Antimicrobials from herbs, spices, and plants

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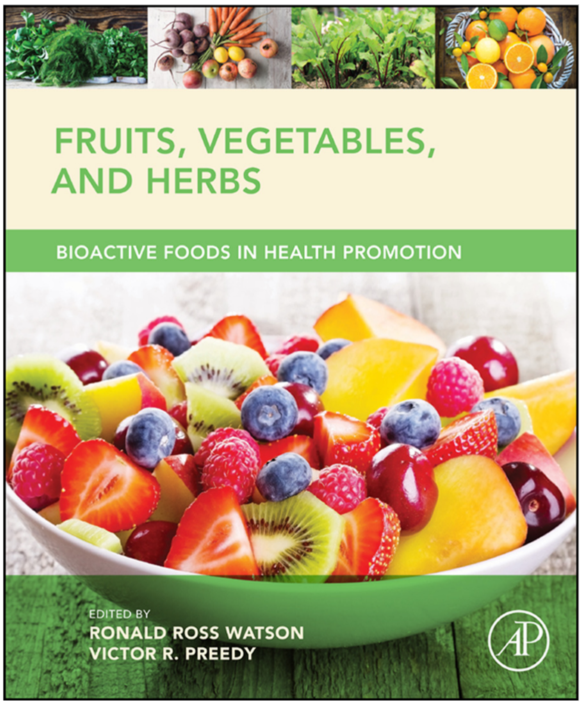

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Chapter 26

Antimicrobials from herbs, spices, and plants

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CHAPTER OUTLINE

INTRODUCTION

Consumers increasingly demand high quality, safe food. Due to the increase in the number of foodborne outbreaks caused by pathogen microorganisms, there has been a corresponding increase in concerns over food safety. Consumers are especially concerned about the use of chemical and artificial antimicrobial compounds and preservatives that inhibit the growth of pathogens (Tajkarimi et al., 2010). Consumers' demand for maintaining nutrition and quality while continuing to provide food safety has increased the need for using alternative preservation methods to inactivate pathogen microorganisms and enzymes in food products. Foods preserved with natural additives have become popular. As a result, new methods for using natural preservatives obtained from plants, animals, or microflora have come along (Holley and Patel, 2005; Tiwari et al., 2009).

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Natural antimicrobials are now gaining attention as a way to control microorganisms. These antimicrobials play an important role in food control by preventing microbial contamination by pathogens and by extending shelf life due to the removal of undesirable pathogens. The antibiotic resistance of pathogens can also be decreased and the immune system can be boosted through the use of natural antimicrobials (Tajkarimi et al., 2010). There are two major reasons for using antimicrobials in food models: to prevent natural spoilage (preservation) and to control the growth of microorganisms (safety). Animals, plants, and microorganisms are the major sources of natural antimicrobials. In addition to their seasoning benefits, some herbs and spices have antimicrobial effects against human and plant pathogens (Brandi et al., 2006). Food processing techniques that only employ chemical preservatives are not completely successful because the chemicals do not totally kill foodborne pathogens or stop microbial spoilage (Gutierrez et al., 2009). Recent food preservation methods such as pulsed light, high pressure pulsed electric and magnetic fields have been used for controlling pathogen and spoilage microorganisms in food (Tajkarimi et al., 2010). In this chapter, we review the antimicrobial properties of different types of herbs, spices, and their extracts. Major antimicrobial compounds of herbs and spices and their chemical composition are also discussed. The use of plant antimicrobials against pathogens in liquid and solid food models is also highlighted.

Herbs, spices, and plant extracts

Herbs and spices have been used as food additives all over the world. Not only do they improve the organoleptic properties of food, they can also increase the shelf life of food by reducing or entirely removing the number of foodborne pathogens (Lai and Roy, 2004).

Plant-derived natural antimicrobials have actually long been used for the preservation of foods. Some spices and essential oils (EOs) were used by the early Egyptians and have been used for centuries in China and India. Spices such as clove, cinnamon, mustard, garlic, ginger, and mint are still used as alternative health remedies. Spices and plant extracts have antimicrobial effects against Gram-positive pathogen microorganisms such as *Listeria monocytogenes*. These antimicrobial compounds can increase storage time and stability with active ingredients such as phenols, alcohols, aldehydes, ketones, ethers, and hydrocarbons, specifically in cinnamon, clove, garlic, mustard, and onion. The first scientific study on preservative effects of spices was conducted in the 1880s and showed the antimicrobial effect of cinnamon oil against *Bacillus anthracis* spores (Tajkarimi et al., 2010).

The majority of herbs and spices exerts antimicrobial activity against different bacteria, yeasts, and molds (Tajkarimi et al., 2010; Friedman et al., 2004, 2002; Raybaudi-Massilia et al., 2008). Phenolic compounds obtained from herbs and spices show biological activity and can be potentially used as food preservatives (Lai and Roy, 2004).

The extract of *Capsicum annuum* bell pepper prevented the growth of *Salmonella typhimurium* at 1.5 mL/100 g concentration in minced beef. For *Pseudomonas aeruginosa*, bell pepper extract showed bacteriostatic effect at 0.3 mL/100 g and bactericidal effect at 3 mL/100 g concentrations (Careaga et al., 2003). The inhibitory effects of the hydrosols of thyme, black cumin, sage, rosemary, and bay leaf against *S. typhimurium* and *Escherichia coli* O157:H7 were shown in carrots and apples. Thyme hydrosol had the highest antibacterial effect on both *S. typhimurium* and *E. coli* O157:H7 counts (Tornuk et al., 2011).

Different types of spices were grouped based on their antimicrobial activities as strong (cinnamon, clove, mustard), medium (allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage, thyme), and weak (black pepper, red pepper, ginger) (Zaika, 1988). Ethanol and hexane extracts of oregano, clove, sage, rosemary, and celery exerted relatively strong antimicrobial activities against *E. coli, Listeria innocua, Staphylococcus aureus*, and *Pseudomonas fluorescens*, but water extracts showed weak or no antimicrobial activity (Witkowska et al., 2013). Aqueous extracts of *Mentha* spp. have antimicrobial activities against *Bacillus fastidiosus*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella choleraesuis*, *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *Serratia odorifera* at different concentrations (1:1, 1:5, 1:10, and 1:20) (Al-Sum and Al-Arfaj, 2013).

Green tea was particularly effective against *Bacillus cereus, S. aureus*, and *L. monocytogenes*, and rosemary had a strong inhibitory effect against *B. cereus* and *S. aureus* in tryptic soy broth (TSB). When green tea or rosemary was added to rice cakes at 1–3% concentration, the growth of *B. cereus* and *S. aureus* was significantly reduced in rice cakes after 3 days of storage at 22°C (Lee et al., 2009). Mixed extracts of *Scutellaria, Forsythia*, honeysuckle, cinnamon, and rosemary and clove oil showed strong antimicrobial effects in vacuum-packaged fresh pork from 1.81 to 2.32 log reductions in *E. coli*, *P. fluorescens*, and *Lactobacillus plantarum* counts during 28 days of storage when compared with the control (Kong et al., 2007).

Rosemary extracts and dry powders of orange and lemon were effective in controlling bacterial spoilage during 12 days of storage at 8°C (Fernandez-Lopez et al., 2005). Activin (grape seed extract) and Pycnogenol (pine bark extract) can be used as food additives to maintain the quality and safety

of cooked beef (Ahn et al., 2007). Green, jasmine, and black tea aqueous extracts suppress the growth of *S. aureus* by 5.0 log CFU/mL and *L. monocytogenes* by 3.0 log CFU/mL in brain heart infusion broth (Kim et al., 2004).

The plants *Pelargonium purpureum* and *Sideritis scardica* exerted the strongest antimicrobial effects against *E. coli* O157:H7 NCTC 12900, *Salmonella enteritidis* PT4, *S. aureus* ATCC 6538, *L. monocytogenes* ScottA, *Pseudomonas putida* AMF178, and *B. cereus* FSS134 but especially on *E. coli* and *L. monocytogenes* (Proestos et al., 2013). The methanol extract of *Peganum harmala*, the EO of *Satureja bachtiarica*, the ethanol extract of *Juglans regia*, and *Trachyspermum copticum* with MIC of 105, 126, 510, and 453 μg/mL, respectively, are the most active plant extracts against *Lactococcus garvieae* from diseased *Oncorhynchus mykiss*, whereas some extracts such as *Quercus branti* Lindley and *Glycyrrhiza glabra* L had lower activity against *L. garvieae* with MIC values of 978 and 920 μg/mL, respectively (Fereidouni et al., 2013).

The addition of 5% olive or apple skin extracts reduced *E. coli* O157:H7 populations to below the detection limit and by 1.6 log CFU/g, respectively, and 1% lemongrass oil reduced *E. coli* O157:H7 to below detectable limits, whereas clove bud oil reduced pathogen bacteria by 1.6 log CFU/g. The formation of carcinogenic heterocyclic amines was reduced with the addition of olive and apple extracts by 76–85% and from 35% to 53% with clove bud oil (Rounds et al., 2012).

The aqueous extracts of *Psidium guajava*, *Citrus limonium*, *Allium sativum*, and *Zingiber officinale* were found to be active against *S. aureus*, *E. coli*, *Bacillus subtilis*, *P. aeruginosa*, and *Salmonella* species. Guava leaves had a stronger antibacterial effect against *B. subtilis* whereas *S. aureus* was inhibited more effectively with garlic cloves. Lemon leaves extract and juice inhibited the growth of *P. aeruginosa* and *E. coli*, respectively, to a high degree (Kumar et al., 2012b).

Cinnamon, clove, star anise, picklyash peel, and common fennel water extracts were mixed with chitosan at 0.2, 3.0, 0.7, 2.2, and 0.6 g/L concentrations, respectively, to design a new food preservative formula. Chilled steak samples treated with this preservative formula remained fresh after 9 days of storage (Wang et al., 2012).

Rosemary, sage, peppermint, and spearmint inhibited the growth of *B. cereus*, *Micrococcus luteus*, and *S. aureus*. Rosemary and sage showed stronger antibacterial effect than green and black teas of *Camellia sinensis* (Chan et al., 2012). *Laurus nobilis* (L.), *Rosmarinus officinalis* (L.), *Equisetum arvense* (L.), *Lavandula officinalis* (L.), and *Lavandula*

stoechas (L.) leaves also showed antibacterial activity. Only aqueous extracts of *L. nobilis* showed anticandidal activity. The most sensitive bacterium was *S. aureus* and the most resistant one was *B. cereus* (Ceyhan et al., 2012).

The antimicrobial activity of cinnamon, clove, and mustard against mycotoxigenic *Aspergillus parasiticus*, *Salmonella enterica*, *L. monocytogenes*, *E. coli*, *Shigella sonnei*, *Shigella flexneri* was shown (Lai and Roy, 2004), and the application of garlic-derived organosulfur compounds in meat could enhance microbial safety (Yin and Cheng, 2003).

Clove was used to be use to prevent spoilage in meat, sirups, sauces, and sweetmeats. Cinnamon and mustard were identified as preservatives in applesauce in the 1910s. It has been reported that allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage, and thyme have strong bacteriostatic effects (Tajkarimi et al., 2010). EOs extracted from plants and spices have an antimicrobial effect against *E. coli* O157:H7, *S. typhimurium*, *Shigella dysenteriae*, *L. monocytogenes*, *B. cereus*, and *S. aureus* at concentrations between 0.2 and 10 μL/mL (Burt, 2004). Two percent of citric acid or up to 0.1% of cinnamon bark oil added into tomato juice and then treated with high intensity pulsed electric fields (HIPEF) caused a 5-log or greater reduction (Mosqueda-Melgar et al., 2008a,b).

In broth model systems 0.25–1.0% clove and garlic showed bacteriostatic and bactericidal activities against *E. coli* O157 and *S. enterica* serovar Enteritidis. Garlic at a 1% concentration reduced the viable cells of *S. enteritidis* in mayonnaise (Leuschner and Zamparini, 2002). The addition of fresh garlic (30 g/kg) or garlic powder (9 g/kg) into raw chicken sausage significantly reduced the aerobic plate counts and extended the shelf life of the product to 21 days (Sallam et al., 2004). Fresh garlic extract combined with ampicillin or ciprofloxacin can synergistically increase the zone of inhibition (Ankri and Mirelman, 1999; Gaekwad and Trivedi, 2013).

Allicin, an active compound, extracted from fresh garlic, in its pure form exerts antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including drug-resistant Enterotoxigenic *E. coli* strains, antifungal (*Candida albicans*), antiparasitic (*Entamoeba histolytica* and *Giardia lamblia*), and antiviral activities (Ankri and Mirelman, 1999). Aqueous extract of garlic and garlic shoot juice in combination with nisin showed synergistic bactericidal activity against nisin resistant *L. monocytogenes* (Kim et al., 2008; Singh et al., 2001).

Turmeric (*Curcuma longa* L.) is a tropical herb which belongs to the Zingiberaceae family and is indigenous to southern Asia. Turmeric is used

in foods as a condiment and in medicine as a carminative, anthelmintic, laxative and as a cure for liver ailments (Negi et al., 1999). The ethanolic extract of turmeric showed high antimicrobial activity at 3.11 and 5.65 mg gallic acid/mL extract against *E. coli*, *S. aureus*, *Saccharomyces sake*, and *Aspergillus oryzae* (Falco et al., 2011).

The total six extracts of black pepper (*Piper nigrum*) and turmeric (*C. longa*) showed antibacterial activity against *B. subtilis*, *Bacillus megaterium, Bacillus sphaericus, Bacillus polymixa, S. aureus, E. coli* and 11 molds, *Aspergillus luchuensis, A. flavus*, *Penicillium oxalicum*, *Rhizopus stolonifer*, *Scopulariopsis* sp., *Mucor* sp. (Pundir and Jain, 2010).

The ethanolic extracts of unripe banana, lemongrass, and turmeric were effective against *E. coli* ATCC25922, *E. coli*, *P. aeruginosa*, *Salmonella paratyphi*, *S. flexneri*, *K. pneumoniae*, *S. aureus*, *S. aureus* ATCC 25921, and *B. subtilis*. MIC ranged from 4 to 512 mg/mL while MBC ranged from 32 to 512 mg/mL depending on bacterial isolates and extracting solvent. *S. aureus* ATCC 25921 was killed in less than 2 h with unripe banana extract, *E. coli*, in less than 3 h with turmeric, *S. paratyphi* (Fagbemi et al., 2009), in just over 3 h with lemongrass.

Hexane extract eluted with 5% ethylacetate was most active fraction of curcumin, the yellow color pigment of turmeric, against *B. cereus*, *Bacillus coagulans*, *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* (Negi et al., 1999). Turmeric extract (1.5%, v/v) alone or combined with shallot extract (1.5%, v/v) enhanced quality characteristics and extended the shelf life of vacuum-packaged rainbow trout (*O. mykiss*) during 20 days of storage at 4±1°C (Pezeshk et al., 2011).

Cinnamon is extracted from cinnamon bark, fruit, leaf, and their EOs and many *Cinnamomum* species produce a volatile oil on distillation with different compositions and aroma characteristics (Jayaprakasha et al., 1997; Kaul et al., 2003; Negi, 2012). Bioactive fraction obtained from fruits of *Cinnamomum zeylanicum* has been used as antibacterial agent (Jayaprakasha et al., 2003). Oil of cinnamon (*C. zeylanicum*) was highly effective against *Bacillus* sp., *L. monocytogenes*, *E. coli*, *Klebsiella* sp., and the fungus *Rhizomucor* sp. (Gupta et al., 2008).

Extract of *Cinnamomum cassia* Blume (cassia bark, Chinese cinnamon) bark exerted antibacterial activity (7–29 mm/20 µL inhibition zone) against 13 bacterial species by in vitro agar diffusion method. Alcohol extracts showed a 7 mm/20 µL inhibition zone against *B.* megaterium and *Enterococcus faecalis.* Alcohol extracts of *Pimpinella anisum* (L.) (anise, aniseed) seeds were effective against *M. luteus* and *Mycobacterium*

smegmatus (8 mm/20 μL inhibition zone), and *G. glabra* root extract showed various antibacterial activities (7–11 mm/20 μL inhibition zone) against tested microorganisms (Ates and Erdogrul, 2003).

The antibacterial activity of EOs extracted from nutmeg, mint, clove, oregano, cinnamon, sassafras, sage, thyme, or rosemary was determined against *B. cereus* INRA L2104 at different temperatures. Tyndallized carrot broth was used as a food model and the addition of cinnamon EO at 0.05% at refrigeration temperature (≤8°C) inhibited the growth of tested strain for at least 60 days in tyndallized carrot broth model (Valero and Salmeron, 2003). The mixture of Chinese chives (*Allium tuberosum*), cinnamon (*C. cassia*), and corni fructus (*Cornus officinalis*) extracts exhibited better inhibitory effects on the growth of *E. coli* than potassium sorbate at 2–5 mg/mL and inhibited growth of *Pichia membranaefaciens* at levels as low as 2 mg/mL concentration. The antimicrobial activity of Eos from corni fructus, cinnamon and Chinese chive was slightly enhanced with the addition of food additives such as polyphosphate and butylated hydroxyanisole (BHA). This mixed extract was effective in several food models including orange juice, guava juice, pork, milk and tea (Hsieh et al., 2001; Mau et al., 2000).

The antimicrobial activity of polyphenols, tannins, and flavonoids is well known. Pomegranate (*Punica granatum* L.) is rich in tannins which show strong antimicrobial activity. Fruits, peels, leaves, flowers, seeds, and roots of pomegranate have been widely used in herbal remedies and medicine in many countries (Negi, 2012; Al-Zoreky, 2009). The 80% methanolic extract of pomegranate peels had an inhibitory effect against *L. monocytogenes*, *S. aureus*, *E. coli*, and *Yersinia enterocolitica* and showed a more than 1 log reduction of *L. monocytogenes* in fish during refrigerated storage (Al-Zoreky, 2009). Pomegranate fruit fractions showed antimicrobial activity (inhibition zone) against *S. aureus* and *P. aeruginosa* (Opara et al., 2009). Pomegranate aril extracts had antimicrobial effects on *B. megaterium* DSM 32, *P. aeruginosa* DSM 9027, *S. aureus* Cowan 1, *Corynebacterium xerosis* UC 9165, *E. coli* DM, *E. faecalis* A10, *M. luteus* LA 2971, and three fungi, *Kluyveromyces marxianus* A230, *Rhodotorula rubra* MC12, and *C. albicans* ATCC 1023 with 30–>90 μg/mL MIC values (Duman et al., 2009). Pomegranate extract at 1% (v/v) eliminated the growth of *S. aureus* FRI 722 and inhibited enterotoxin (SE) production at 0.05% (v/v) concentration (Braga et al., 2005). The addition of pomegranate peel extract to chicken meat products extended their shelf life by 2–3 weeks during chilled storage (Kanatt et al., 2010).

Garcinia kola, commonly known as Bitter Kola, is a medium-sized tree. Different species of *Garcinia* possess antimicrobial, antioxidant,

antitumorogenic, and cytotoxic activities through their secondary metabolites (Negi, 2012; Akerele et al., 2010). The crude ethanol extract of *G. kola* Heckel showed inhibitory activity against clinical bacterial isolates of *S. aureus*, *B. subtilis*, *Streptococcus viridians*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* and fungi like *Penicillium notatum*, *Aspergillus niger*, and *C. albicans*. The MIC values ranged between 2.5 and 7.5 mg/mL (Akerele et al., 2010).

Compounds extracted from *Garcinia brasiliensis* which are active against *S. aureus* and *B. cereus* were pericarp hexane extract 4.0 and 2.4 μg/mL and seed ethanol extract 10.0 and 12.6 μg/mL, respectively (Naldoni et al., 2009). Methanolic crude extract of the stem bark of *Garcinia lucida* Vesque exerted good inhibitory effect against *C. albicans* (MIC value was 64 μg/mL). Only cycloartenol obtained from dichloromethane fraction exhibited antimicrobial activity against *E. coli* and *P. aeruginosa* (Momo et al., 2011).

Pericarp extract of *Garcinia mangostana* Linn. (mangosteen) was effective against *S. aureus*, *Staphylococcus albus*, and *Micrococcus lutus* (Priya et al., 2010). The crude methanolic extracts of *Garcinia atroviridis* had an inhibitory effect against *B. subtilis* B28 mutant, *B. subtilis* B28 wild type, methicillin resistant *S. aureus, E. coli*, and *P. aeruginosa* UI 60690 at a minimum inhibitory dose of 15.6 μg/disc (Mackeen et al., 2000). The ethanol extracts of *G. kola* produced inhibition zones ranging from 8 to 18 mm against *S. aureus, P. aeruginosa, E. coli*, and *C. albicans* (Ezeifeka et al., 2004). *G. mangostana* had a strong inhibitory effect against *Propionibacterium acnes* and *Staphylococcus epidermidis* and can be used as an alternative treatment for acne (Chomnawang et al., 2005). The natural xanthones isolated from the fruit hulls of *G. mangostana* showed good inhibitory activity against phytopathogenic fungi, *Fusarium oxysporum vasinfectum*, *Alternaria tenuis*, and *Dreschlera oryzae* (Gopalakrishnan et al., 1997). The MIC exerted by the seed extracts of *G. kola* against *E. coli*, *S. aureus*, and *P. aeruginosa* ranged between 3.125 and 25 mg/mL and inhibition zones were between 4.0 and 10.5 mm. Crude ethanol extract was found to be most effective against all bacteria at different treatment regimens (Ghamba et al., 2012).

The antimicrobial activities of seven Turkish spice [cumin, *Helichrysum compactum* Boiss (HC), laurel, myrtle, oregano, sage, and thyme] methanolic extracts were investigated against *E. coli* O157:H7. Thyme and oregano showed higher inhibitory effects and their antibacterial effects varied in proportion to the concentration of extracts used (Sagdic et al., 2002). High concentrations of rosmarinic acid in methanolic extract of

Orthosiphon stamineus were closely related with the antibacterial activity of plant extract against *Vibrio parahaemolyticus*, and a similar inhibitory effect was obtained with 5% lactic acid (Ho et al., 2010).

Tea (*C. sinensis*) is a popular nonalcoholic beverage consumed by over two-thirds of the world's population due to its taste and medicinal properties. Among all tea polyphenols, epigallocatechin-3-gallate is responsible for antiinflammatory, antimicrobial, antitumor, antioxidant activities and protection from cardiovascular disease, antiobesity, and antiaging properties. Tea and its polyphenols possess antibacterial activity against *S. aureus*, *E. coli*, *Helicobacter pylori*, *Bacillus* spp., *Clostridium* spp., *Streptococcus* spp., and other bacteria, antiviral activity against influenza virus, human immunodeficiency virus (HIV), Epstein-Barr virus, Hepatitis B virus, herpes simplex virus (HSV), and other viruses, and antifungal activity against *C. albicans* and other fungi (Bansal et al., 2013). Methanolic extracts of green tea (*Camellia assamica*) at 10, 20, and 30 μL concentrations had significant antibacterial activity against *Staphylococcus* sp., *Streptococcus* sp., and *Bacillus* sp., and they were least active against *Pseudomonas* sp. and *Proteus* sp. (Kumar et al., 2012a). Ethanolic extract from green tea had potent antimicrobial activity against *Streptococcus mutans*, *Streptococcus sobrinus*, *L. monocytogenes, S. flexneri*, and *S. enterica* (Oh et al., 2013).

The green tea kombucha exhibited the highest antimicrobial activity against *S. epidermidis* (22 mm), *L. monocytogenes* (22 mm), and *M. luteus* (21.5 mm). The anticandidal activity of green tea kombucha was revealed by the reaction against *Candida parapsilosis* (Battikh et al., 2013). Aqueous green tea leaf extract was effective against *S. epidermidis*, *P. fluorescens*, *M. luteus*, *Brevibacterium linens*, and *B. subtilis* by disc diffusion assay (zone of inhibition ≥7 mm). MIC was determined via nitro blue tetrazolium assay (0.156–0.313 mg/mL). The extract was not toxic to Vero cell line up to a concentration of 500 μg/mL (Sharma et al., 2012).

Incorporation of green tea extract into chitosan film enhanced the antimicrobial activity of the film and therefore maintained the sensory quality and extended shelf life of pork sausages (Siripatrawan and Noipha, 2012). Aqueous and methanolic extracts of Lipton black tea in Nigeria contain tannin and reduced sugar and have antimicrobial activity against *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa* at 2–10% concentrations (Funmilayo et al., 2012). Black tea and tea with milk beverages significantly reduced cariogenic oral *S. mutans* and *Lactobacillus* sp. counts (43.6–83.3%) and are thus recommended as natural anticariogenic beverages (Abd Allah et al., 2012).

Among the different teas tested, green and white tea extracts were found to be the most effective against *Campylobacter jejuni*, *E. coli* O157:H7, *S. enteritidis*, and *S. aureus*, and white tea killed all tested bacteria except *C. jejuni* 81176 within 48 h of incubation (Murali et al., 2012). Longjing tea extract reduced the *V. parahaemolyticus* count by 0.8 log MPN/g in shucked Pacific oysters and green tea treatment of oysters reduced *V. parahaemolyticus* while retarding the growth of total bacteria in oysters at 5±1°C storage (Xi et al., 2012).

Caffeine (1,3,7-trimethylexanthine) is a methylated xanthine alkaloid derivative present in plant species and has shown significant inhibitory effect against *E. coli* O157:H7 at a concentration of 0.5% (Gyawali and Ibrahim, 2012; Ibrahim et al., 2006), *Serratia marcescens*, *Enterobacter cloacae*, and *S. enterica* (Almeida et al., 2006), *S. aureus* and *Vibrio* sp. (MIC values of 192±91 and 162±165 μg/mL, respectively) (Taguri et al., 2004). Processed and fresh forms of sloe berry purees reduced *Salmonella* spp. to range of 4.24–6.70 log units within 24 h at 25°C (Gunduz, 2013).

Major antimicrobial compounds of herbs and spices

Plants have a nearly endless potential to synthesize mostly phenolic aromatic compounds or their oxygen-substituted analogs (Geissman, 1963). The majority of these compounds are secondary metabolites and approximately 12,000 have already been isolated; however, this number does not even correspond to 10% of the total number of metabolites (Schultes, 1978). In general, these materials allow plants to defend themselves against microorganisms, insects, and herbivores. Some of these materials, for example, terpenoids give the plants their characteristic scent whereas quinones and tannins are related to the plant pigment. Most of these metabolites give the plants their flavor such as the terpenoid from chili peppers and some herbs and spices used by people to marinate food produce useful medicinal compounds.

Phenolics and polyphenols

Simple phenols and phenolic acids

Simple phenols and phenolic acids are composed of a single-substituted phenolic ring (Tajkarimi et al., 2010; Gyawali and Ibrahim, 2012; Cowan, 1999). Cinnamic and caffeic acids are communal substitutes of a large group of phenylpropane-derived compounds. Tarragon and thyme both consist of caffeic acid and they are efficient against bacteria, viruses, and fungi. Catechol and pyrogallol are hydroxylated phenols proved to be toxic to microorganisms (Cowan, 1999).

Quinones

Quinones are aromatic rings with two ketone substitutions. These quinones are responsible for the browning reaction in peeled fruits and vegetables. The compound responsible for dyeing in henna is quinone. Coenzyme Q (ubiquinone) has an important role in mammalian electron transport system. Vitamin K is a naphthoquinone. Besides providing a source of free radicals, quinones bind irreversibly with nucleophilic amino acids in proteins generally causing the protein inactivation and loss of function (Cowan, 1999). Therefore quinones have potentially great antimicrobial effects. Possible targets in cells are surface-exposed adhesions, cell wall polypeptides, and membrane bound enzymes. Furthermore the quinones may also make the substrate unavailable for microorganism. Anthraquinones have antibacterial and antidepressant effects (Cowan, 1999).

Flavones, flavonoids, and flavonols

Flavones have phenolic structure containing one carbonyl group. Flavonols have extra 3-hydroxyl groups. Flavonoids are also hydroxylated phenolic compounds, but there is a C_6–C_3 unit bound to the aromatic ring. Flavonoids have antimicrobial activity through their ability to form complexes with proteins and cell walls, as explained for quinones (Cowan, 1999). Catechins inhibited *Vibrio cholerae*, *S. mutans, Shigella*, and other bacteria and microorganisms. Flavonoids show inhibitory effects against some viruses such as HIV, respiratory syncytial virus, herpes simplex virus type 1 (HSV-1), poliovirus type 1, and parainfluenza virus type 3. Hesperetin reduces intracellular replication of viruses. Galangin has antimicrobial activity against a lot of different Gram-positive bacteria, fungi, and viruses. Alpinumisoflavone stops schistosomal infection when administered topically (Cowan, 1999).

Tannins

They are a group of polymeric phenolic compounds which have the ability to tan leather or sedimentation of gelatin from solution, a feature known as astringency and they are present in bark, wood, leaves, fruits, and roots of the plants. Beverages containing tannins especially green teas and red wines can cure several diseases. Their mechanism of action may be related to the inactivation of microbial adhesions, enzymes, and cell envelope transport proteins. Tannins can bind to polysaccharides as well and can be toxic to filamentous fungi, yeasts, and bacteria (Cowan, 1999).

Coumarins

Coumarins are phenolic substances composed of fused benzene and α-pyrone rings. At least 1300 different coumarins have been identified.

Coumarins have antithrombotic, antiinflammatory, and vasodilatory activities. Warfarin is the most popular one and it is used as an oral coagulant and rodenticide. Coumarins may also have antiviral effects and are highly toxic in rodents. Some of the coumarins compounds have antimicrobial effects such as an inhibitory effect against *C. albicans* and thus can be used to cure vaginal candidiasis. In addition, coumarins can induce macrophages which exert a negative effect on bacterial infections. Hydroxycinnamic acids can inhibit Gram-positive bacteria and phytoalexins are assumed to have effects against fungi (Cowan, 1999).

Terpenoids and EOs

EOs have the odor of plants and are secondary metabolites that are highly enriched in substances which are called terpenes. These terpenes have predicated isoprene rings on chemical structures (Tajkarimi et al., 2010; Gyawali and Ibrahim, 2012; Cowan, 1999). Their general chemical conformation is $C_{10}H_{16}$, and their subcategories are diterpenes, triterpenes, tetraterpenes, hemiterpenes, and sesquiterpenes. When EOs are comprised of additional elements, mostly oxygen, they are called terpenoids and they are synthesized from acetate units and share their origins with fatty acids. Terpenoids are chemically in cyclic form and extensively branched. Well-known terpenoids are methanol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenes). Artemisin has been recently used as an antimalarial agent (Cowan, 1999).

Terpenoids are effective against bacteria, fungi, viruses, and protozoa. Betulinic acid can inhibit HIV. The mode of action for terpenes is not exactly understood, but it is assumed that they disrupt the membrane by the action of lipophilic compounds. Terpenoids in EOs are used to control *L. monocytogenes*. Capsaicin is an analgesic terpenoid that is present in chili peppers and is involved in a series of biological activities related to the nervous, cardiovascular, and digestive systems of human beings (Cowan, 1999).

Capsaicin can inhibit a lot of different bacteria and has bactericidal effects against *H. pylori*. Aframodial is a strong antifungal. Petalostemumol showed strong antimicrobial activity against *B. subtilis*, *S. aureus* and less activity against Gram-negative bacteria alongside *C. albicans*. Terpenoids are useful in preventing the formation of ulcers and in decreasing the severity of existing ulcers (Cowan, 1999).

Alkaloids

Alkaloids are heterocyclic nitrogen compounds. Morphine was isolated from the opium poppy *Papaver somniferum* in 1805 and used in

medical applications. Codeine and heroin are both morphine derivatives. Diterpenoid alkaloids have antimicrobial effects. Solamargine is a glycoalkaloid and can be useful against HIV together with other alkaloids. Berberine is one of a considerable group of alkaloids that are effective against trypanosomes (Cowan, 1999).

Lectins and polypeptides

Peptides that could inhibit microorganisms were first discovered in 1942. These peptides generally have positive charges and are comprised of disulfide bonds. Their mode of action can be explained by the formation of ion channels in the mitochondrial membrane. Thionins are commonly present in barley and wheat and are toxic against yeasts, Gram-negative and Gram-positive bacteria whereas thionins AX1 and AX2 are only effective against fungi. Fabatin is a peptide isolated from fava beans and inhibits *E. coli*, *P. aeruginosa*, and *Enterococcus hirae*. The larger lectin molecules contain mannose-specific lectins from different plants. MAP30 from bitter melon, GAP31 from *Gelonium multiflorum*, and jacalin have inhibitory effects against viruses (Cowan, 1999).

Mixtures

In African countries, a chewing stick is used instead of a toothbrush for maintaining oral hygiene. These chewing sticks are obtained from a variety of species and they may contain different chemical compounds such as alkaloids. *Serinda werneckei* can inhibit periodontal pathogen microorganisms *Porphyromonas gingivalis* and *Bacteroides melaninogenicus* (Cowan, 1999).

Papaya secretes a milky sap called latex and included a mixture of different chemical compounds such as papain (proteolytic enzyme), carpaine alkaloid, and terpenoids. Latex is bacteriostatic to *B. subtilis*, *E. cloacae*, *E. coli*, *Salmonella typhi*, *S. aureus*, and *P. vulgaris*. Ayurveda is a type of healing practice in India that relies on single plant or mixture preparations. Preparations such as Ashwagandha, Cauvery 100, and Livo-vet have antimicrobial, antidiarrheal, immunomodulatory, anticancer, and psychotropic effects. They are potentially toxic and can cause an accumulation of high levels of lead in human blood. Propolis, or bee glue, is a crude extract with a complex chemical composition. Bee glue is collected from different trees by honeybees and contains terpenoids, flavonoids, benzoic acids and esters, and substituted phenolic acids and esters. It has antiviral activity against HSV-1, adenovirus type 2, vesicular stomatitis virus, and poliovirus (Cowan, 1999).

Other compounds

Polyamines, isothiocyanates, thiosulfinates, and glucosides have antimicrobial effects. Cranberry and blueberry juices competitively inhibit the attachment of pathogenic *E. coli* to urinary tract epithelial cells (Cowan, 1999).

Commonly used antimicrobials in the food industry include more than 1340 plants containing clearly defined antimicrobial compounds and more than 30,000 components isolated from plant oil compounds that have phenolic groups. However, characterization of features which are important in the preservation of food is only available for a few EOs (Tajkarimi et al., 2010).

In food systems, more evaluation of EOs is required. Spices and their EOs are increasingly being used for the preservation of food as natural antimicrobials in order to increase the shelf life of foods, eliminate foodborne pathogens, and improve the overall quality of food products (Tajkarimi et al., 2010).

The most common method for the production of plant-origin antimicrobials is hydro distillation, or steam distillation. Supercritical fluid extraction, as an alternative method, allows higher solubility with better mass transfer rates. Additionally, the modification of parameters like temperature and pressure allows for the extraction of different antimicrobial compounds (Burt, 2004). Medicinal and herbal plants and spices such as sage, basil, oregano, thyme, rosemary, turmeric, garlic, ginger, clove, mace, nutmeg, savory, and fennel have been used in food preservation either alone or in combination with other preservation techniques. These natural products increase the shelf life of food products or have antimicrobial effects against a wide variety of Gram-negative and Gram-positive bacteria. Factors affecting these natural additives' efficacy include pH, storage temperature, amount of oxygen, and the level of concentration and active ingredients of EOs (Burt, 2004).

In food manufacturing, spices and EOs are used as natural antimicrobials to increase the shelf life of foods, improve food quality, and remove foodborne pathogens. The aromatic oily liquids of flowers, seeds, leaves, bark, buds, twigs, herbs, wood, fruits, and roots of plants contain plant-origin antimicrobials that are extracted with different methods. EOs are composed combinations of various compounds. Some EOs such as those extracted from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage, and vanillin have strong antimicrobial effects, whereas ginger, black pepper, red pepper, chili powder, cumin, and curry powder have weaker antimicrobial effects (Holley and Patel, 2005; Burt, 2004).

Chemical components of EOs

EOs can contain 20–80 different constituents at different concentrations. The main group of constituents is terpenes and terpenoids (Burt, 2004).

The monoterpenes constitute 90% of EOs and have very different structures. Oxygenated monoterpenes are more active than hydrocarbon ones (Carson and Riley, 1995). EOs also consist of terpenoids, sesquiterpenes, and diterpenes with aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones (Fisher and Phillips, 2006). Plant extracts and EOs are mainly responsible for antimicrobial activities, and they can be extracted from plants and spices with different methods (Ceylan and Fung, 2004; Bajpai et al., 2008). The primary compounds of EOs can comprise 85% of the product while other compounds are generally at trace levels (Burt, 2004; Grosso et al., 2008). Plant-origin antimicrobials contain chemicals such as saponin and flavonoids, thiosulfinates, and glucosinolates. Saponin and flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis, and honey and usually generate soapy foam after being shaken in water. They have antimicrobial effects when they are obtained from roots, stem bark, leaves, and wood of plants such as *Avena sativa* (oat) (Tajkarimi et al., 2010). Thiosulfinates are extracted from garlic through mild procedures and have extreme antimicrobial effects against Gram-negative bacteria. Glucosinolates are found in broccoli, Brussels sprouts, cabbage and mustard powder, and account for the sharp acidic flavor of mustard and horseradish. Glucosinolates also show a broad range of activity against bacteria and fungi with direct or synergistic effects when used with other substances (Tajkarimi et al., 2010).

Commonly, the phenolic components of EOs such as citrus oil, olive oil, tea-tree oil, orange, and bergamot have a wide range of antimicrobial effects and are not classified as spices. Oils extracted from citral, garlic, clove, oregano, coriander, cinnamon, rosemary, parsley, lemongrass, sage, purple, and bronze muscadine seeds have some nonphenolic compounds as well and are effective against Gram-negative and Gram-positive bacteria (Tajkarimi et al., 2010).

Uses of plant-origin antimicrobials

Food spoilage can happen at any step from food processing to distribution. Sources of spoilage can be microbiological, physical, and chemical. Food preservation methods to control microbiological spoilage have improved remarkably recently to eliminate pathogenic microorganisms (Gould, 1996). There has been a concomitant increase in research related to the utilization of EOs and spices as natural food preservatives to improve quality and extend shelf life of food products, and reduce or remove pathogenic microorganisms (Burt, 2004; Moreira et al., 2007; Simitzis et al., 2008). Table 26.1 lists natural ingredients that have antimicrobial activity against pathogen microorganisms.

Table 26.1 Natural Ingredients Which Possess Antimicrobial Activity Against Target Pathogens

Type of Antimicrobial	MIC Value	Target Organism(s)	Reference(s)
Mint EO from *Mentha piperita*	1.2% (v/v)	*Staphylococcus aureus* and *Salmonella enteritidis*	Tassou et al. (2000)
Carvacrol, thymol, TC	1–3 mM	*Escherichia coli and Salmonella typhimurium*	Helander et al. (1998)
Carvone	10 mM		
Fingerroot extract	0.2% (v/v), 0.4% (v/v)	*Listeria monocytogenes*, *Bacillus cereus*, and *S. aureus*	Aktug and Karapinar (1986)
Galangal extract	8% (v/v)	*E. coli* O157:H7	Aktug and Karapinar (1986)
Fingerroot extract	10% (v/v)		
Clove, cinnamon, and mustard extracts	1% (v/v)	*E. coli* O157:H7, *S. aureus*, and *B. cereus*	Sofia et al. (2007)
Garlic	3% (v/v)		
α-Pinene, 1,8-cineole, (+)-limonene, linalool, and geranyl acetate	1 mg/mL	*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis*, and *Staphylococcus aureus*	Sandasi et al. (2008)
Lonicera japonica Thunb. EO	62.5 µg/mL	*Listeria monocytogenes*, *B. subtilis*	Rahman and Kang (2009)
	125 µg/mL	*S. aureus*, *Salmonella enteritidis*	
	250 µg/mL	*Bacillus cereus*, *S. typhimurium*	
	500 µg/mL	*Enterobacter aerogenes*, *E. coli*	
Thyme	1000 ppm	*Vibrio parahaemolyticus*	Amiri et al. (2008)
Bay leaves	5000 ppm		
Mint	6000 ppm		
Thyme	0.05% (v/v)	*S. aureus*	Kwon et al. (2008)
Bay leaves	0.5% (v/v)		
Phenolic contents from dried fruits of cinnamon	5 mg/plate	*Salmonella typhimurium* TA100	Jayaprakasha et al. (2007)
Carvacrol	1 mM	*E. coli* O157:H7 ATCC 43895	Burt et al. (2007)
EO from *Nandina domestica* Thunb	62.5– 1000 µg/mL	*B. subtilis* ATCC6633, *L. monocytogenes* ATCC19166, *S. aureus* KCTC1916 and ATCC6538, *P. aeruginosa* KCTC2004, *S. typhimurium* KCTC2515, *S. enteritidis* KCCM12021, *E. coli* O157-Human, *E. coli* ATCC8739, *E. coli* O157:H7 ATCC43888, and *Enterobacter aerogenes* KCTC2190	Bajpai et al. (2008)
Different organic extracts of hexane, chloroform, ethylacetate, and methanol	250– 2000 µg/mL		
Carvacrol, p-cymene	7.8–800 µg/mL	*Campylobacter* spp.	Aslim and Yucel (2008)
EO and extracts from clove	0.5–5.5 mg/mL	*L. monocytogenes*	Hoque et al. (2008)
EO and extracts from cinnamon	1.0–5.0 mg/mL		

Using natural antimicrobials against pathogens in liquid food models

Clove oil and mint extract exerted strong activity at low concentrations and mild heat without pulsed electric field in tomato juice (Nguyen and Mittal, 2007). The effect of cinnamon (0% and 0.3%) in pasteurized apple juice was investigated against *S. typhimurium*, *Y. Enterocolitica*, or Enterotoxigenic *S. aureus* at 4 log CFU/mL and stored at 5°C and 20°C. Cinnamon was effective against *S. aureus* (1.2 log CFU/mL at 3 days) and *Y. enterocolitica* (0.3 log CFU/mL at 1 day). Morbidity levels were significantly greater at 20°C for all three pathogens with ~4 log CFU/mL reductions in 1 day in *Y. enterocolitica* inoculated samples with and without cinnamon, and in *S. aureus* inoculated samples with cinnamon (Yuste and Fung, 2003).

Four EOs from clary sage, juniper, lemon, and marjoram were tested against wild-type isolates of the food-related yeasts *Geotrichum candidum*, *Pichia anomala*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* in malt extract medium, apple juice and milk. *S. pombe* was most sensitive yeast (MICs of 0.0625–0.125 μL/mL) and *G. candidum* was least sensitive (MICs of 0.5–2 μL/mL). Significant reduction was achieved only with the highest EO concentrations (Tserennadmid et al., 2011).

The effect of thermal treatment, applied previously, on the growth of *B. megaterium* cells was investigated in a culture medium with and without the presence of nisin, carvacrol, and thymol. When thymol and carvacrol were combined at high doses (0.6 mM), the result was a further increase in the lag phase and significant decrease in the growth rate. The combination of carvacrol and/or thymol with a previous thermal treatment killed 90% of the population, and the growth of survivors was inhibited for at least 7 days (Periago et al., 2006).

The effect of HIPEF with or without the combination of citric acid or cinnamon bark oil in apple, pear, orange, and strawberry juices against *S. enteritidis* and *E. coli* 0157:H7 was evaluated. Both of these pathogens were reduced more than 5.0 $\log_{10}$ units in orange juice by only HIPEF although strawberry, apple, and pear juices were pasteurized by a combination of HIPEF with citric acid at 0.5%, 1.5%, 1.5%, respectively, or cinnamon bark oil at 0.05% and 0.1%, respectively (Mosqueda-Melgar et al., 2008a).

The effects of different concentrations (0.01–15%) of thyme (*Thymus vulgaris*), peppermint (*Mentha piperita* L.), caraway seed (*Carum carvi*), fennel (*Foeniculum vulgar*), tarragon (*Artemisia dracunculus*), and

pennyroyal (*Mentha pullegium*) EOs against *S. aureus* and *E. coli* were investigated in a nutrient broth medium. Thyme EOs exerted the broadest spectrum of antibacterial effects while EOs of peppermint, caraway seed, pennyroyal, and fennel showed a moderate effect on tested microorganisms while, in contrast, tarragon EO was less efficient against both microorganisms (Mohsenzadeh, 2007).

After inoculation at a level of 4 log CFU/mL in unpasteurized apple juice *E. coli* O157:H7 could survive up to 3 days at 25°C and 19 days at 4°C. The addition of 1.25 mM carvacrol or *p*-cymene into the juice decreased the number of *E. coli* O157:H7 to undetectable levels in 1–2 days for both storage temperatures (Kisko, 2005). The effects of garlic, bay, black pepper, Origanum, orange, thyme, tea tree, mint, clove, and cumin EOs on *L. monocytogenes* AUFE39237, *E. coli* ATCC25922, *S. enteritidis* ATCC13076, *P. mirabilis* AUFE43566, *B. cereus* AUFE81154, *Saccharomyces uvarum* UUFE16732, *Kloeckera apiculata* UUFE10628, *C. albicans* ATCC10231, *Candida oleophila* UUPP94365, and *Metschnikowia fructicola* UUPP23067 and effects of thyme oil at a concentration of 0.5% on *L. monocytogenes* and *C. albicans* in apple and carrot juices during +4°C (1–5th day) were investigated. Thyme, Origanum, clove, and orange EOs had the most effective antibacterial and antiyeast activities against these microorganisms. Cumin, tea tree, and mint oils effectively inhibited the yeasts (Irkin and Korukluoglu, 2009).

EOs extracted from lemon balm, marjoram, oregano, and thyme and their MICs were investigated against *Enterobacter* spp., *Lactobacillus* spp., and *Pseudomonas* spp. through the use of agar dilution method and/or the absorbance based microplate assay in food model media based on lettuce, meat, and milk. MICs were significantly lower in beef and lettuce media than in TSB. Thyme and oregano were the most active EOs and *Listeria* strains were more sensitive to EOs than other bacteria (Gutierrez et al., 2009).

The antimicrobial effect of cinnamon was investigated against *E. coli* O157:H7 in apple juice at 8°C and 25°C. *E. coli* O157:H7 was reduced by 1.6 log CFU/mL at 8°C and 2.0 log CFU/mL at 25°C by 0.3% cinnamon (Ceylan et al., 2004). The antibacterial effect of low concentrations of *trans*-cinnamaldehyde (TC) was determined against five *E. coli* O157:H7 strains in apple juice and apple cider. The initial inoculum level for a five strain mixture was ~6.0 log CFU/mL in apple juice or cider, followed by the addition of TC at 0%, 0.025%, 0.075%, and 0.125% (v/v) levels. The inoculated apple juice samples were incubated at 23°C and 4°C for 21 days, although the cider samples were stored only at 4°C. At 23°C, 0.125%

and 0.075% (v/v) TC completely inactivated *E. coli* O157:H7 in apple juice on days 1 and 3, respectively. At 4°C, 0.125% and 0.075% (v/v) TC decreased the counts in the juice and cider to undetectable levels on days 3 and 5, respectively (Baskaran et al., 2010).

Using natural antimicrobials against pathogens in solid food models

The antimicrobial activity of EOs of oregano, thyme, basil, marjoram, lemongrass, ginger, and clove was tested *in vitro* by agar dilution method and MIC determination against Gram-positive (*S. aureus* and *L. monocytogenes*) and Gram-negative strains (*E. coli* and *S. enteritidis*). $MIC_{90\%}$ values were determined against bacterial strains in irradiated minced meat and against natural microbiota in minced meat samples. $MIC_{90\%}$ values varied from 0.05% (v/v; lemongrass oil) to 0.46% (v/v; marjoram oil) to Gram-positive bacteria and from 0.10% (v/v; clove oil) to 0.56% (v/v; ginger oil) to Gram-negative strains. Nevertheless, the $MIC_{90\%}$ values on minced meat and natural microbiota were 1.3 and 1.0, respectively, against tested microorganisms (Barbosa et al., 2009).

The physical properties and antimicrobial activities of allspice, cinnamon, and clove bud oils against *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* in apple puree film forming solutions formulated into edible films at 0.5–3% (w/w) concentrations. The antibacterial activities against these three pathogens were in the following order: cinnamon oil > clove bud oil > allspice oil. The results showed that apple-based films with allspice, cinnamon, or clove bud oils were active against these three main foodborne pathogens either with direct contact with the pathogens or by vapors emanating from films (Du et al., 2009).

Oregano and thyme incorporating soy protein edible films (SPEF) showed similar antibacterial activity against *E. coli*, *E. coli* O157:H7, *S. aureus, P. aeruginosa*, *and L. plantarum* in inhibition zone test. Although *E. coli, E. coli* O157:H7, and *S. aureus* were significantly inhibited by these films, *L. plantarum* and *P. aeruginosa* seemed to be more resistant bacteria. SPEF with oregano, thyme, and oregano plus thyme did not have significant effects on total viable counts, lactic acid bacteria, and *Staphylococcus* spp. when applied to ground beef patties, although reductions ($P < 0.05$) in coliform and *Pseudomonas* spp. counts were determined (Emiroglu et al., 2010).

The EO of cloves (10%) and cinnamon (5%) were tested against a cocktail of five strains of *L. monocytogenes* in ground chicken meat. EOs from cloves reduced all tested strains to undetectable levels within 1 day

of exposure whereas EOs from cinnamon could only reduce levels by 2.0 log CFU/g within 1 day with only slight reductions or no additional decline in cell numbers during 15 days of incubation (Hoque et al., 2008).

The effect of carvacrol, cinnamaldehyde, thymol, and oregano oil on *Clostridium perfringens* growth and spore germination was investigated during the chilling of cooked ground beef. The addition of test compounds (≥0.1%) into beef completely inhibited *C. perfringens* spore germination and outgrowth ($P \leq 0.05$) during cooling of the cooked beef over 12 h. Greater concentrations were required for longer chilling times. Cinnamaldehyde was significantly more effective at a lower concentration (0.5%) at the most abusive chilling rate tested (21 h) than other compounds (Juneja et al., 2006).

The addition of clove and tea tree EOs at highest concentrations (three and four times MICs) was required to inhibit the growth of *E. coli* O157:H7 in blanched spinach and minced cooked beef, respectively. The antimicrobial effect was dependent on the oil concentration, the food composition, and storage temperature (Moreira et al., 2007). The ethanolic extract of cinnamon bark (2%, w/v) inhibited the growth of *E. coli* O157:H7 and *L. innocua* by 94% and 87%, respectively. When combined with commercial antibrowning dipping solution (FreshExtend), cinnamon bark extract (1%, w/v) reduced the microbial growth significantly on apple slices stored for 12 days at 6°C in comparison to the control. The major constituent of this extract was cinnamic aldehyde (Muthuswamy et al., 2008).

The antibacterial effect of clove oil applied to the surface of ready to eat chicken frankfurters was evaluated on seven strains of *L. monocytogenes* at low (2–3 log) or high (4–6 log) inoculum levels and stored 2 weeks at 5°C or 1 week at 15°C. The growth of all seven *L. monocytogenes* strains was inhibited under either storage conditions with the addition of 1% or 2% clove oil (Mytle et al., 2006).

The effect of malic acid and EOs of cinnamon, palmarosa, and lemongrass and their main active antimicrobial substances attached to an alginate-based edible coating on shelf life and safety of fresh-cut "Piel de Sapo" melon was tested. Malic acid was effective in increasing the shelf life of fresh-cut melon from microbiological (up to 9.6 days) and physicochemical (>14 days) aspects compared with noncoated melon. The microbiological and physicochemical shelf life was up to 3.6 days and <14 days, respectively. When palmarosa oil was integrated into edible coatings at a 0.3% concentration, it seemed to serve as a good alternative for fresh-cut melon because it has been accepted by panelists, preserved fruit quality properties, inhibited the native microflora growth, and provided a

significant reduction in the number of *S. enteritidis* (Raybaudi-Massilia et al., 2008).

The effect of EOs of oregano and nutmeg and storage temperature on survival and growth of *E. coli* O157:H7 has been investigated in ready to cook traditional Iranian barbecued chicken. The EOs of oregano and nutmeg had no significant inhibitory effect against *E. coli* O157:H7 in broth and ready to cook barbecued chicken (Shekarforoush et al., 2007). The effect of oregano EO supplementation on lamb meat was also tested. The results showed that incorporation of oregano EO into dietary supplements showed promising antioxidant activity by retarding lipid oxidation in lamb meat during refrigerated and frozen storage (Simitzis et al., 2008).

Thyme EOs at 0.3% concentration had a weak antibacterial activity against *E. coli* O157:H7 in TSB, negative impact on organoleptic properties of minced meat at 0.9%, and showed inhibitory activity at 0.6% against *E. coli* O157:H7 during storage at 10°C but not at 4°C. The addition of nisin into minced beef or TSB at 500 or 1000 IU/g had no effect on pathogens (Solomakos et al., 2008).

CONCLUSION

Natural antimicrobial extracts from herbs, spices, and plants can be used in food industry to prevent growth of foodborne pathogens and food spoilage microorganisms and to enhance the shelf life and stability. Due to the antioxidant and antimicrobial effects of natural ingredients, they can be a good alternative to classical food preservation methods and usage of chemical preservatives and food additives. These plant products have shown to reduce the growth of both Gram-positive and Gram-negative microorganisms including foodborne pathogens. Besides possessing antimicrobial effect on pathogenic microorganisms, herbs and spices also improve flavor, aroma, and texture of food products without affecting their sensory properties. Thus the use of plant-derived antimicrobials has a greater potential to improve the safety and quality of food products. Natural ingredients can be used in combination with other traditional food preservation methods to improve antimicrobial efficacy and ensure safety of food products.

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