraminana, Rota, Burillo, & Herrera, 2008). The activity of oregano EOs against *Clostridium botulinum* spores in combination with low levels of sodium nitrite enhanced the delay of growth of bacteria more than the sodium nitrite alone, depending on the number of inoculated spores (Burt, 2004). EOs extracted from the aerial parts of cultivated *Salvia officinalis* L. were effective against *Salmonella enteritidis* (Hayouni et al., 2008). Winter savory (*Satureja montana*) EOs in combination with other preservation methods such as reduced temperature, pulsed light, high pressure, pulsed electric and magnetic fields, irradiation, or packaging under a modified atmosphere can be utilized as economical natural antibacterial substance to control growth of food-borne bacteria and improve quality of minced pork (Carraminana et al., 2008). Chinese cinnamon and winter savory EOs were used successfully to increase radio sensitivity in ground beef (*Turgis, Borsa, Millette, Salimieri, & Lacroix, 2008*). Combinations of EOs and nisin showed enhanced antimicrobial activities against *L. monocytogenes* (Solomakos, G ovaris, Koidis, & Botsoglou, 2008). Rancidity of heat-treated turkey-meat products was inhibited by aqueous extract of rosemary, sage and thyme (*Mielnik, Sem, Egelandsdal, & Skrede, 2008*). Treatments (homogenization) of meat with oregano and sage EO significantly reduced oxidation, while the heat treatment and storage time significantly affected the antioxidant activity of the meat (*Fessas, Mountzouris, Tarantilis, Polissiou, & Zervas, 2007*). Milk protein-based edible films containing oregano, pimento, or oregano/pimento were effective against *Escherichia coli* O157:H7 or *Pseudomonas* sp. on meat surfaces (Mosqueda-Melgar et al., 2008a; Mosqueda-Melgar et al., 2008b; Oussalah et al., 2004). Chlorophyll-containing films were tested against *Staphylococcus aureus* and *Listeria monocytogenes*, showing that it was possible to reduce growth of these micro-organisms inoculated in cooked frankfurters by covering the frankfurters with sodium magnesium chlorophyllin-gelatin films and coatings (*López-Carballo, Hernández-Muñoz, Gavara, & Ocío, 2008*). Marjoram (*Origanum majorana*) EOs were effective against several species of bacteria in fresh sausage (*Busatta et al., 2008*). Chlorophyllins are semi-synthetic porphyrins obtained from chlorophyll. Chlorophyllin-gelatin films and coating applications successfully reduced *Staphylococcus aureus* and *L. monocytogenes* in cooked frankfurter (*López-Carballo et al., 2008*). Individual extracts of clove, rosemary, cassia bark and liquorice demonstrated strong antimicrobial activity; but the mixture of rosemary and liquorice extracts was the best inhibitor against all four types of microbes (*L. monocytogenes, Escherichia coli, Pseudomonas fluorescens* and *Lactobacillus sake*) in modified atmosphere-packaged fresh pork and vacuum-packaged ham slices stored at 4 °C (*Zhang, K kong, Xiong, & Sun, 2009*). *Santalum album, Cinnamomum cassia*, and *Artemisia capillaris* were the most effective antimicrobials.
in raw sheep meat (Luo et al., 2007). There are potential bio-preservative capabilities for application of clove and tea-tree oils to control *Escherichia coli* O157:H7 on blanched spinach and minced cooked beef (Moriera et al., 2007).

### 6.2. Fish

The high fat content of some fish, as with meat products, reduces the antibacterial effect of EOs against various micro-organisms; however, some of the EOs had positive effects even in the high-fat-content fishes. For example, oregano oil is more effective against the spoilage organism *Photobacterium phosphoreum* on cod fillets than on salmon, which is a fatty fish. Oregano oil is more effective than mint oil in/on fish, even in fatty-fish dishes. Application of EOs on the surface of whole fish or as coating for shrimps inhibited *Salmonella Enteritidis*, *L. monocytogenes* and natural spoilage flora (Burt, 2004; Hayouni & Chraief et al., 2008).

There is significant preservative and increased shelf-life effects of wild thyme (*Thymus serpyllum*), about 15 to 20 days with freshwater fish (Oral, Gulumz, Venateswar, & Guven, 2008). Strong antioxidant activity of oregano has been shown in a study by Goulas and Kontominas (2007). Shelf life of carp fillets was extended four-fold by application of combined carvacrol + thymol with some other additives, compared to sterile 0.2% agar solution as a control (Mahmoud, Kawai, Yamazaki, Miyashita, & Suzuki, 2007). Antimicrobial activity studies of garlic oil and nine compounds of EOs against bacterial isolates from carp showed that thymol, carvacrol and cinnamaldehyde had the strongest antimicrobial activities, followed by isoeugenol, eugenol, garlic oil, and then citral for increasing the shelf life of carp fillets, respectively (Mahmoud et al., 2004). Essential oils of *Aloysia sellowii* were successfully screened against a variety of Gram-positive and -negative micro-organisms and two yeasts in brine shrimp (Simonatto et al., 2005). The freshness indicator of tuna slices showed even better results after coating with an edible solution containing 0.5% eugenol plus 0.5% linalool, compared to controls (Abou-taleb & Kawai, 2008). Basil, clove, garlic, horseradish, marjoram, oregano, rosemary, and thyme have been successfully used to implement hurdle technology for protecting seafood from the risk of *Vibrio parahaemolyticus* contamination (Yano et al., 2006). A synergistic effect of treatment with anodic electrolyzed NaCl solution, combined with eugenol and linalool, has been found to enhance shelf-life extension of coated semi-fried tuna (Abou-taleb & Kawai, 2008).

### 6.3. Dairy products

It has been shown that extract of mango seed kernel could reduce total bacterial count, inhibit coliform growth, exert remarkable antimicrobial activity against an *Escherichia coli* strain and extend the shelf life of pasteurized cow milk (Abdalla et al., 2007). High water activity positively affects the application of EOs in milk by speeding the transfer and movement of EOs toward the targeted micro-organisms (Cava, Nowak, Taboada, & Marin-Iniesta, 2007).

The antimicrobial activity of terpenes is not or is only marginally involved in the explanation of the influence of the botanical composition of meadows on the sensory properties of pressed cheeses (Tornambe et al., 2008). *Satureja ciliaca* essential oil can serve in butter as both a natural antioxidant and aroma agent (Ozkam, Simsek, & Kulecian, 2007). EOs from oregano, thyme, rosemary, sage, cumin and clove were tested on the growth of six bacteria, four of them used as starters and two of them as spoilage-causing bacteria (Vucla-Martos et al., 2008). Cinnamon, cardamom and clove oils inhibit the growth of yoghurt starter cultures more than mint oil; however, in other study mint oil was effective against *Salmonella enteritidis* in low-fat yoghurt and cucumber salad (Burt, 2004). An in vitro study on twelve ethanol extracts of propolis showed antimicrobial and antifungal activity especially at low levels against pathogenic micro-organisms to protect starter culture strains in fermented products (Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009). EOs of *Melaleuca armillaris* are effective against lactic acid bacteria and may serve to improve the quality of food products, depending on the LAB strain, as well as on the concentration of the EOs (Hayouni, Bouix, Abedrabba, Leveau, & Hamdi, 2008). EOs of clove, cinnamon, bay and thyme were tested against *L. monocytogenes* and *Salmonella enteritidis* in soft cheese; clove oil was found more effective against *Salmonella enteritidis* in full-fat cheese than in cheese slurry (Burt, 2004).

### 6.4. Vegetables

Application of antimicrobial activity of EOs in vegetables has shown more successful results against natural spoilage flora and food-borne pathogens in washing water, due to the low fat content of the products. It can be enhanced by decreasing the pH of the food and/or temperature. For example, oregano oil was effective against *Escherichia coli* O157:H7 and reduced final populations in eggplant salad (Burt, 2004). Cinnamaldehyde and thymol were effective against six *Salmonella* serotypes on alfalfa seeds. Increasing temperature reduced the effectiveness of these antimicrobials, perhaps because of volatility decreasing permeability of the antibacterial compounds in the liquid media (Burt, 2004; Cava et al., 2007; Goni et al., 2009).

### 6.5. Rice

Leaves of five different varieties of *Ocimum basilicum* were effective against three stored-rice pests (*Sitophilus oryzae*, *Rhizopertha dominica* and *Cryptolestes pusillus*) (Lopez, Jordan, & Pascual-Villalobos, 2008). Sage oil and carvacrol were successfully used against *Bacillus cereus* in rice (Burt, 2004). *Ocimum gratissimum* and *Thymus vulgaris* have been used successfully against two major seed-borne fungi of rice in Cameroun (Nguefack et al., 2007).

### 6.6. Fruit

Natural flora on kiwi fruit (pH 3.2–3.6) was effectively inhibited by carvacrol and cinnamaldehyde but less effectively on honeydew melon (pH 5.4–5.5), which may result from the difference of pH between the fruits (Burt, 2004). Natural fungicidal plant volatiles have been found more effective, compared to similar synthetic preservatives, against post-harvest fungal disease caused by *Botrytis cinerea* in stored grapes (Tripathi et al., 2008). EOs incorporated into an alginate-based edible coating of fresh-cut Fuji apples showed more than 4-log reduction in the population of *E. coli* O157:H7. A total inhibition of native microflora for 30 days at 5 °C, for preservation, taste, quality and pathogen control of the product has been shown. The most effective antimicrobials were lemongrass and cinnamon EOs (0.7%, vol/vol), cinnamaldehyde (0.5%, vol/vol), and citral (0.5%, vol/vol) (Raybaudi, Rojas-Grau et al., 2008). Cinnamon, clove, and lemongrass EOs and their active compounds cinnamaldehyde, eugenol, and citral have also shown promising antimicrobial and quality effects on fresh-cut melon (Raybaudi, Mosqueda-Melgar et al., 2008). Application of a combination of cinnamon and eugenol was tested for control of the germination of *Alycyclobaccillus* spores. The application of 40 ppm cinnamaldehyde with 40 ppm of eugenol or 80 ppm eugenol by itself preserved apple juice for 7 days (Bevilacqua, Corbo, & Siniggila, 2010). Polyphenols are present in green, white and commercial tea and are key antimicrobial and antioxidant components for fresh-cut apple (Abou-taleb & Kawai, 2008). Combination of cinnamaldehyde and eugenol as an apple juice preservative against *Alycyclobaccillus acidoterrestris* showed more acceptable results in the test panels (Bevilacqua et al., 2010).
6.7. Animal feed

Application of thymol in animal feed shows effects on the bacterial community in animal feed (Janczyk, Trevisi, Souffrant, & Bosi, 2008). EO compounds showed limited effects on nutrient utilization in studies of alfalfa silage and corn silage as the sole forage source in ruminants (Benchaar et al., 2007). Effects of cinnamon leaf oil on total volatile fatty acid (VFA) concentration have been studied. It was concluded that it inhibits propionate-producing bacteria and might have an adverse effect on metabolism and productivity of ruminants (Fisher & Phillips, 2008).

7. Effectiveness determinations

Comparison of published data regarding different methods is complicated: according to some investigators there is increased variation based on extraction method, culture medium, size of inoculum, choice of emulsifier and basic test method (Sofia, et al. 2007). Antimicrobial activity in most experiments has been quantified on two bases, MIC and MBC (minimum bactericidal concentration), which is presumably greater than the MIC. MIC is defined in different terms; some of them are “lowest concentration resulting in maintenance or reduction of inoculum’s viability,” “lowest concentration

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<tr>
<td><em>Salmonella typhimurium</em>, <em>Pseudomonas aeruginosa</em>, <em>Escherichia coli</em>, <em>Staphylococcus aureus</em>, <em>Bacillus cereus</em>, <em>Bacillus subtilis</em> and <em>Salmonella typhimurium</em></td>
<td>Agar disc-diffusion method (Nair, Kalariya, &amp; Chanda, 2005)</td>
<td>Methanol and acetone extracts of 14 plants belonging to different families</td>
<td>Vagahassiya and Chanda (2007)</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em> and <em>Escherichia coli</em></td>
<td>Screening growth or survival of these two bacteria at different temperatures in nutrient-rich medium and defined pH</td>
<td>18 Plant species</td>
<td>Yano et al. (2006)</td>
</tr>
</tbody>
</table>
required for complete inhibition of test organism up to 48 h incubation,” “lowest concentration inhibiting visible growth of test organism,” or “lowest concentration resulting in a significant decrease in optical density” (Brandi et al., 2007). MBC is defined as “concentration at which no growth is observed after sub-culturing into fresh broth.” The MIC method is cited by most researchers, but some quote MBC as a measure of antibacterial performance (Brandi et al., 2006; Romeo et al., 2008). MICs for carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinnamic acid were in the range of 0.05–5 μM (in vitro) (Burt, 2004). The in vitro EO studies using agar to determine MICs are generally the same order of magnitude. The optical density (OD) (turbidity) measurement and the enumeration of colonies by viable count are the most-used methods. The redox indicator resazurin has been used recently in a new microdilution method (Burt, 2004). New approaches and advances in extraction methods, such as crude extraction, high-intensity ultrasound-assisted (HI-US) in combination with proper solvent selection, can improve these approaches (Burt, 2004; Ibrahim, Tse, Yang, & Fraser, 2009; Romeo et al., 2008; Thongson, Davidson, Maharkanchanakul, & Weiss, 2004). Thirty-eight strains of Salmonella have been tested with some modifications in the diffusion technique to evaluate antimicrobial activity of chive extract (Ibrahim et al., 2009). The most-used methods for quantitative and qualitative evaluation of EOs of plants and spices are in vitro or exploratory (end-point and descriptive methods) and applied (inhibition curves and endpoint methods). Diffusion, dilution or bio-autographic methods are categories of antimicrobial activity tests (Brandi et al., 2006). However, there are some observed anomalies in screening of antimicrobial activity against Staphylococcus aureus, with a greater reduction of growth at the MIC than at the MBC (Becerril et al., 2007). Moreover, there are differences among publications between definitions and among procedures used in preparation of the tested samples (Brandi et al., 2006; Burt et al., 2007; Kim et al., 2006). Methods such as solvent-free microwave extraction (SFME) and application of crude extracts and enzyme conversions of herbs and spices will improve efficacy and extraction rates, with shorter extraction times and higher levels of active antimicrobial components. Crude extracts of herbs and plants have demonstrated oxidative degradation and antioxidant properties (Bendahou et al., 2008; Ibrahim et al., 2009; Kahkonen et al., 1999; Mandalari et al., 2007). In SFME method, a microwave is used and a plant material is inserted into an extraction vessel with hexane, more detailed information is provided in Bendahou et al. (2008). Vapor-phase experiments are more reliable methods in determining antimicrobial properties; in this method the concentration is expressed as weight per unit volume (mg/l air), and the sterile blank filter disc is placed in the center of the lid of the Petri dish (Goni et al., 2009).

The disc-agar-diffusion method, drop-agar diffusion method and direct-contact technique in agar are the usual techniques in the screening approach, which has been described in detail in other references (Burt, 2004).

Identification of antimicrobial activity is significantly affected by the method of assay and may be interfered with by various food components and/or addition of compounds such as bovine serum albumin (BSA) (Burt, 2004; Davidson & Naidu, 2000). High-pressure extraction (with pure CO2 and with co-solvent) was more effective method than low pressure to obtain extracts (Kitzberger et al., 2007). Other techniques in antimicrobial determination are: diffusion of the EO in agar or broth which showed similar approaches between methods and definitions, broth-dilution studies with bacterial enumeration by optical density (OD) (turbidity) measurement, and application of the redox indicator resazurin as a visual indicator of the MIC (Brandi et al., 2006; Holley & Patel, 2005).

Table 6
Antibacterial activity of various concentrations of spices, as a function of the screening method.*

<table>
<thead>
<tr>
<th>Spice</th>
<th>Concentration (%)</th>
<th>Bacillus cereus</th>
<th>Escherichia coli</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disc</td>
<td>Well/cup</td>
<td>Disc</td>
<td>Well/cup</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>0.5</td>
<td>10.3 ± 0.5</td>
<td>–</td>
<td>8.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11.0 ± 0.0</td>
<td>10 ± 0</td>
<td>10.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.3 ± 0.5</td>
<td>11.6 ± 0.5</td>
<td>12.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.0 ± 0.0</td>
<td>13.3 ± 0.5</td>
<td>14.3 ± 0.5</td>
</tr>
<tr>
<td>Clove</td>
<td>0.5</td>
<td>10.3 ± 0.5</td>
<td>–</td>
<td>11.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11.3 ± 0.5</td>
<td>14.6 ± 0.5</td>
<td>13.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.6 ± 0.5</td>
<td>23.6 ± 0.5</td>
<td>15.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16.0 ± 0.0</td>
<td>25.6 ± 0.5</td>
<td>19.3 ± 0.5</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.5</td>
<td>11.6 ± 0.5</td>
<td>–</td>
<td>10.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12.6 ± 0.5</td>
<td>–</td>
<td>10.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.0 ± 0.0</td>
<td>–</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>14.3 ± 0.5</td>
<td>10.6 ± 0.5</td>
<td>12.3 ± 0.5</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.5</td>
<td>9.6 ± 0.5</td>
<td>–</td>
<td>9.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10.3 ± 0.5</td>
<td>–</td>
<td>10.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.0 ± 0.0</td>
<td>10.3 ± 0.5</td>
<td>10.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.6 ± 0.5</td>
<td>11.3 ± 0.0</td>
<td>11.3° ± 0.5</td>
</tr>
<tr>
<td>Mint</td>
<td>0.5</td>
<td>11.3 ± 0.5</td>
<td>13.3 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>14.6 ± 1.1</td>
<td>14.0 ± 0.0</td>
<td>13.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.6° ± 0.5</td>
<td>14.6° ± 0.5</td>
<td>15.0° ± 1.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.3° ± 1.1</td>
<td>16.6° ± 0.5</td>
<td>16.3° ± 0.5</td>
</tr>
<tr>
<td>Mustard</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>11.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10.3 ± 0.5</td>
<td>9.6 ± 0.5</td>
<td>19.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.6 ± 0.5</td>
<td>10.3 ± 0.5</td>
<td>21.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15.6 ± 0.5</td>
<td>11.3 ± 0.5</td>
<td>25.6 ± 0.5</td>
</tr>
</tbody>
</table>

Differences between different concentrations within a spice found to be significant at P < 0.05.

* All readings are mean of triplicates ± SD, in mm diameter.
* All controls (0% spice) showed negligible inhibition.
* Diffusion from paper disc on inoculated agar.
* Diffusion from cup or well into inoculated agar.
* Unclear zones of inhibition.

Adapted from Winward et al. (2008).
demonstrates some different screening methods for evaluating the antimicrobial activity of different plant-origin antimicrobials against different food-borne pathogenic micro-organisms.

7.1. Disc-diffusion method

The Food and Drug Administration (FDA) has approved this method as a standard for the National Committee for Clinical Laboratory Standards (Turker & Usta, 2008). The disc-diffusion method is the most often-used technical method for antimicrobial screenings of EOs. Antimicrobial activity is generally evaluated by this method for preliminary studies. In this method, a paper disc soaked with the EO is placed on the inoculated surface of an agar plate and the zone of microbial inhibition is measured. Different parameters in this test could affect the result, such as the volume of EO on the paper discs, the thickness of the agar layer and the solvent. For example, some of reported solvents are ethanol, methanol, Tween-20, Tween-80, aceton in combination with Tween-80, polyethylene glycol, propylene glycol, n-hexane and dimethyl sulfoxide, which could result in difficulties when comparing different studies (Brandi et al., 2006; Burt et al., 2007). More precise data regarding extract antimicrobial activity have been obtained with this method (Kil et al., 2009). Table 6 shows antimicrobial activities against Escherichia coli, Bacillus cereus, and Staphylococcus aureus measured by the paper disc-diffusion technique and well or cup method.

7.2. Drop-agar-diffusion method

The drop-agar-diffusion method has been described in detail (Cruz et al., 2007; Hammer, Carson, & Riley, 1999; Hill, Evans, & Vness, 1997). The inhibition zones of EOs against food-borne and other pathogens have been measured by this method (Saif Mokbel & Suganuma, 2006). This was the method used for testing antimicrobial activity of extracts from aerial parts of seven wild sages from Western Canada – Artemisia absinthium L., Artemisia biennis Willd, Artemisia cana Pursh, Artemisia dracunculus L., Artemisia frigida Wild, Artemisia longifolia Nutt. and Artemisia ludoviciana Nutt. – against bacteria, yeasts and fungi, including Escherichia coli, Staphylococcus aureus, Candida albicans and Aspergillus niger (Lopes-Lutz et al., 2008).

7.3. Broth microdilution method

The broth microdilution method was used for antimicrobial activity evaluation of aerial parts of fresh Plectranthus cymbifolius oil against Staphylococcus aureus and Bacillus subtilis (Mohsenson, 2007). According to Davidson and Naidu (2000), this method showed lower MICs (%) v/v of different essential oils extracted from bay, clove, peppermint and thyme against Escherichia coli, Staphylococcus aureus and Candida albicans, compared to agar-dilution assay. This method was used by Radusiene et al. (2007) to evaluate the antimicrobial activity of Acorus calamus against 17 species of bacteria, yeasts and fungi.

7.4. Direct-contact technique in agar

The direct-contact technique in agar has been used for screening the antimicrobial activity of carvacrol, the EO produced from Thymus ciliatus sp. eu-ciliatus, against Staphylococcus aureus and Escherichia coli (Boushma-Marroki, Atik-Bekkara, Tomi, & Casanova, 2007).

8. Conclusions

Plant-origin antimicrobials are present in a variety of plants, spices and herbs. Spices and herbs are used for both flavoring and preservation purposes. Spices and herbs, which were originally added for improving taste, can also naturally and safely improve shelf life of food products (Holley & Patel, 2005). There are a variety of methods for testing the antimicrobial activities of spices, herbs and their components. These methods strongly affect the observed level of inhibition; there is a difference in activity against micro-organisms between each individual and or combination of spices and herbs. Various reasons may contribute in the differences between results, including inconsistency between analyzed plant materials even in the same leaves (Radusiene et al., 2007). The low pH level might work as a result of direct effect of the pH or the effect on EOs on the lipid phase of the bacterial membrane (Abou-taleb, & Kawai, 2008; Akhoundzadeh Basti et al., 2007). Organoleptic and safety issues in application of the EOs in food must be addressed. Application of traditional and natural antimicrobials has been recently outlined by regulatory agencies in the US. Based on Code of Federal Regulation 21 CFR part 182.20, EOs of cinnamon, clove, lemon grass and their respective active compounds (cinnamaldehyde, eugenol and citral, respectively) are generally recognized as safe (GRAS) (Raybaudi, Rojas-Grau et al., 2008; Turgis et al., 2009). Application of Vapor phase combination of EOs showed successful results for an antimicrobial packaging development with less active components of cinnamon and clove (Goni et al., 2009).

Changes in flavor due to application of antimicrobial components are a major issue. However, flavor has not been affected after application of eugenol oil (Eugenia caryophyllata) against four apple pathogens in fresh-cut apple (Amiri et al., 2008). Application of edible and bio-base films with different EOs in the packaging process of sausage casing and processed cheese slices will need further investigation (Seydim & Sarikus, 2006). However, some materials, such as thymol, have not been recognized as food-grade additives by European legislation (Falcone, Speranza, Del Nonile, Corbo, & Sinigaglia, 2005).

In the EU countries, EOs as flavorings have been registered and recognized as safe-to-use materials: carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol and thymol are in this group; however, two EO components, estragole and methyl eugenol, have been deleted from the safe list in 2001. Application of whole EOs seems to be more feasible for most countries, due to the costs of procedures to evaluate safety of any added flavoring materials (Burt, 2004). Synergism and antagonism of these materials need to be further investigated before their broad application in the food industry. It is necessary to define a target and effective range for each EO, including MIC and safety data (toxicity, allergenicity) in food materials (Svoboda et al., 2006).

Application of plant-origin antimicrobials needs to be addressed by regulatory authorities for most parts of these compounds. Assessment of the effect of high doses of some EOs on intestinal cells, as well as cost and odors created by high concentrations of these materials, also should be considered seriously. Several successful combined methods of application of these EOs in conjunction with new approaches such as hurdle technology and modified-atmosphere packaging created pleasant odor with longer shelf life (Burt, 2004; Davidson, 2006; Fisher & Phillips, 2008; Friedman, 2007; Goulas & Kontominas, 2007; Naidu, 2000a; Proestos et al., 2008; Silva et al., 2007). According to Lanciotti et al. (2004), other possible ways to reduce the organoleptic impact include:

1. Minimizing perception of the presence of spices/herbs and EOs in food by optimizing food formulation.
2. Application of combined methods.
3. Enhancing a calibrated vapor pressure capacity in order to increase interaction between EO and the bacterial cell membrane.
Evaluation of new preservatives such as natural antimicrobials in food, evaluating food structure, composition and interaction between natural microflora and food-borne disease agents could be made much more precise by application of predictive models (Koutsoumanis et al., 1999).

Several studies have been focused on the application of individual EOs derived from plants. Some studies showed whole EOs have more antimicrobial activity compared to the mixture of major components (Burt, 2004). However, information on the effects of these natural compounds in combination and or as crude extracts against food-borne micro-organisms is limited (Ibrahim et al., 2009; Mandalari et al., 2007).

The future will see much-needed investigation of food applications of the naturally occurring antimicrobials, especially the effectiveness of EOs, individually and in combination with other parts of plant extract, other effective EOs and other food-processing techniques.

References


Abou-taleb, M., & Kawai, Y. (2008). Shelf life of semi fried tuna slices coated with special EOs derived from plants. Some studies showed whole EOs have more antimicrobial activity compared to the mixture of major components (Burt, 2004). However, information on the effects of these natural compounds in combination and or as crude extracts against food-borne micro-organisms is limited (Ibrahim et al., 2009; Mandalari et al., 2007).


Bendahou, M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J. M., Abou-taleb, M., & Kawai, Y. (2008). Shelf life of semi fried tuna slices coated with special EOs derived from plants. Some studies showed whole EOs have more antimicrobial activity compared to the mixture of major components (Burt, 2004). However, information on the effects of these natural compounds in combination and or as crude extracts against food-borne micro-organisms is limited (Ibrahim et al., 2009; Mandalari et al., 2007).


