Chapter 7
Phytochemicals as Anti-microbial Food Preservatives

Mehrdad Tajkarimi and Salam A. Ibrahim

Abstract Phytochemicals containing essential oils (EOs) in the range of 0.05–0.1% have demonstrated inhibitory activity against pathogens, such as *Salmonella* Typhimurium, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*, in food systems. Three major limitations for the broad application of phytochemicals in food are: limited data about their effects in food, strong odor, and high cost. New techniques and synergistic effect of compounds have been successfully applied in several food and *in-vitro* experiments. Several in-food and *in-vitro* applications of essential oils, phenolic and other components are discussed in this chapter.

Keywords Phytochemicals • Food preservatives • Food spoilage bacteria • Essential oils • Phenolic compounds

7.1 Introduction

Increasing occurrence of food-borne disease outbreaks and growing interest in consumer demand for safe, fresh, ready to eat and high-quality foods, raises considerable challenges (Chana-Thaworn et al. 2011; Tajkarimi et al. 2010). Application of chemical preservatives, synthetic antimicrobials and several processing techniques are commonly used to inactivate or inhibit the growth of spoilage and pathogenic microorganisms. However, they have not been considered as a comprehensive control method (Tajkarimi et al. 2010; Chana-Thaworn et al. 2011; Xing et al. 2010). As a consequence, naturally derived compounds such as plant extracts in food are receiving a good deal of attention as control agents for microorganisms.

M. Tajkarimi (✉) • S.A. Ibrahim
Food and Nutritional Science Program, Department of Family and Consumer Science,
North Carolina A&T State University, Greensboro, NC 27410, USA
e-mail: tajkarimi@gmail.com

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The potential use of plant extract as a natural antimicrobial agent in food preservation forms the basis for many applications (von Staszewski et al. 2011; Chana-Thaworn et al. 2011; Zouari et al. 2010; Thembo et al. 2010). Antimicrobials of plant origin are generally secondary metabolites in plants, that could act separately or jointly against food borne pathogens, in addition to contributing to the taste and flavor (Sotelo et al. 2010; Park et al. 2010). Strong odor and flavor of EOs such as thiosulfinates is a major issue, however, there are promising reports about the application of some EO constituents such as eugenol (*Eugenia caryophylata*), a major component of clove oil, against pathogens in fresh-cut apple without flavor change (Teng et al. 2010). Antimicrobials control and prevent natural spoilage processes (food preservation) and growth of microorganisms, including pathogens (food safety). Phytochemicals and plant origin materials such as spices and herbs have antimicrobial effects on plant and human pathogens in addition to their flavoring effects (Tajkarimi et al. 2010; Romeo et al. 2010).

There are new techniques such as pulsed light, high pressure pulsed electric, magnetic fields, incorporation of natural antimicrobials into packaging materials, micro emulsion, micro- and nanoencapsulation that could provide protection conditions to deliver bioactive compounds into food systems for food preservation and controlling pathogens and spoilage microorganisms in food (Nori et al. 2011; Tajkarimi et al. 2010; Zhang et al. 2010a; Khanzadi et al. 2010). For example, *Gelidium corneum* (GC), a type of agarose-containing red algae as an edible film packaging material, has successfully demonstrated antimicrobial activity by the addition of grape seed extract or thymol against *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Lim et al. 2010a, 2010b; Bisha et al. 2010). Grape skin extracts of Riesling *Vitis vinifera* L. grapes showed strong preservative effects against Gram-positive foodborne pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium* (Corrales et al. 2010). Table 7.1 illustrates recent reports on the application of phytochemicals against Gram positive bacteria. However, some of the new technologies are not sufficiently effective for eliminating pathogens or delaying microbial spoilage. A growing body of data indicates considerable potential for the utilization of EOs derived from spices and herbs against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Shigella dysenteria*, *Bacillus cereus* and *Staphylococcus aureus* at levels ranging between 0.2 and 10 $\mu l\ ml^{-1}$. There are a number of examples indicating more than 5 log10 CFU reduction in pathogenic microorganisms, using a combination of phytochemicals with common processing techniques (Tajkarimi et al. 2010). Phytochemicals could also improve the storage stability by means of active components including phenols, alcohols, thiosulfinates, aldehydes, ketones, ethers and hydrocarbons, especially in spices such as cinnamon, clove, garlic, mustard, and onion (Santas et al. 2010).

The purpose of this chapter is to provide an overview of some phytochemicals that have been reported to be effective against spoilage or pathogenic microorganisms, and practical methods used for screening these compounds.

Table 7.1 Inhibitory activities of plant-origin antimicrobials against gram positive pathogenic bacteria, listed alphabetically representative studies conducted within the last 10 years

Organism	Adverse effects	Inhibitors	References
Bacillus cereus	Food poisoning; emesis	Tea, eugenol, leaf essential oil, bark essential oil; bark oleoresin, E-cinnamaldehyde, clove, mustard, cinnamon, guarana extract, EOs of *Syzygium gardneri* leaves, supercritical fluid extract of the shiitake mushroom Lentinula edodes, hydro-distilled fresh leaves of *Pittosporum neelgherrense* Wight et Arn, Melianthaceae Bersama engleriana, leaves, bark and fruits of Neolitsea fischeri , EOs of the flower heads and leaves of *Santolina rosmarinifolia* L. thymol and carvacrol thyme, cinnamic aldehyde and eugenol extracted from cinnamon and clove, aerial parts of Ammoides atlantica, leaves, bark and fruits of Neolitsea fischeri, hydro distillation of Syzygium gardneri leaves and dried and oil samples of Thymus caramanicus EOs from Anzer teas and wild-grown leaves of Acorus calamus, aerial parts of fresh Plectranthus cylindraceus oil, EOs of Actinidia macrosperma, fruits of Eucalyptus globules, oil-macerated garlic, Petiveria alliacea L. root extract, Aristolochia indica L., tannins, dried and commercial garlic products, garlic oil, marjoram and basil essential oils, citral, linalool and bergamot vapor, methanol and acetone extracts of 14 plants belonging to different families, ethanolic extract from Rhodomyrtus tomentosa (Ait.) Hassk. Leaves, Salvia pisidica Boiss. and Heldr. ex Bentham (Lamiaceae) essential oil; relative inhibitory effects on Bacillus cereus: clove > mustard > cinnamon > garlic > ginger > mint. Hydro distilled extract of *Bunium persicum, Cuminum cyminum and Carum copticum.* EOs and methanol extracts of sweet basil *Ocimum basilicum* L. (Lamiaceae)	Tajkarimi et al. (2010), Ozkan et al. (2010), Oroojalian et al. (2010), and Hossain et al. (2010)

(continued)

Table 7.1 (continued)

Organism	Adverse effects	Inhibitors	References
Bacillus subtilis	Food poisoning	Teas, leaf essential oil, leaf oleoresin, eugenol, bark essential 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, *Eruca sativa* (aerial and root). EOs and methanol extracts of sweet basil *Ocimum basilicum* L. (Lamiaceae). Ponkan (*Citrus reticulata* Blanco). Seeds of *Zizyphus jujube.*	Tajkarimi et al. (2010), Khoobchandani et al. (2010), Hossain et al. (2010), Gao et al. (2010), and Al-Reza et al. (2010)
Clostridium spp.	Food poisoning; diarrhea	Clove EOs, *Zataria multiflora*	Tajkarimi et al. (2010), and Khanzadi et al. (2010)
Listeria monocyto-genes	Food poisoning; listeriosis	Tea, pure essential oils, oregano in whey protein isolate (WPI) films containing garlic essential oil, cabbage juice, cinnamon bark, cinnamon leaf, and clove, Brassica oleracea juice, lemon balm and sage essential oils, cassia bark-derived substances, citral, linalool and bergamot vapor, *Rosmarinus officinalis* oil, Thymol and carvacrol EOs, borneol extracted from sage and rosemary. Hydro distilled extract of *Bunium persicum, Cuminum cyminum and Carum copticum.* Asari Radix, the roots of *Asarum heterotropoides* F. Maekawa var. manshuricum F. Maekawa. *Alpinia galanga* (Linn.) Swartz. EOs and methanol extracts of sweet basil *Ocimum basilicum* L. (Lamiaceae). Raw state of brown Irish edible seaweeds, *Himanthalia elongata, Laminaria sachharina* and *Laminaria digitata*(100% inhibition). ethanol extracts of *Artemisia Herba Alba, Lavandula officinalis* L., *Matricaria Chamomilla, Eugenia caryophylata, Cistus salvifolius, Mentha suaveolens subsp. Timija, Thymus serpyllum* L., *Lippia citriodora, Cinnamomum Zeylanicum, Rosa centifolia, Thymus vulgaris* L, *Rosmarinus officinalis and Pelargonium graveolens.* Seeds of *Zizyphus jujube.*	Tajkarimi et al. (2010), Oroojalian et al. (2010), Oh et al. (2010), Hsu et al. (2010), Hossain et al. (2010), Gupta et al. (2010), Castano et al. (2010), Bayoub et al. (2010), and Al-Reza et al. (2010)

Mycobac-terium tuberculosis	Tuberculosis	Catechins, Bersama engleriana (Melianthaceae),	Tajkarimi et al. (2010)
Spore forming bacteria	Food poisoning	Catechins	Tajkarimi et al. (2010)
Staphylo-coccus aureus	Food poisoning; infection	Theasinensin, tea, cinnamon, oregano (Origanum vulgare), pure essential oils, leaf essential oil, leaf oleoresin, eugenol, bark essential oil, bark oleoresin, E-cinnamaldehyde, [oregano 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 piperata), and oregano (Origanum vulgare), Bersama engleriana (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 vapour, methanol and acetone extracts of 14 plants belonging to different families. Hydro distilled extract of *Bunium persicum, Cuminum cyminum and Carum copticum.* Methanol extract of *Salvia leriifolia* leaf. Steam distilled Herba Moslae. Hydro distilled extract of *Bunium persicum, Cuminum cyminum and Carum copticum.* Lantana camara Linn. (Verbenaceae), Ageratum houstonianum Mill. (Asteraceae) and Eupatorium adenophorum Spreng. (Asteraceae), *Eruca sativa* (aerial and root). *Alpinia galanga* (Linn.) Swartz. EOs from peel of Ponkan (*Citrus reticulata* Blanco). Seeds of *Zizyphus jujube. Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.	Tajkarimi et al. (2010), Oroojalian et al. (2010), Mehr et al. (2010), Li et al. (2010), Kurade et al. (2010), Khoobchandani et al. (2010), Hsu et al. (2010), Gao et al. (2010), Castano et al. (2010), Al-Reza et al. (2010), and Abalaka et al. (2010)

7.2 Historical Overview of Plant Antimicrobials as a Food Preservative

Natural antimicrobial agents derived from plant oils have been recognized and used for centuries by the early Egyptians and Asian countries such as China and India. Spices such as clove, cinnamon, mustard, garlic, ginger and mint are still applied as an alternative health remedy in India. Processing and production of EO's can be traced back over 2,000 years to the Far East, with the beginnings of more modern technology occurring in Arabia in the ninth century. Spices have eastern origin and some of them such as chili peppers, sweet peppers, allspice, annatto, chocolate, epazote, sassafras, and vanilla have been recognized after discovery of the New World. In 1880s, the first scientific report about the potential of spices as preservatives was published. In the 1910s, the preservative effect of cinnamon and mustard in applesauce was reported. Other spices, such as allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage and thyme, have been reported by many researchers to have significant bacteriostatic properties since then (Tajkarimi et al. 2010). Hemp (Cannabis sativa L.), a therapeutic phytochemical, has been used in Asia 5,000 years ago (Nissen et al. 2010)

7.3 Major Plant Antimicrobials

Phytochemicals are used as natural components for extending the shelf life of foods, reducing or eliminating pathogenic bacteria, and increasing the overall quality of food products. Herbs, spices and fruits can be divided into subgroups based on their chemical structures. The most important groups include phyto-phenolics in herbs and spices, flavonoids and acids in fruits and berries, and glucosinolates in cruciferous vegetables, mustard, cabbage, and horseradish (Schirmer and Langsrud 2010). There are various parts of the plant are used to obtain phytochemicals including flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots. Phytochemicals, generally are mixtures of several components; some phytochemicals such as components in oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage, vanillin and lichens exhibit antimicrobial activity (Shukla et al. 2010; Tajkarimi et al. 2010; Schirmer and Langsrud 2010). The essential oils extracted from some of the above-stated spices contain chemical compounds such as carvacrol, cinnamaldehyde, eugenol and camphor, that play a major role as antimicrobial compounds (Xing et al. 2010; Tajkarimi et al. 2010; Weerakkody et al. 2010; Qiu et al. 2010; Mihajilov-Krstev et al. 2010). Other spices, such as ginger, black pepper, red pepper, chili powder, cumin oil and curry powder, also have antimicrobial properties (Hajlaoui et al. 2010; Holley and Patel 2005). Zingiberaceous plants, galangal (*Alpinia galanga*), turmeric (*Curcuma longa*), and finger root (*Boesenbergia pandurata*) extracts have been found to be effective against Gram-positive and Gram-negative pathogenic bacteria at 0.2–0.4% (v/v) for finger root and 8–10% (v/v) for all of the other spices (Chen et al. 2008; Tajkarimi et al. 2010). Table 7.2 shows the use of phytochemicals against gram negative bacteria.

It has been well demonstrated that more than 1,340 plants with defined antimicrobial compounds have been isolated and used in the food industry. However, there are only few commercial EOs available with useful characterizations of preservative properties. Different methods including steam distillation (SD) and hydro distillation (HD) methods, cold, dry and vacuum distillation, solvent free microwave extraction (SFME) , supercritical carbon dioxide extraction and supercritical fluid extraction (SFE) are available in the production of commercial EOs (Okoh et al. 2010; Tajkarimi et al. 2010; Zhang et al. 2010b). Some of these methods enable better extraction properties (Okoh et al. 2010). Manipulating the parameters such as temperature, pressure and bioengineering contributes the number of commercially available products. Varieties of edible medicinal and herbal plants, and spices, have been successfully used alone or in combination with other preservation methods to extend the shelf life of foodstuffs or as antimicrobial agents against a variety of Gram-positive and Gram-negative bacteria. The efficacy of the components depends on the pH, storage temperature, the amount of oxygen, the EO concentration and active components presented (Tajkarimi et al. 2010; Rao et al. 2010).

7.3.1 Chemical Components Present in Plant-Origin Antimicrobials

There are various chemical components present in plant-origin antimicrobials including, saponin and flavonoids, thiosulfinates and glucosinulates. EOs may contain different components including terpenoids, sesquiterpenes and possibly diterpenes with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones.

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 or could be extracted from roots, stem bark, leaves and wood of the selected plants. *Thiosulfinates* are hydrolysis products of garlic and onion, formed when the bulb damages. They have a strong antimicrobial activity against pathogenic microorganisms such as *Listeria monocytogenes* (Santas et al. 2010). *Glucosinulates* are present in broccoli, brussels sprouts, cabbage, and mustard powder and cause the pungent flavor of mustard and horseradish; they exhibit a wide range of antibacterial and antifungal activity with direct or synergistic effect in combination with other compounds. Generally, phenolic compounds extracted from lemon, olive oil (oleuropein) and tea-tree oil (terpenoids), orange and bergamot have broader antimicrobial effects. Phenolic compounds present in eugenol oil (*Eugenia caryophylata*) and selected clonal herb species of the Lamiaceae family show inhibitory effect against a variety of microorganisms. Meanwhile, there are increasing reports of non-phenolic

Table 7.2 Inhibitory activities of plant-origin antimicrobials against gram negative pathogenic bacteria, listed alphabetically representative studies conducted within the last 10 years

Organism	Adverse effects	Inhibitors	References
Aeromonas hydrophila	Gastrointestinal diseases	Linalool and methyl chavicol vanillin extracted from sweet basil vanilla	Tajkarimi et al. (2010)
Campylobacter jejuni	Food poisoning; diarrhea	Black and green tea, cinnamaldehyde and carvacrol, marigold, ginger root, jasmine, patchouli, gardenia, cedar wood, carrot seed, celeray seed, mugwort, spikenard, orange bittle oils and coriander(*Coriandrum sativum*) oil, Linalool vapor of bergamot and linalool oils, cinnamic aldehyde and eugenol extracted from cinnamon and clove, linalool and methyl chavicol vanillin extracted from sweet basil vanilla , EO of *Origanum minutiflorum*	Tajkarimi et al. (2010), and Rattanachaikunsopon and Phumkhachorn (2010b)
Escherichia coli	Food poisoning; diarrhea	Cinnamon, oregano oil (Oreganum vulgare), pure essential oils, leaf essential oil, eugenol, bark essential oil, bark oleoresin, E-cinnamaldehyde, carvacrol, oregano oil, citra, lemongrass oil, cinnamaldehyde, cinnamon oil, Oregano in whey protein isolate (WPI) films containing garlic essential oil at, lemongrass, thyme, carvacrol, cinnamaldehyde, citral, and thymol, clove (Eugenia caryophyllata), glucosinolates naturally present in mustard powder, mustard, Bersama engleriana (Melianthaceae), chrysanthemum extracts, cabbage juice, Petiveria alliacea L. roots, Aristolochia indica L., catechin, chlorogenic acid and phloridzin, 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), ion extracts, lemongrass, crude extracts of Lycoris chinensis (bulbs), Nandina domestica/Mahoniafortunei/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, Guarana extract, chloroform extract of the plant of *Abrus precatorious* L. roots, EOs from leaves, stems and flowers of *Salvia reuterana* (Lamiaceae), linalool vapor of bergamot and linalool oils, water-distilled EO from leaves and flowers of *Micromeria nubigena* H.B.K. (Lamiaceae), hydro distillation leaf oil of *Cinnamomum chemungianum and Cinnamomum zeylanicum* Blume, *Rosmarinus officinalis* oil, Anzer tea EOs, EOs of *Actinidia macrosperma,* oleuropein extracted from olive oil, clove EOs, thymol and carvacrol EOs, aerial parts of *Ammoides atlantica,* mango	Tajkarimi et al. (2010), Trajano et al. (2010), Ozkan et al. (2010), Oroojalian et al. (2010), Kurade et al. (2010), Gao et al. (2010), Castano et al. (2010), Al-Reza et al. (2010), and Abalaka et al. (2010)

		seed kernel, EOs of the flower heads and leaves of *Santolina rosmarinifolia* L. (Compositae), *Salvia pisidica* Boiss. and Heldr. ex Bentham (Lamiaceae) extract, sorghum extracts and fractions, ydro-distilled fresh leaves of *Pittosporum neelgherrense* Wight et Arn, relative inhibitory effects on *Escherichia coli* as follows: mustard > clove > cinnamon > garlic > ginger > mint. Hydro distilled extract of *Bunium persicum, Cuminum cyminum and Carum copticum.* Lantana camara Linn. (Verbenaceae), Ageratum houstonianum Mill. EOs from peel of Ponkan (Citrus reticulata Blanco) (Asteraceae) and Eupatorium adenophorum Spreng. (Asteraceae). Seeds of *Zizyphus jujube. Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.	
Pseudomonas aeruginosa	Food spoilage	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, *Eruca sativa* (aerial and root). Raw state of brown Irish edible seaweeds, *Himanthalia elongate*(98% inhibition), *Laminaria sachharina (93%)*	Tajkarimi et al. (2010), Khoobchandani et al. (2010), and Gupta et al. (2010)
Pseodomonas fluorescens	Food spoilage	Teas, [Milk protein-based edible films containing oregano, 1.0% (w/v) pimento, or 1.0% oregano pimento(1:1)], tannins, polymers of flavanols, *Salvia pisidica* Boiss. and Heldr. ex Bentham (Lamiaceae) essential oil	Tajkarimi et al. (2010), and Ozkan et al. (2010)
Shigella spp. Tea	Diarrhea	Tea, Bersam engleriana(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), *Eruca sativa* (aerial and root). EOs and methanol extracts of sweet basil *Ocimum basilicum* L. (Lamiaceae)	Tajkarimi et al. (2010) Khoobchandani et al. (2010), and Hossain et al. (2010)

(continued)

Table 7.2 (continued)

Organism	Adverse effects	Inhibitors	References
Salmonella spp.	Food poisoning; Salmonellosis	Teas, leaf essential oil; leaf oleoresin; eugenol; bark essential oil; bark oleoresin, E-cinnamaldehyde, [oregano in whey protein isolate (WPI) films containing at 2% level, garlic essential oil at 3% and 4%], oregano (Origanum vulgare), and cinnamon (Cinnamomum zeylanicum), lemongrass, thyme (Thymus vulgaris), (carvacrol, cinnamaldehyde, citral, and thymol), methanol extract of Aspilia mussambicensis (Compositae), Bersama engleriana (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, EOs extracted from the aerial parts of cultivated *Salvia officinalis* L. Hydro distilled extract of *Bunium persicum, Cuminum cyminum and Carum copticum. Alpinia galanga* (Linn.) Swartz. EOs and methanol extracts of sweet basil *Ocimum basilicum* L. (Lamiaceae). lemon balm (*Melissa officinalis*), aqueous garlic extract (*Allium sativum*). Seeds of *Zizyphus jujube.*	Tajkarimi et al. (2010), Oroojalian et al. (2010), Hsu et al. (2010), Hossain et al. (2010), Dikbas et al. (2010), Belguith et al. (2010), and Al-Reza et al. (2010)
V. cholerae	Cholera	Tea extract	Tajkarimi et al. (2010)
V.parahaemolyticus	Mild gastroenteritis	Basil, clove, garlic, horseradish, marjoram, oregano, rosemary, thyme, cassia bark-derived substances. EOs and methanol extracts of sweet basil *Ocimum basilicum* L. (Lamiaceae). *Cuminum cyminum* L.	Tajkarimi et al. (2010), Hossain et al. (2010), and Hajlaoui et al. (2010)
Yersinia enterocolitica	Diarrhea	Teas, pure essential oils, *Salvia pisidica* Boiss. and Heldr. ex Bentham (Lamiaceae) essential oil	Tajkarimi et al. (2010), and Ozkan et al. (2010)

compounds such as allyl isothiocyanate, carvacrol, isoterpinolene, caryophyllene, camphene, pinene, and thymol, being effective against both Gram-positive and Gram-negative groups (Szabo et al. 2010; Schirmer and Langsrud 2010). EOs could be extracted from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, purple (cultivar Ison) and bronze (cultivar Carlos) muscadine seeds, clary sage, juniper , lemon, marjoram and herb infusions such as *Ilex paraguaiensis* (Vaquero et al. 2010; Tajkarimi et al. 2010; Tserennadmid et al. 2010). Antimicrobial activity of Clove is higher than rosemary and lavender EOs (Gomez-Estaca et al. 2010). Terrenes, carvacrol, *p*-cymene*,* and thymol present in oregano, savory and thyme EOs, have demonstrated antifungal and antimicrobial activity that has attracted attention recently because of their potential in food safety applications (Mihajilov-Krstev et al. 2010).

7.4 Uses of Plant-Origin Antimicrobials

Food spoilage can occur through the whole production chain from raw food materials to process and distribution. Preserving food from spoilage and/or pathogenic microorganisms using plant origin compounds have dramatically increased since the 1990s. Plant origin antimicrobials have relatively low molecular weight, (Padovan et al. 2010) and strong antimicrobial activity as potential food preservatives (Dikbas et al. 2010). Water extracts of plants may have greater antimicrobial potential and less undesirable gastric disorders, for example water-phase and decoction extract of bamboo shavings and *Piliostigma reticulatum* (DC.) The bark of *Piliostigma reticulatum* showed antimicrobial activity against different pathogenic and spoilage microorganisms with minimum inhibitory concentrations (MICs) ranging from 0.28 to 32 mg/ml (Zhang et al. 2010b; Zerbo et al. 2010). The MIC values essential oil ranged between 0.039 and 10 mg/ml for *Hypericum scabrum*, *Myrtus communis*, *Pistachia atlantica*, *Arnebia euchroma*, *Salvia hydrangea*, *Satureja bachtiarica*, *Thymus daenensis* and *Kelussia odoratissima* against *Escherichia coli* O157:H7, *Bacillus cereus*, *Listeria monocytogenes* and *Candida albicans* (Pirbalouti et al. 2010). Terpinen-4-ol, linalool, nerol, geraniol, β-pinene, limonene, α-pinene, sabinene, γ-terpinene and myrcene, cineole, and geranyl acetate are antimicrobial compounds that have been effective against some food-borne pathogens and antibiotic-resistant *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Campylobacter jejuni* (Tserennadmid et al. 2010; Tajkarimi et al. 2010; Park et al. 2010). Olive leaves (*Olea europaea*), rich in phenolic compounds, demonstrated strong antimicrobial effects and potential use in food processing including turkey breast packaging (Erbay and Icier 2010; Botsoglou et al. 2010). The crude extract of *Sorghum bicolor* Moench showed antimicrobial properties and showed variable antimicrobial properties (Tajkarimi et al. 2010; Lee and Lee 2010; Erbay and Icier 2010)

The influence of seasonal harvest of plants and geographical location still has to be investigated in detail in order to be able to draw the utmost benefit for industrial use. Seasonal variations showed some effects on EOs of the cerrado species;

however, they did not have a significant effect on *Myrcia myrtifolia* EOs. Different harvest locations of *Thymus pallescent* resulted in different antimicrobial activities; However, differences in antimicrobial/antioxidant activity of the Tunisian *Thymus capitatus* have not been attributed to location. Essential oil of the aerial parts of *Satureja hortensis* L. containing carvacrol showed strong antimicrobial effect against a variety of gram positive and gram negative pathogenic microorganisms and molds (Adiguzel et al. 2007; Razzaghi-Abyaneh et al. 2008).

Oregano and thyme, oregano with marjoram, and thyme with sage were the most effective EOs against pathogenic microorganisms. Phenolic compounds of spices and plants such as hydroquinone, thymol, carvacrol, BHA, as well as octyl gallate and tannic acid might be primarily responsible for bacteriocidal/bacteriostatic properties (Xia et al. 2011; Tajkarimi et al. 2010; Rua et al. 2010).

Tables 7.1, 7.2 and 7.3 presents the antimicrobial activities and components of various spices and herbs.

7.4.1 Mechanism of Action

The antimicrobial effect of EOs are basically demonstrated by causing structural and functional damages and altering the bacterial cell membrane, including the phospholipid bilayer as well as the toxicity caused by the optimum range of hydrophobicity. This antimicrobial function causes swelling and increases permeability, loosing cellular pH gradient (Mihajilov-Krstev et al. 2009; Serrano et al. 2011). Other mechanisms include disrupting enzyme systems, compromising the genetic material of bacteria, and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids. For example, tea tree oil changes the respiratory enzyme or metabolic event inhibition or leakage in potassium ions transfer. Mustard derived EOs showed multi-targeted mechanisms of action in metabolic pathways, membrane integrity, cellular structure and statistically significant higher release of the cell components of *Escherichia coli* O157:H7. Carvacrol extract increases the heat shock protein 60 HSP 60 (GroEL) and inhibits the synthesis of flagellin significantly in *E. coli* O157:H7. It has been suggested that essential oil constituents such as eugenol might inhibit the mechanism of virulence *agr* two-component system gene expression by interactive, hierarchical regulatory cascade among the *agr*, *sar*, and other regulatory gene products in *Staphylococcus aureus* (Qiu et al. 2010). Cytotoxic activity of cumin EOs might contribute to its antimicrobial effect (Allahghadri et al. 2010).

It has been well demonstrated that the antimicrobial efficacy of plant-origin antimicrobials depends on several factors including the EO extraction method, the inoculum volume, growth phase, culture medium used, and intrinsic or extrinsic factors of the food such as pH, fat, protein, water content, antioxidants, preservatives, incubation time/temperature, packaging procedure, and physical structure (Tajkarimi et al. 2010).For example, in galangal flowers, oven-dried samples extracted with ethanol was more effective compared to the freeze-dried samples extracted with

Table 7.3 Inhibitory activities of plant-origin antimicrobials against protein toxins and fungi, listed alphabetically representative studies conducted within the last 10 years

Organism	Adverse effects	Inhibitors	References
Botulinum	Neurotoxin botulism	Black tea	Tajkarimi et al. (2010)
Cholera	Cholera	Catechins, theaflavins	Tajkarimi et al. (2010)
Molds			
Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus	Myco-toxicosis, Ochratoxins	Pure essential oils, leaf oleoresin, leaf essential oil, eugenol, bark essential oil, bark oleoresin, E-cinnamaldehyde, cassia bark-derived substances, naringin, hesperidin and neohesperidin, and enzymatically-modified derivatives of these compounds, higher levels of phenolic compounds in thyme, cinnamon and clove, Cinammon oil, Methanol extract of *Aspilia mussambicensis* (Compositae), Guarana extract , EO and methanol extract of *Satureja hortensis, Satureja hortensis* L. containing carvacrol and thymol, water-distilled EO from leaves and flowers of *Micromeria nubigena* H.B.K. (Lamiaceae), oleuropein extracted from olive oil, EOs from thymol, cinnamic aldehyde and eugenol extracted from cinnamon and clove, *Thymus vulgari* and *Citrus aurantifolia*, *Mentha spicata* L., *Foeniculum miller*, *Azadirachta indica* A. Juss, *Conium maculatum* and *Artemisia dracunculus*, *Carum carvi* L.; linalool and methyl chavicol vanillin extracted from sweet basil vanilla, hydro-distilled EOs of stems, leaves (at vegetative and flowering stages) and flowers of *Eugenia chlorophylla* O. Berg. (Myrtaceae), thyme, oleoresin extracted from cinnamon and cinnamic aldehyde and eugenol extracted from cinnamon and clove , allyl isothiocyanate and citralon in mustard and lemongrass, marjoram oil, essential oil of *Ageratum conyzoides.* Thyme and rosemary EOs isolated from hazelnut. EOs of *Ageratum conyzoides* L. Ocimum sanctum essential oil. *Eruca sativa* (aerial and root). EOs of oregano (*Origanum vulgare* L.) = citrus (*Citrus sinensis* L. Osbeck) > savory (*Satureja thymbra* L.) > laurel (*Latin's nob ills* L.) > myrtle (*Myrtus communis* L.). EOs from peel of Ponkan (Citrus reticulata Blanco). Mexican oregano (Lippia berlandieri Schauer) essential oil added to amaranth, chitosan, or starch edible films. *Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.	Tajkarimi et al. (2010), Salas et al. (2011), Xia et al. (2011), Xing et al. (2010), Patil et al. (2010), Ozcakmak et al. (2010), Nogueira et al. (2010), Kumar et al. (2010), Khoobchandani et al. (2010), Gunduz et al. (2010), Gao et al. (2010), Avila-Sosa et al. (2010), and Abalaka et al. (2010)

(continued)

Table 7.3 (continued)

Organism	Adverse effects	Inhibitors	References
Fusarium semitectum		Naringin, hesperidin and neohesperidin, and enzymatically-modified derivatives of these compounds, higher levels of phenolic compounds in thyme, cinnamon and clove, methanol and hexane extracts of weedy plant species Vigna unguiculata and Amaranthus spinosus, allyl isothiocyanate and citralon in mustard and lemongrass, marjoram oil	Salas et al. (2011), Xia et al. (2011), and Thembo et al. (2010)
Penicillium expansum		Naringin, hesperidin and neohesperidin, and enzymatically-modified derivatives of these compounds , higher levels of phenolic compounds in thyme, cinnamon and clove, Cinammon oil, linalool and methyl chavicol vanillin extracted from sweet basil vanilla, hydro-distilled EOs of stems, leaves (at vegetative and flowering stages) and flowers of *Eugenia chlorophylla* O. Berg. (Myrtaceae), thyme, oleoresin extracted from cinnamon and cinnamic aldehyde and eugenol extracted from cinnamon and clove, allyl isothiocyanate and citralon in mustard and lemongrass, marjoram oil	Salas et al. (2011), Xia et al. (2011), Xing et al. (2010), and Avila-Sosa et al. (2010)
		Mexican oregano (*Lippia berlandieri* Schauer) EOs added to amaranth, chitosan, or starch edible films	

ethanol (Hsu et al. 2010). Other parameters such as lower pH level inside the bacterial cell such as *E. coli* and *Salmonella* are important. Gram negative bacteria are less sensitive to the plant origin antimicrobials because of the lipopolysaccharide outer membrane of this group, which restricts diffusion of the hydrophobic compounds (Tajkarimi et al. 2010). Gram positive bacteria are more sensitive to plant essential oils compared to gram negative bacteria (Wanner et al. 2010a, b).

Some spices have stronger antimicrobial activity than others. Generally, spices, herbs and their essential oils are used in food systems within the range of 0.05–0.1% (500–1,000 ppm). Application of higher amount of the spices of herbs raises concerns as it releases its aroma and taste into the food product. For example, there are concerns regarding the application of 1% oregano in food systems. Some studies suggest application of certain combinations of EOs in order to reduce the adverse sensory impact in food (Bassole et al. 2010). Different exposure methods of EOs also have effect on the mechanism of action. For example, application of mustard and clove EOs in vapor phase compared to the direct contact method, showed considerable difference in results. The slight modifications in stereochemistry, lipophilicity and other factors affected the biological activity of these compounds positively or negatively.

7.4.2 Synergistic and Antagonistic Effects of Components

Synergy is defined when the combined effect of substances is higher than the sum of the individual effects. When a combination shows less effect compared to the individual application, it means antagonism. Synergistic effects might happen by combination of different compounds and/or combination of compounds and techniques. The synergistic effect of different components and methods could offer a way to prevent possible off flavor caused by different plant origin materials, spices and herbs. Combination of a variety of plant extracts with nitrite ($NaNO_2$) shows stronger antimicrobial effect against *Clostridium botulinum* in meat without compromising the organoleptic properties (Cui et al. 2010). The synergic effect of some compounds such as organic acids and nicin, in addition to the major components in EOs, have been shown in some studies (Tyagi and Malik 2010; Tajkarimi et al. 2010; Tserennadmid et al. 2010). Combination of carvacrol- thymol and carvacrol-*p*-cymene can improve the efficacy of EOs against pathogenic microorganisms. Antimicrobial activity of a combination of cinnamon and clove EOs in vapor phase showed promising antimicrobial effect with less active concentration in the vapor phase compared to the liquid phase (Tyagi and Malik 2010; Tajkarimi et al. 2010). Oregano EOs showed higher antimicrobial activity in combination with low levels of sodium nitrite against *Clostridium botulinum* spores compared to sodium nitrite alone, depending on the number of inoculated spores. Combinations of EOs, oregano with thyme, oregano with marjoram and thyme with sage had the strongest effect against different pathogenic microorganisms (Gutierrez et al. 2008a). Synergistic and antagonistic effects of thymol and carvacrol have been observed in different combinations of cilantro, coriander, dill and eucalyptus EOs and mixtures of cinnamaldehyde and eugenol, against different pathogenic microorganisms (Tajkarimi et al. 2010). Chinese cinnamon and winter savory EOs were used successfully used to increase the radio sensitivity in ground beef. Combinations of EOs and nisin showed enhanced antimicrobial activity against *Listeria monocytogenes* (Tajkarimi et al. 2010). Combination of EDTA–lysozyme–rosemary oil and EDTA–lysozyme–oregano oil were most effective against the growth of Gram-negative, Gram-positive and to a lesser extent on yeasts on the shelf-life of semi cooked coated chicken fillets stored under controlled condition (Ntzimani et al. 2010). Synergic effect of compounds in combination with processing techniques such as vacuum packing, negative air ions, high-hydrostatic pressure and modified atmosphere packaging (MAP), increasing temperature from 8ºC to 30ºC and increasing light exposure to plants, showed interesting results against different pathogenic microorganisms with a variety of plant compounds such as thymol, carvacrol, Oregano, hexane, ethyl acetate, n-butanol. Sensitivity of antimicrobials increases by lowering the exposure of microorganisms to the available oxygen (Tajkarimi et al. 2010). Application of both extracts and EOs of plant-origin such as floral parts of *Nandina domestica* Thunb could be used as potential alternative for synthetic preservatives (Tajkarimi et al. 2010; Joung et al. 2010). Table 7.3 summarizes the results of various experiments regarding the application of phytochemicals against molds and yeasts.

7.5 Review of Some Findings of *In-Vitro* Experiments

In-vitro experiments of plant-origin antimicrobials are well described in the literature; however, the antimicrobial activity of herbs and spices might vary based on the tested organism.

Significant antibacterial activity was shown on ethanolic, methanolic and acetonic extracts by the *Calligonum Comosum*, a Medicinal Plant from Tunisia by the agar-well diffusion method against *Listeria ivanovii*. (Riadh et al. 2011). Cardiac glycosides, polyphenols, saponins and tannins presented in ethanolic extracts of leaves of two species of genus Ziziphus showed strong antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* at MIC 1 mg and 5 mg/l respectively (Abalaka et al. 2010). Phenolics and flavonoids present in pomegranate fruit peels demonstrated strong antimicrobial activity against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Yersinia enterocolitica*. The minimum inhibitory concentration (MIC) against Salmonella enteritidis was 4 mg/ml (Al-Zoreky 2009). Phenolic, flavonoid, carotenoid, terpenoid and triterpene present in Psidium guajava demonstrated strong antimicrobial activity (Gutierrez et al. 2008b). Antimicrobial activity of non-volatile ethanol extract of *Satureja montana* L. might be attributed to the presence of phenolic compounds such as carvacrol, carvacrol methyl ether, and thymol and terpenes (γ-terpinene) molecules (Serrano et al. 2011). Antimicrobial activity of phenolic compounds have also been demonstrated in several studies including edible seeds extract of Chinese Mei (Prunus mume Sieb. et Zucc) (Xia et al. 2011), different Argentinean green tea varieties (von Staszewski et al. 2011), *Piliostigma reticulatum* (DC.) Hochst extracts (Zerbo et al. 2010), soybean extracts enriched for phenolic content via dark-germination sprouting or solid-state bioprocessing by the dietary fungus Rhizopus oligosporus or Lentinus edodes (McCue et al. 2005), dark germinated fenugreek sprouts phenolic compounds (Randhir et al. 2004) and wood smoke (Niedziela et al. 1998).

Flavonoids, extracted from Citrus species, such as naringin, hesperidin and neohesperidin, and enzymatically-modified derivatives of these compounds, also demonstrated strong antifungal activity (Salas et al. 2011) Black raspberry and Chardonnay seed extracts showed antibacterial activity against *Escherichia coli* and growth inhibition against *Listeria monocytogenes,* under experimental conditions (Luther et al. 2007).

7.6 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 and might not have an impact on the bacterial growth in complex food systems (Schirmer and Langsrud 2010). The difference is mainly because only a small percentage of EOs is tolerable in food materials. Factors such as type, effects on organoleptic properties, composition/concentration and biological properties of the antimicrobial and the target microorganism, processing and storage conditions of the targeted food product are also important. *In vitro* experiments using microbiological medium are more common. Consequently, the effectiveness of EOs when applied in food has been less understood due to their unacceptable organoleptic properties (Romeo et al. 2010). Generally, higher concentrations of EOs are necessary in food, compared to *in-vitro* trials: for example, two-fold increase compare to laboratory medium in semi-skim milk, ten-fold in pork liver sausage, 50-fold in soup and 25- to 100-fold in soft cheese. Generally, effective EOs in decreasing order of antimicrobial activity is: oregano > clove > coriander > cinnamon > thyme > mint > rosemary > mustard > cilantro/sage. The antimicrobial potential has shown some inconsistencies in some experiments; for example in a study, mint showed lower antimicrobial effect compared to mustard (Tajkarimi et al. 2010).

Odors created mostly by high concentrations, and the cost of these materials are the two major issues restricting the application of plant-origin antimicrobials in food. However, there is promising news about the application of agricultural by-products such as the hulls of Antep pistachio (*Pistacia vera* L.) as an effective antimicrobial against *E. coli* O157:H7 and *Listeria monocytogenes* (Ozturk et al. 2010; Tajkarimi et al. 2010).

7.6.1 Meat and Poultry Products

Plant extracts are useful for reducing pathogenic microorganisms of meat origin however; some studies demonstrated low antimicrobial effects of plant origin antimicrobials against meat pathogens. The low antimicrobial effect might be caused by the interaction of meat fat and greater solubility of EOs in lipids compared to aqueous parts of food. For example, a combination of 1% clove and oregano in broth culture did not show similar inhibitory effect against *Listeria monocytogenes* in meat slurry. A 5–20 μl g^{-1} level of eugenol and coriander, clove, oregano and thyme oil inhibits the growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and autochthonous spoilage flora in meat products (Tajkarimi et al. 2010). According to Tables 7.1 and 7.2, in recent studies, certain oils such as eugenol existing in clove, oregano and thyme oils showed high effect against *Listeria monocytogenes*, *Aeromonas hydrophila* and autochthonous spoilage flora in meat products; however, mustard, cilantro, mint and sage oils were less effective or ineffective (Tajkarimi et al. 2010). Combination of water soluble extracts of oregano and cranberry, at a ratio of 50:50 and a concentration of 750 ppm, with 2% sodium lactate showed the best inhibitory effect against *Listeria monocytogenesis* in fresh and frozen meat and poultry (Apostolidis et al. 2008). Antimicrobial effect of Roselle (*Hibiscus sabdaridda* L.) against both susceptible and antibiotic-resistant Campylobacter strains were similar on *in-vitro* and in contaminated ground beef. Better EO delivery systems such as encapsulation showed better antimicrobial effect against *Listeria monocytogenes* in pork liver sausage (Tajkarimi et al. 2010; Ruiz et al. 2009).

Basil oil showed 3 log CFU reductions on *Salmonella enteritidis* population after 3 days of storage (Rattanachaikunsopon and Phumkhachorn 2010a). Combination of different methods such as cold temperature, pulsed light, high pressure, pulsed electric and magnetic fields, irradiation, or modified atmosphere packaging with the application of Winter savory (*Satureja montana*) EOs demonstrated to be economically reasonable as a natural antibacterial substance to control the growth of food-borne bacteria and improve the quality of minced pork. Radiation at 25 kGy in a cobalt-60 irradiator did not show changes in antimicrobial effect of *Glycyrrhiza glabra* roots (Khattak and Simpson 2010). Aqueous extract of rosemary, sage and thyme inhibited the rancidity of heat-treated turkey-meat products. Milk protein-based, chitosan, or starch edible films containing oregano, pimento, or oregano and pimento was effective against *Escherichia coli* O157:H7 or *Pseudomonas* spp. (Tajkarimi et al. 2010; Avila-Sosa et al. 2010; Aider 2010). Preservation methods using a combination of different techniques and natural plant antimicrobials for fresh poultry meat have been successfully conducted (Ntzimani et al. 2010). Chlorophyll-containing films were successfully tested against *Staphylococcus aureus* and *Listeria monocytogenes*. Marjoram (*Origanum majorana* L.) EO in fresh sausage was effective against several species of bacteria. *Staphylococcus aureus* and *Listeria monocytogenes* in cooked frankfurter were successfully reduced by using Chlorophyllin-gelatin films and coating applications. *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens* and *Lactobacillus sake* in modified atmosphere-packaged fresh pork and vacuum-packaged ham slices stored at 4ºC were reduced using individual extracts of clove, rosemary, cassia bark and liquorice. Clove and tea-tree oils showed potential bio-preservative capabilities to control *Escherichia coli* O157:H7 on blanched spinach and minced cooked beef (Tajkarimi et al. 2010). Methanol leaf extract of *Salvia leriifolia* successfully reduced *Staphylococcus aureus* numbers in hamburger (Mehr et al. 2010).

7.6.2 Sea Food

The antimicrobial effect of EOs against various microorganisms in foods containing high fat, such as meat and some fish products is low; however, some of the EOs such as oregano oil showed effective antimicrobial effect against the spoilage organism *Photobacterium phosphoreum* on cod fillets, which is a fatty fish. Oregano also showed strong antioxidant activity. *Salmonella enteritidis*, *Listeria monocytogenes* and the natural spoilage flora were inhibited using EOs on the surface of whole fish or as coating for shrimps. Wild thyme (*Thymus serpyllum*) application in fresh water fish significantly increased the shelf-life by about 15–20 days. Combined application of carvacrol + thymol with some other additives extended the shelf-life of carp fillets. Thymol, carvacrol and cinnamaldehyde, isoeugenol, eugenol, garlic oil, and citral increased the shelf life of carp fillets. *Aloysia sellowii* EO in brine shrimp was successfully applied against a variety of Gram-positive and -negative bacteria and yeasts. 0.5% eugenol plus 0.5% linalool contributed to the freshness sensation of tuna slices (Tajkarimi et al. 2010). Addition of 0.05% (v/v) thyme oil to packed sea bass could significantly reduce bacterial growth during storage (Schirmer and Langsrud 2010). 1% and 2% clove oil caused a reduction between 1 and 4 $\log_{10}$ CFU/g in *Listeria monocytogenes* on Salmon fish (Miladi et al. 2010). Pre-treatment of mackerel fillets with diluted quince-polyphenolic extract might be used to inhibit spoilage bacteria (Fattouch et al. 2008). One percent cinnamaldehyde or 1% carvacrol showed a 5 $\log_{10}$ CFU/g reduction in microbial populations of tested oysters (Ravishankar et al. 2010). *Vibrio parahaemolyticus* contamination risk has been successfully minimized using allspice, basil, clove, garlic, horseradish, marjoram, oregano, rosemary, and thyme and hurdle technology. A synergistic effect of treatment with anodic electrolyzed NaCl solution, combined with eugenol and linalool, was found to enhance the shelf-life of coated semi-fried tuna (Tajkarimi et al. 2010). Complex gelatin-chitosan film incorporating clove EO in packaged fish developed effective antimicrobial effect below detection limits after 6 days, especially for *Enterobacteriaceae* (Gomez-Estaca et al. 2010). Chitosan is desired in liquid and solid foods due to its convenience as an antimicrobial and an antioxidative-preservative and its stability at pH 6 (Friedman and Juneja 2010; Fernandez-Saiz et al. 2010a, 2010b; Diaz-Visurraga et al. 2010).

7.6.3 Dairy Products

The application of EOs in milk is positively affected by higher water activity in milk. Remarkable antimicrobial activity against *Escherichia coli* and extended shelf-life of pasteurized cow's milk was shown using the extract of mango seed kernel.

The botanical composition of meadows on the sensory properties of terpenes in pressed cheeses was not or only marginally involved. Natural antioxidant and aroma properties were shown using *Satureja cilicica* EO in dairy products. Some EOs such as cinnamon, cardamom and clove oils inhibit the growth of yogurt starter cultures; however, mint oil was effective against *Salmonella enteritidis* in low-fat yogurt. *Salmonella enteritidis* was inhibited using clove oil in full-fat cheese (Tajkarimi et al. 2010). Allyl isothiocyanate isolates from plants showed 3.6 log CFU reduction in yeasts and molds counts in mozzarella packed with the antimicrobial sachet over 15-day storage time (Pires et al. 2009). Application of bay, clove, cinnamon and thyme showed 1 log CFU reduction on *Listeria monocytogenes* in low-fat cheese. However, 1% clove oil showed similar reduction effect on full-fat soft cheese against *Listeria monocytogenes* at cold storage temperatures over a 14-day period (Smith-Palmer et al. 2001). In another study , a mixture of plant essential oils at 2,500 ppm showed bacteriostatic effect against *Listeria monocytogenes* and ineffective against *E. coli* O157:H7 (MendozaYepes et al. 1997). In fermented products such as Ayran, similar responses were obtained using mint, thyme, garlic, salt and their mixture and non treated product against *E. coli* O157:H7 (Simsek et al. 2007). However, combined techniques of High pressure-processing with mint essential oil appeared to be a promising technique to preserve Ayran (Evrendilek and Balasubramaniam 2011).

7.6.4 Fruits and Vegetables

Low fat content, accompanied by lower pH and/or temperature of these products enable more successful application potential for EOs as antimicrobials. For example, oregano oil was effective against *Escherichia coli* O157:H7 in eggplant salad and Cinnamaldehyde and thymol were effective against six *Salmonella* serotypes on alfalfa seeds (Tajkarimi et al. 2010). Carvacrol and cinnamaldehyde effectively inhibited the natural microflora of kiwi fruits (Tajkarimi et al. 2010). Post-harvest fungal disease caused by *Botrytis cinerea* in stored grape was reduced effectively by using natural fungicidal plant EOs. Alginate-based edible coating of fresh-cut Fuji apples using EOs showed more than 4-log $_{10}$CFU/ml reduction in the population of *E. coli* O157:H7 and a total inhibition of native microflora for 30 days at 5°C (Khwaldia et al. 2010). Promising antimicrobial and quality effects on fresh-cut melon was shown using Cinnamon, clove, and lemongrass EOs and their active compounds. 40 ppm cinnamaldehyde with 40 ppm of eugenol or 80 ppm eugenol preserved apple juice for 7 days. *Alicyclobacillus acidoterrestris* was inhibited with more acceptable results in the test panels using a combination of cinnamaldehyde and eugenol in apple juice. The key antimicrobial and antioxidant component for fresh-cut apple and salads were polyphenols which are present in green, white and commercial tea (Tajkarimi et al. 2010; Chiu and Lai 2010). Sumac (*Rhus coriaria* L.) water extract and oregano oil suspension on tomato surfaces significantly reduced *Salmonella* Typhimurium populations without affecting the sensory properties of tomatoes (Gunduz et al. 2010).

7.6.5 Cereals

Alkaloids, tannins and cardiac glycosides found in lemon grass powder and essential oil is believed to have preservative and antimicrobial effects in maize and cowpea samples against moulds like *Aspergillus flavus, A. fumigatus, Microphomina phaseoli and Penicillium chrysogenum* and bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Ps. fluorescens, and Bacillus subtilis* (Adegoke and Odesola 1996). Antifungal and antiaflatoxigenic activity of *Pimpinella anisum* L. (anise), *Peumus boldus* Mol (boldus), *Hedeoma multiflora* Benth (mountain thyme), *Syzygium aromaticum* L. (clove), and *Lippia turbinate* var. integrifolia (griseb) (poleo) essential oils (EOs) at 2,000 and 3,000 μg/g has been demonstrated (Bluma and Etcheverry 2008). Three stored-rice pests (*Sitophilus oryzae*, *Rhyzopertha dominica* and *Cryptolestes pusillus*) were reduced using leaves of five different varieties of *Ocimum basilicum*. *Bacillus cereus* in rice was reduced using sage oil and carvacrol. Two major seed-borne fungi of rice was reduced using *Ocimum gratissimum* and *Thymus vulgaris* (Tajkarimi et al. 2010). It has been suggested that cinnamon and oregano oils could be effective in controlling the growth of Fumonisin B1 production in preharvest conditions (Velluti et al. 2003). However, edible bamboo shoots in Korea were able to inhibit bacterial growth (Park and Jhon 2010).

7.6.6 Animal Feed

Considering restrictions on the application of antibiotics and growth promoters especially in European countries, significant increase in EO use were reported in farm animal feeds. Carvacrol-rich and some other EOs have shown inhibitory effect against *Clostridium perfringens* and necrotic enteritis in poultry. Digestion improvement in ruminants, and improving the stability and palatability of animal feed, were some positive impacts of using EOs for animal health (Franz et al. 2010). Bacterial communities in animal feed were affected by the presence of thymol. There is limited effect on nutrient utilization using EO compounds in alfalfa and corn silage. However, cinnamon leaf oil showed inhibition effects on the total volatile fatty acid (VFA) concentration and prevented adverse effects on metabolism and increased the productivity of ruminants (Tajkarimi et al. 2010). Oregano oil administration at 0.5% ZnO level in feed did not prevent diarrhea in weanling pigs (Henn et al. 2010).

7.7 Conclusions

Plant-origin antimicrobials are present at various levels and at different degrees of effectiveness in a variety of plants, spices and herbs. These compounds can naturally improve shelf life of food products.

Parameters such as low pH or modifying physical conditions such as vapor phase, may improve the inhibitory effects of EOs that might be a because of direct result of acidity or interaction of EOs with the lipid phase of the affected bacterial membrane (Tyagi and Malik 2010; Tajkarimi et al. 2010). Application of EOs in vapor phase, in combination with other techniques showed successful result for antimicrobial packaging development. Direct application of plant essential oils onto food packaging and developing active form of packaging are approaches (Ojagh et al. 2010). Phytochemicals such as eugenol could be used as a structural model for developing antimicrobial agents aimed at the bacterial virulence factors (Qiu et al. 2010). Majority of plant origin antimicrobials need to be addressed by regulatory authorities for most parts of these compounds. The US regulatory agencies have recognized EOs of cinnamon, clove, lemon grass and their respective active compounds (cinnamaldehyde, eugenol and citral, respectively) as generally recognized as safe (GRAS). However, some materials, such as thymol, have not been recognized as food-grade additives by European legislators. Carvacrol, carvone, cinnamaldehyde, citral, *p*-cymene, eugenol, limonene, menthol and thymol have been registered and recognized as safe-to-use materials in the EU countries (Tajkarimi et al. 2010; Qiu et al. 2010). Risk assessment of the effect of high doses of some EOs on intestinal cells should be considered seriously. Application of these compounds in the food industry needs further investigation from a synergism and antagonism point of view. Determination of EOs for target and effective range including MIC and safety data (toxicity, allergenicity) in food materials is a necessary requirement. Several successful application of EOs in conjunction with hurdle technology and modified atmosphere packaging created pleasant odor with longer shelf life. Other possible ways to reduce the organoleptic impact include: Minimizing perception of the presence of plant origin compounds in food by optimizing food formulation; Application of combined methods; Enhancing a calibrated vapor pressure capacity in order to increase the interaction between EO and the bacterial cell membrane.

Several studies showed higher antimicrobial activity of whole EOs present in plants compared to selective components and information on the effects of crude extracts of plant origin compounds against food-borne microorganisms is limited. Future studies are required to conduct a more in-depth review for individual, and in combination application of EOs with extracts from other parts of the plant, other effective EOs and other food-processing techniques.

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