Antimicrobial Natural Products

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Natural antimicrobials are secondary metabolites that can be found in plants, animals, and microorganisms.Plants, especially herbs and spices, are been given more attention in natural antimicrobial research, however; this chapter focuses on the newly discoveries in fruits and vegetables. Microorganisms that are been used in food fermentation also produce different antimicrobial metabolites including organic acids, hydrogen peroxide, ethanol, and diacetyl in addition to bacteriocins. Products of animal origin such as tissues, milk, and eggs contain different antimicrobial agents that are mainly in the form of peptides (polypeptides). In most cases natural antimicrobials are extracted and purified to be tested or applied to food products. Extraction and purification may alter the chemical structure of natural antimicrobials and affect their functionality. Thus, an extraction method with minimal processing such as juice or mechanical direct extraction seems to be more promising to avoid possible alteration or destruction of active ingredients. Activity of natural antimicrobials could be also influenced by the original source, time of harvesting, and stage of development. In food applications, these natural antimicrobial compounds could be influenced by food components, processing, and storage. On the other hand, increasing level of consumer concerns on chemical antimicrobials and increasing resistance of pathogenic microbes have turned the attention of the scientific communities towards studies on the potential antimicrobial activities of natural products. Natural antimicrobials are generally recognized as safe and they appear to be the most promising solution for microbial resistance and could best meet with consumers' demands for healthier foods. This chapter discussed the recent insights regarding the antimicrobial activities of natural products, extraction methods, mechanism of action, and factors influencing the antimicrobial activity.

Keywords antimicrobial, natural products, plant, animal, microorganisms.

1. Introduction

Natural products are chemical compounds or substance produced by a living organism or found in nature that have pharmacological or biological activity [1]. Living organisms produce manifold primary and secondary metabolites. Primary metabolites have essential function in the organism whereas secondary metabolites may have important functions for their producers or they could simply be waste products. However, secondary metabolites may also have properties that are beneficial to humans. In many cases they can be used as drugs against human diseases such as cancer, bacterial infections, inflammation, and many other diseases [2-4]. However, a number of these secondary metabolites have been noted for their antimicrobial activity. Secondary metabolites with antimicrobial activity can be found in most organisms including: (1) plants such as fruits, vegetables, seeds, herb, and spices, (2) animal sources such as milk, eggs, and tissues, and (3) microorganisms such as bacteria and fungi.

Natural antimicrobials are been given more attention due to the increase concerns on chemical preservatives among consumer. Even though chemical preservatives are approved for human consumption by government agencies, many of these preservatives still threaten our health. Thus, the scientific communities have given more attention towards the potential antimicrobial activities of natural products. On the other hand, the increasing antibiotic resistance against chemical preservatives of some pathogens associated with foodborne illness is in increasing rates [5, 6]. Natural antimicrobials seems to be the most promising answer to many of the increasing concerns regarding antibiotic resistance and could yield better results than antimicrobials from combinatorial chemistry and other synthetic procedures [7]. Therefore, novel types of effective and healthy antimicrobial compounds that could protect food against contamination and consumer against infection is highly demanded.

In recent years, a large number of studies have been conducted searching the antimicrobial activity of natural products. Plants, especially herbs and spices, are been given more attention. Nowadays, there are over 1340 plants with defined antimicrobial activities, and over 30,000 antimicrobial compounds have been isolated from plants [8]. However, antimicrobials from microorganisms and animals have also shown a numerous studies. In addition, extensive research has investigated the potential food applications, in food products and animal feeds, for natural antimicrobial agents against foodborne pathogens. This chapter explores the recent findings about natural antimicrobial compounds from plants, animals, and microorganisms which could be used to control spoilage and pathogenic microorganisms in food products. In addition, extraction methods, mechanism of action, and factors influencing the antimicrobial activity are been discussed.

2. Natural antimicrobial products from plants

Plants produce an array of secondary metabolites that can be found in the edible, medicinal, and herbal plants and their derived essential oils (EOs) [9]. Secondary metabolites from plants are extensively studied as promising healthy ingredient or human disease controlling agents. These secondary metabolites possess various benefits including antimicrobial properties against pathogenic and spoilage microbes [7]. Natural antimicrobials derived from plants have been recognized for centuries, but only scientifically confirmed in the last 30 years [3, 10]. Thus an increasing interest in finding natural antimicrobials for application in food products to prevent or inhibit microbial growth and extend shelf life have been noticed [11, 12]. The presence of both antioxidant and antimicrobial properties in a single molecule makes them more effective and better suited as food preservatives. In general plant have much greater inhibition effect against Gram-positive than Gram-negative bacteria. The activity against both types of bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins [13].

The antimicrobial efficacy of components in plants depends on the chemical structure of active components and their concentration. There are various chemical components present in plants with antimicrobial effect including saponin, flavonoids, thiosulfinates, glucosinolates, phenolics, and organic acids. However, the main components in plants with antimicrobial activity are phenolic compounds such as terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids [9]. For example, the antibacterial activity of 46 extracts from spices and herbs was suggested to be associated with the presence of phenolic constituents [14]. The authors have reported that all the tested spices have a strong antibacterial effect against *Bacillus cereus, Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella anatum*. The antimicrobial activity of red cabbage was also suggested to be due to the fact that red cabbage is a rich source of phenolic compounds, anthocyanins [15]. Phenolic compounds of EOs such as citrus oils extracted from lemon, olive oil (oleuropein) and tea-tree oil (terpenoids), orange and bergamot have broader antimicrobial effects. However, there are increasing reports of nonphenolic oil compounds, which are effective against both Gram-positive and Gram-negative groups, from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, muscadine seeds, and sage [16-20]. Some nonphenolic constituents of essential oils such as allyl isothiocyanate and garlic oil are more effective or quite effective against Gram-negative bacteria.

Saponin and flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers, and leaves [21]. The antimicrobial activity of saponins and flavonoids derived from plants like *Bersama engleriana* (Melianthaceae) have been proven when extracted from roots, stem bark, leaves, and wood [21-24]. Thiosulfinates have also shown a strong antimicrobial effect against Gram-negative bacteria. Glucosinolates are secondary metabolites that occur in many species of plants include mustards, cabbage, cauliflower, brussel sprouts, broccoli, kohlrabi, kale, horseradish, and radishes [10, 25]. Glucosinolate hydrolysis products were reported to have significant antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungi with direct or synergistic effect in combination with other compounds [10].

Antimicrobial compounds in plant materials are commonly found in herbs and spices (rosemary, sage, basil, oregano, thyme, marjoram, cardamom, and clove), fruits and vegetable (guava, xoconostle, pepper, cabbage, garlic, and onion), seeds and leaves (grape seeds, caraway, fennel, nutgem, parsley, and olive leaves) [8, 9].

2.1. Herbs and spices

Herbs and spices have been used by humans since ancient times for different purposes including antimicrobial. Herbs and spices and their extracts are the most commonly used natural antimicrobials by the food industry. They are generally containing EOs that are well known to possess antimicrobial activity. EOs from plants usually have a relatively high vapor pressure and are capable of reaching microbial pathogens through the liquid and gas phases [26]. Several studies have shown the antimicrobial effect of EOs against different foodborne pathogens and spoilage microorganisms. However, the efficacy of EOs depends on the pH, storage temperature, amount of oxygen, EO concentration, and active components [16, 18, 27, 28]. EOs of lemongrass, cinnamon, and geraniol were found to be the most effective in inhibiting the growth of *S. enteritidis*, *E. coli*, and *Listeria innocua* [29]. Mustard EOs can be used against *E. coli* O157:H7 and *S. typhi* [30]. Allyl isothiocyanate, a non-phenolic volatile compound found in the crucifereae family at low concentration, effectively inhibits several pathogenic microorganisms [30]. Oregano EOs showed antimicrobial effects against *E. coli*, *S. aureus*, *B. subtilis*, and *Saccharomyces cerevisiae* [4].

Herbs and spices can exert direct or indirect effects to extend foodstuff shelf life as antimicrobial agents against variety of Gram-positive and Gram-negative bacteria. Edible and herbal plants and spices such as oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage and vanillin, have been successfully used alone or in combination with other preservation methods [16, 17, 31, 32]. Other spices, such as ginger, black pepper, red pepper, chili powder, cumin, and curry powder showed lower antimicrobial properties [18]. The degree of antibacterial action of various spices tested against *Salmonella* and other enterobacteria was reported in the order of clove > kaffir lime peels > cumin > cardamom > coriander > nutmeg > mace > ginger > garlic > holy basil > kaffir lime leaves [33]. Oregano and thyme EOs possess significant bacteriostatic and bactericidal properties, and this bactericidal concentration of oregano EOs irreversibly damaged *E. coli* O157:H7 cells within 1 min [34]. Oregano and thyme EO were found to exhibit stronger antimicrobial properties than clove and bay [34]. However, natural antimicrobial compounds from herbs and spice have been the most studied natural antimicrobials, and were also widely reviewed [8, 13, 26, 35-37]. Thus, in this chapter more attention will be given to other plant products.

2.2. Fruits and vegetables

While herbs and spices have been well studied and documented for antimicrobial activity, recently fruits and vegetable are being given more attention. Thus, many fruits and vegetables are nowadays well known to have antimicrobial effect against different pathogenic and spoilage microbes. Fruits and vegetables generally contain phenolics and organic acids that are well known to possess antimicrobial activity. For example, the antimicrobial activity in *Capsicum* was reported to be due to the phenolic compound and 3-hydroxycinnamic acid (coumaric acid) [38]. Favonoids from bergamot peel, a byproduct of citrus fruit processing, was found to be active against Gram-negative beacteria (*Escherichia coli*, *Pseudomonas putida*, *Salmonella enterica*) and the antimicrobial potency of favonoids was increased after enzymatic deglycosylation [20].

The antibacterial activity of group of fruits and vegetables including: aronianberry, asparagus, bell pepper, beet, blackberry, blueberry, broccoli, carrot, cucumber, cherry, cranberry, garlic, ginger, grape, red onion, red cabbage, rhubarb, rutabaga, raspberry, pomegranate, spinach, strawberry, and green tea have been investigated [39]. The authors reported that all green vegetables have no antibacterial activity on *Staphylococcus epidermidis* and *Klebsiella pneumonia* whereas all purple and red vegetable and fruit juices have showed antibacterial activities. Pomegranate juice was reported to inhibit the growth of *E. coli O*157:*H*7 [40]. The methanolic extract of pomegranate peels was also reported to have antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungi due to the presence of phenolics and flavonoids [41]. Antibacterial effects of grape pomace extracts (cultivars Emir and Kalecik karasi) against spoilage and pathogenic bacteria including *Aeromonas hydrophila, B. cereus, Enterobacter aerogenes, Enterococcus faecalis, E. coli, E. coli* O157:H7, *Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, S. typhimurium*, *S. aureus* and *Yersinia enterocolitica* was evaluated [42]. All tested bacteria were inhibited by extract concentrations of 2.5, 5, 10 and 20%, except for *Y enterocolitica* which was not inhibited by the 2.5% concentration. *E coli* O157:H7 was the most sensitive among the tested bacteria to grape pomace extracts.

Direct extract from guava was reported to have a significant inhibition effect against the growth of *E. coli* O157:H7 and *Salmonella* [43]. Direct extract from xoconostle pears was reported to yield an active natural antimicrobial effect against *E. coli* O157:H7 in laboratory medium [44]. The antimicrobial activity in xoconostle pears was reported to be due to the phenolic compound, ascorbic acid, and betalains. Betalains is active compounds in the opuntia plant with will document antioxidant activity [45-47] but their antimicrobial effect has not been confirmed yet. Antimicrobial activity of *Capsicum annuum* extract against *S.* Typhimurium (ATCC 14028) in beef meat was reported at1.5 mL/100 g concentration [48]. *C. annuum* extracts was also reported to inhibit the growth of *L. monocytogenes*, *S. aureus*, *S.* Typhimurium, and *B. cereus* [38]. The aqueous acetone extract of quince (*Cydonia oblonga* Miller) fruit pulp and peel showed higher antimicrobial effect against bacteria growth compared to quince pulp due to the high concentration of phenolic content, mainly chlorogenic acid [11].

Garlic (Allium sativum) extract also has a broad range of antimicrobial activity. Allicin, an organosulfur compound present in garlic, acts as a growth inhibitor for both Gram-positive and Gram-negative bacteria including *E. coli*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus*, and *Helicobacter pylori* [49, 50]. The antibacterial activity of garlic could be due to the action of allicin, diallyl thiosulfinic acid, or diallyl disulfide [51] . In addition, organosulfur compounds present in garlic may have higher antimicrobial activity than those of garlic phenolic compounds [3]. The bactericidal property of garlic-derived organosulfur compound was found to be more effective against *Campylobacter jejuni* compared to phenolic compound [4]. Garlic extract has excellent antibacterial activity against *E. coli*, *Salmonella*, and *Aeromonas hydrophila*. Garlic extract showed antibacterial activity against all serogroups of *E. coli* whereas enterohemorrhagic *E. coli* (serogroup O157) and enterotoxigenic *E. coli* (serogroup O8) were found to be more sensitive to garlic extract [52]. Chinese chive that belongs to the same family as garlic is an important ingredient in Asian cooking. Chinese chive was reported to have a strong antimicrobial effect against *E. coli* and yeast (*Pichia membranaefaciens* CCRC 20859) [53]. The crude extract of Chinese chive contains sulfur compounds that found effective against the growth of *Salmonella* and could be used in food products to prevent the growth of this pathogen [54].

Srinivasan and others (2001) tested 50 different plants against Gram-positive bacteria, Gram-negative bacteria, and fungi. Among all tested plants, the authors reported that *Allium sativum*, a species in the onion genus, to be very promising showing a significant inhibition effect against all tested pathogenic bacteria and fungi. The volatile oils of black pepper, clove, geranium, nutmeg, oregano, and thyme possessed antibacterial activity against 25 different genera of tested bacteria with various degrees of growth inhibition [35]. The methanolic extracts of spinach (*Spinacea oleracia*), pumpkin (*cucurbita pepo*), suran (*Amorphophalus campanulatus*) and ghuiya (*Colocasia esculenta*) showed moderate to high antimicrobial activity against different Gram-positive and Gram-negative bacteria [55]. Methanolic extract of spinach was most effective among all extracts against *E. coli*. Methanolic extracts were more effective than their aqueous extracts. Red cabbage was reported to have antibacterial and the antifungal activities against Methicillinresistant *S. aureus*, *E. coli O157:H7*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *S. aureus*, and *S. enterica* serovar Typhimurium and against human fungal pathogens, *Trichophyton rubrum* and *Aspergillus terreus* [15]. Thus, fruits and vegetables rich with phenolics and organic acids are most likely to have high antimicrobial activity.

2.2. Seeds and leaves

Seeds and leaves containing EOs and different phenolic compounds can also show antimicrobial activity. For example, crude EOs from dill (*Anethum graveolens* L.), coriander (seeds of *Coriandrum sativum* L.), cilantro (leaves of immature *C. sativum* L.) and eucalyptus (*Eucalyptus dives*) were found effective against several bacteria and one yeast at concentrations ≤ 0.5% (vol/vol) [56]. Grape seed extract is generally regarded as a safe food additive and is known for its antioxidant and antimicrobial activities [57-59]. The partial hydrophobic nature of the grape seed phenolic compounds in grape seed extract is responsible for the antimicrobial activity [59, 60]. The inhibitory effect of of grape seed extract and pine bark extract against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* have been studied in ground beef. The results suggest that these natural extracts have the potential to be used with other preservative methods to reduce pathogenic population and improve the quality of ground beef [57]. Grape seed extract with nisin, malic acid, and EDTA showed strong antimicrobial activity against the growth of *L. monocytogenes* and *E. coli* O157:H7 [60]. Water extracts from muscadine seeds were also found to have a high concentration of phenolic compounds and showed a strong antimicrobial activity against *E. coli* O157:H7 [61].

Olive leaf was tested against pathogenic bacteria and fungi. The water extract of olive leaf at 0.6% (w/v) killed almost all bacteria cells within 3 h, whereas 1.25% and 15% (w/v) olive leaf extract were required to kill Dermatophytes and *Candida albicans* respectively [62]. The extract of olive leaves was also reported to possess a strong antibacterial and antifungal action [63]. The authors also indicated that this antibacterial and antifungal actions of olive leaves are due to the phenolic compounds including: caffeic acid, verbascoside, oleuropein, luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside and luteolin 4'-*O*-glucoside [63]. The authors thus suggested a potential use of extract from olive leaves as nutraceuticals, particularly as a source of phenolic compounds.

Methanolic extract of flower, stem, and leaf of Tagetes minuta L was found effective against all E. coli, Klebsiella pneumoniae, Pseudomonas auregnosa, S typhi, *S.* aureus, Streptococcus viridian, B. licheniformis, B. subtilis, and Pasteurella multocida with MIC in the range of 4-100mg/mL [64]. The authors suggested that this antimicrobial effect is due to the presences of alkaloids, tannins, saponins, and flavonoids. The antimicrobial activity of Rubus chamaemorus leaf was evaluated against Gram-positive bacteria, Gram-negative bacteria, and Candida albicans [65]. The authors suggested that leaves of Rubus chamaemorus are active against some Gram-positive bacteria. Extract of leaves from *Cassia alata* were reported to show activity against *S. aereus* but not to affect *E. coli* [66]. Methanolic extracts of the Orthosiphon stamineus plant demonstrated inhibitory activity against Vibrio parahaemolyticus [67]. The antimicrobial effect of Orthosiphon stamineus leaf extract was suggested to be due to the high concentration of rosmarinic acid. Leaves of parsley and dill were also found to exhibite antibacterial activity against natural microflora, coliforms, yeast, molds, and S. aureus in Kareish cheese [68].

Coffee contains different phenolic compounds including caffeic acid, chlorogenic acid, and protocatechnic acid that are thought to have antibacterial properties [69]. Caffeine (1,3,7-trimethylexanthine) is a methylated xanthine alkaloid derivative present in plant species. Caffeine from coffee has shown significant growth inhibition against *E. coli* O157:H7 at a concentration of 0.5 % [70]. Similarly, polyphenols (epicatechin, catechin, caffeine, chlorogenic acid, gallic acid, theobromine, theophylline, gallocatechin, epigallocatechin gallate, catechin gallate, epicatechin gallate, and theaflavin) from tea have also been found to have antimicrobial activity against againt Gram-positive and Gram-negative bacteria [71]. The antimicrobial activity of crude water-soluble arrowroot (*Puerariae radix*) tea against *E. coli* O157:H7, *S enterica*, *L. monocytogenes*, and *S. aureus* was reported [71]. The antimicrobial activity of arrowroot tea could be due to the presence of catechins. Catechins present in green tea extract, epigallocatechin gallate and epigallocatechin, showed a strong antimicrobial activity due to the galloyl moiety present in their structures [72]. Green tea was also reported to have significant antibacterial activity against a spectrum of pathogens including resistant strains such as methicillin- and ciprofloxacin-resistant staphylococci, vancomycin-resistant enterococci, and ciprofloxacin-resistant *Pseudomonas aeruginosa* [39]. Thus, phenolic compounds can be extracted from different seeds and leaves and could be used to enhance the safety of food products and extend food shelf life.

3. Natural antimicrobial products from animals

Animals have a numerous antimicrobial systems that often evolved as part of host defense mechanisms. Many of the antimicrobial agents inherent to animals are in the form of antimicrobial peptides (polypeptides). Antimicrobial peptides have been found in every living organism including bacteria, fungi, plants, and animals [73]. Antimicrobial peptides are widely distributed in nature and used by the host as essential components of nonspecific defense systems [9, 73]. They form a promising solution to antibiotic resistance because of the fact that the specific molecular sites are not targeted and peptides also can destruct the membranes rapidly which does not allow sufficient time for even fast-growing bacteria to mutate [9]. Antimicrobial peptides kill both Gram-positive and Gram-negative bacteria, and most peptides have also shown antifungal and antiviral activities [10,11]. Several peptides with potential antimicrobials from animal origin such as Pleurocidin, Lactoferrin, Defensins, and Protamine have been well documented.

Pleurocidin was found to be effective against foodborne organisms including *Vibrio parahemolyticus*, *L. monocytogenes*, *E. coli* O157:H7, *Saccharomyces cerevisiae*, and *Penicillium expansum* [74]. However, the antimicrobial effect of pleurocidin was inhibited by magnesium and calcium [75], which may limit the use of these antimicrobial peptides in environments rich in these cations, magnesium and calcium. Pleurocidin, isolated from the skin mucus membrane of the winter flounder (Pleuronectes americanus), found active against Gram-positive and Gram-negative bacteria [75]. Defensins are another group of antimicrobial peptides that are widely found in nature including mammalian epithelial cells of chickens and turkeys. This antimicrobial peptides are abundant in cells and tissues and has been reported to have antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and viruses [9]. Other antimicrobial peptides such as protamine and magainin have also been reported to be effective against Gram-negative and Gram-positive bacteria, yeasts, and molds [9, 76, 77]. Magainin showed to kill Gram-positive bacteria and reduce the adhesion of bacteria at the surface [77]. However, components of food may interfere with the antibacterial effects of protamine whereas this undesirable interferences can be reduced by altering the electrostatic properties of protamine [76].

Antimicrobial peptides can be also found naturally in milk. For example, lactoperoxidase is an abundant enzyme in milk that showed a strong antimicrobial effect against bacteria, fungi, and viruses [78]. Lactoperoxidase can present in cow milk, ewe milk, goat milk, buffalo milk, pig milk, and human milk [78]. Lactoperoxidase in human milk may contribute to the defense against infection already in the mouth and upper gastrointestinal tract [79]. In cow milk, lactoperoxidase system is used by the dairy industry to preserve microbial quality. Gram-negative bacteria are generally more sensitive to lactoperoxidase mediated food preservation than Gram-positive species [78]. Lactoferrin is another antimicrobial peptide that showed to be active against Gram-positive and Gram-negative bacteria, fungi, and parasites [9, 80]. The antimicrobial mechanism of lactoferrin has been studied but not clearly defined [80]. *Lysozyme,* a bacteriolytic enzyme, is present in egg white, *milk,* and blood and has been found to be effective against food spoilage microorganisms [9]. Lysozyme was also reported to inhibit the growth of *Clostridium tyrobutyricum* spores in cheeses [81].

In addition to peptides, some polysaccharides and lipids from animals were also reported for antimicrobial effect. Chitosan, a natural linear polysaccharide obtained from the exoskeletons of crustaceans and arthropods, is known for its unique polycationic nature and has been used as an active material for its antifungal activity [82] and antibacterial activity [83]. Antibacterial activity of chitosan was observed against *S. aureus* and *E. coli*, while its molecular weight appeared to be a significant parameter defining its activity [83]. Lipids of animal origin were also reported for antimicrobial activity against different microorganisms [84]. Milk lipids could inactivate Gram-positive and Gram-negative bacteria and fungi [9, 85]. Lipids in food may serve to inhibit the proliferation and prevent the pathogenic and spoilage microorganisms in food matrixes. In addition, other components in animals such as eicosapentaenoic acid and docosahexaenoic acid exhibited antibacterial activities against Gram-positive and Gram-negative bacteria [85].

4. Natural antimicrobial products from microbial sources

Microorganisms such as bacteria, fungi, and mold produce different compounds that could be active against other microorganisms. Secondary metabolites from bacteria and fungi such as penicillins, cephalosporins, tetracyclines, aminoglycosides, chloramphenicol, and macrolides are well known in pharmaceutical use as antibiotics [86]. In the food industry, microorganisms produce many antimicrobial compounds, especially those produced during food fermentation, as end products metabolites. These antimicrobial metabolites include organic acids, hydrogen peroxide, ethanol, and diacetyl in addition to bacteriocins [87].

Organic acids can be effective at low concentration without affecting the desirable sensory properties of food products. The antibacterial effectiveness of organic acids has been reported in the following order: formic acid > lactic acid > acetic acid > propionic acid. Organic acids are more effective against Gram positive bacteria compared to that against Gram-negative bacteria. For example, organic acids were found more pronounced in cultures of *Clostridium perfringens*, a Gram positive, than in *E. coli* and *Salmonella* sp., a Gram negative [88]. However, a combination of two organic acids or organic acid with other natural antimicrobial compounds could have a stronger inhibitory effect compared to the effect of single acid. For example, combinations of acetic and formic, lactic and formic, and propionic and formic acids showed a higher inhibition effect compared to single acid [89]. Formic acid was reported to be a good antibacterial agent for decontaminating animal carcass surfaces especially when mixed with lactic acid [89]. The synergistic effect of ascorbic acid and lactic acid against *E. coli* O157:H7 was also confirmed [90]. Lactic acid in combination with copper sulfate was also found more effective against *E coli* O157:H7 and *Salmonella* compared to lactic acid alone [91, 92].

Hydrogen peroxide has a strong antimicrobial activity due to its strong oxidizing effect on the bacterial cell and the destruction of basic molecular structures of cell proteins [93-95]. Hydrogen peroxide production is considered to be the main metabolite of lactic acid bacteria (LAB) and could protect against urogenital infections, especially in the case of bacterial vaginosis [96]. Diacetyl, (2,3-butanedione) is another antimicrobial compound produced by heterofermentative lactic acid bacteria (LAB) during fermentation. However, the buttery aroma of this compound as well as high concentration that required for food preservation have limited the use of diacetyl as a food preservative [97]. Similarly, acetaldehyde, produced by heterofermentative LAB, has shown antimicrobial activity against several pathogenic bacteria.

Bacteriocins are proteinaceous antibacterial compounds that constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides and they are commonly produced by different species of LAB [98]. Both Gram-negative and Gram-positive bacteria produce bacteriocins. The detection and identification of bacteriocins produced by LAB have received much attention whereas a large number of bacteriocins have been isolated and characterized and some are used in food preservation [9, 99]. Bacteriocins are cationic peptides that display hydrophobic or amphiphilic properties whereas is in most cases they target the bacterial membrane. Bacteriocins can be categorized into three classes: class I, the lantibiotics; class II, the small heat stable non lantibiotics; and class III, large heat labile bacteriocins [98]. The majority of bacteriocins fall into classes I and class II. Class I bacteriocins are small peptides that contain several unusual amino acids whereas class II bacteriocins are small, nonmodified, heat stable peptides [9]. Many bacteriocins are active against food borne pathogens and spoilage bacteria and being used in different food application as biopreservatives [99].

The most important bacteriocins include nisin, diplococcin, acidophilin, pediocin, bulgarican, helveticin, lactacin, and plantaricin [9, 98, 99]. Nisin, produced by various *Lactococcus lactis* strains, is the most thoroughly studied bacteriocin to date and is applied as food additive in over 50 countries in the word [98, 99]. Nisin is produced by fermentation of a modified milk medium by species of LAB, mainly *Lactococcus lactis*. Nisin is the only natural antimicrobial peptide approved by FDA for food applications. However, nisin has a limited spectrum of activity, does not inhibit Gram-negative bacteria or fungi, and is only effective at low pH [100, 101]. Pediocin is a bacteriocin produced by *Pediococcus acidilactici* and *P. pentosaceus* and is commonly used in fermented sausage. Pediocins are thermostable proteins that can function over a wide range of pH values with a strong bactericidal action against Gram-positive food spoilage and pathogenic bacteria such as *L. monocytogenes*, *Enterococcus faecalis*, *S. aureus*, and *C. perfringens* [102, 103]. Pediocin and nisin are the most studied bacteriocins produced by LAB due to their broad spectrum and stability in food. Natamycin, another bacteriocin, is a polyene antifungal produced by *Streptomyces natalensis* that is effective against molds and yeasts but has little or no effect on bacteria or viruses [104]. Natamycin has a very low solubility in water and is effective at very low levels.

Reuterin and reutericyclin produced by *Lactobacillus reuteri* are known to be active antimicrobials toward Gram-positive bacteria. Reuterin was also shown to have antimicrobial activity against Gram-negative bacteria, yeasts, moulds, and protozoa [105]. Reutericyclin is a tetramic acid derivative and reuterin is a mixture of monomeric, hydrated monomeric and cyclic dimeric forms of β-hydroxypropionaldehyde [106, 107]. Reuterin (β-hydroxypropionaldehyde) is a water soluble nonproteinaceous metabolite of glycerol [105, 107]. Reuterin was reported to exhibit high antimicrobial activity against *L. monocytogenes*, *E. coli* O157:H7, *S. choleraesuis* subsp. *Choleraesuis*, *Yersinia enterocolitica*, *Aeromonas hydrophila* subsp. *hydrophila*, and *Campylobacter jejuni* [108]. When reuterin was tested in combination with nisin, the antimicrobial activity of reuterin against Gram-negative bacteria was not enhanced [108].

5. Mechanism of actions of natural antimicrobials

Phenolic compounds are the main antimicrobial agents in plants [9]. Even though the exact antimicrobial mechanism of phenolic compounds is not clear, phenolic compounds are commonly known for their antimicrobial effects [9, 109]. The ability of phenolic compounds to alter microbial cell permeability, thereby permitting the loss of macromolecules from the cell interior, could help explain some of the antimicrobial activity [110]. Another explanation might be that phenolic compounds interfere with membrane function and interact with membrane proteins, causing deformation in structure and functionality [110]. A combination of phenolic compounds can provide synergistic antimicrobial effects and can contribute to a better antimicrobial reaction as compared to the reaction of an individual compound [111]. In addition, the effect of phenolic compounds can be concentration dependent; at low concentration, phenols affect enzyme activity while at high concentrations they cause protein denaturation. It has been reported that the antimicrobial activity of isothiocynates derived from onion and garlic is related to the inactivation of extracellular enzymes through oxidative cleavage of disulfide bonds and that the formation of the reactive thiocyanate radical was proposed to mediate the antimicrobial effect [112].

For peptides, the mechanism of action of antimicrobial peptides seems to involve multiple targets. The plasma membrane is the most cited target by peptides whereas recent studies have suggested intracellular targets to be more likely for some peptides [113]. Most antimicrobial peptides have nonspecific mechanisms and they may display some selectivity between different microorganisms. Antimicrobial peptides can assume amphipathic structures, which are able to interact directly with the microbial cell membrane. This action rapidly disrupt the membrane in several locations and result in the leaching out of vital cell components [75]. Studies on the mechanism of action of pleurocidin revealed that this peptide exhibits a strong membrane translocation and pore-formation ability reacting with both neutral and acidic anionic phospholipid membranes [114].

The antimicrobial activity of each fatty acid can be influenced by the fatty acids structure and shape including the length of carbon chain and number of double bounds. Fatty acids with longer carbon chains usually exhibit a stronger inhibitory effect than those with shorter chains. Unsaturated fatty acids also tend have higher inhibitory effect than saturated ones [84]. Medium and long chain unsaturated fatty acids are more active against Gram positive bacteria than Gram negative [84]. The most active saturated fatty acids have 10 or 12 carbons in the chain and antibacterial efficacy tends to decrease as the chain length gets longer or shorter [84]. Even though the antimicrobial effect of fatty acids is well studied, the mechanism in which fatty acids inhibit the bacterial growth is not well defined [84, 115]. In general, lipids inactivate microorganisms mainly by disruption of the bacterial cell wall or membrane, inhibition of intracellular replication, or inhibition of an intracellular target.

The antimicrobial action of bacteriocins is based on pore formation in the cytoplasmic membrane of the target microorganism. This leads to a loss of small intracellular molecules and ions and a collapse of the proton motive force [116]. This pore formation in the cytoplasmic membrane explains why nisin is less effective on Gram-negative bacteria since the outer membrane disables the entry of this molecule to the site of action [74, 117, 118]. In regard to nisin's mechanism of action, nisin first passes through the cell wall of Gram-positive bacteria by diffusion. However, the Gram-positive cell wall can act as a molecular sieve against nisin depending on its composition, thickness, or hydrophobicity [119]. After passing the cell wall, nisin will associate with the cytoplasmic membrane of the target microorganism. Then nisin will interact electrostatically with the negatively charged phosphate groups of surface membrane phospholipids [120, 121]. Nisin functions by interacting with the phospholipids in the cytoplasmic membrane of bacteria, thus disrupting membrane function and preventing outgrowth of spores by inhibiting the swelling process of germination. Nisin is highly active against many of the Gram-positive bacteria and is used by the cheese industry to control the growth of Clostridium spp. [120, 121].

6. Factors influencing the antimicrobial activity of natural products

The antimicrobial activity of natural compounds could be influenced by number of factors including botanical source, time of harvesting, stage of development, and method of extraction in addition to the composition, structure, and functional groups of the natural compounds [9]. In addition, food components and additives, which may interact with the natural antimicrobial compounds, play an important role in the use of natural components as food preservatives. For example, nonspecific binding of the cationic antimicrobial peptide to negatively charged food particles may reduce the effect of antimicrobial peptide in food systems [76]. In addition, most studies related to the antimicrobial efficacy of natural compounds have been conducted in vitro using microbiological media [8, 9, 56]. Thus, there is less understanding of the efficacy of natural compounds when applied to complex food systems. Foods are not sterile, so the results that are obtained by antagonistic assays can be influenced by a mixed microbial population. The heat-treatments which are necessary to kill microbial flora in foods might also modify the chemical composition of food matrixes and thus affect the efficiency of natural antimicrobials. Thus the areas of interactions between natural antimicrobials and food components required more knowledge to ensure the efficiency of natural antimicrobials when applied to food products.

The impact of food components, processing, and storage conditions need to be considered when applying natural antimicrobials to food products [9, 16, 37, 76]. For example, EOs of thyme, clove, and pimento were tested in peptone water and found effective against *L. monocytogenes*. However, when applied in a food system, the efficacy of EOs was reduced due to interaction with food components [37]. In vitro studies have shown EOs to have antibacterial properties against *L monocytogenes*, *S. typhimurium*, *E. coli*, *B. cereus*, and *S. aureus* [122]. However, higher concentrations of EOs were found to be required in foods compared to concentrations in laboratory media. In general higher concentrations of natural antimicrobials in food compared to that in laboratory media could be required to produce a similar effect when used in food systems [36]. In addition, combinations of natural antimicrobials such as combinations of EOs or organic acids could be more effective in food than single EO [16, 89]. The pH value also has a significant impact on the efficiency of natural antimicrobials. Low pH value (around 5) seemed to have the highest impact on the increase of EOs antimicrobial effect against *L. monocytogenes* [16]. This low pH value may increase the hydrophobicity of EOs which could enable easier dissolution in the cell membrane lipids of the target bacteria.

Bacteriocin antimicrobials can be influenced by various factors including the emergence of bacteriocin-resistant bacteria, conditions that destabilize the biological activity of proteins such as proteases or oxidation processes, binding to food components or inactivation by food additives, poor solubility, and uneven distribution in the food matrix in addition to food pH [9, 123]. The hurdle application of bacteriocins in combination with other bacteriocins or natural preservations could reduce resistance to bacteriocins in the target microorganism [124]. For example, the antimicrobial efficacy of bacteriocins LABB and LABP from appam batter and vegetable pickles *Lactobacillus* isolates, respectively, and nisin individually and in combinations was evaluated. Bacteriocins LABB and LABP have been shown to exhibit greater potency in combination with nisin or in combination with other bacteriocins has shown more potent than alone and these dual bacteriocin combinations were also able to inhibit *E. coli* and *Pseudomonas* spp [124].

7. Extraction methods and purification

Utilization of natural antimicrobial agents has recently been focused on extending the shelf life of foods, reducing or eliminating pathogenic bacteria, and increasing overall quality of food products [8, 16, 125]. However, for commercial applications of natural antimicrobial an effective extraction and purification methods are required. Commercially based plant-origin antimicrobials are most commonly produced by SD (steam distillation) and HD (hydro distillation) methods, and alternative methods such as SFE (supercritical fluid extraction) provide higher solubility and improved mass transfer rates. However, the manipulation of parameters such as temperature and pressure leads to the extraction of different components when a particular component is required. Bioengineering of the EO components also provides more available commercial products [36]. EOs and other plant extracts are principally responsible for antimicrobial activities in plants. These extracts can be obtained from plants and spices by various methods, such as steam, cold, dry and vacuum distillation [8, 110].

Common extraction procedures include chemical or heat treatments that could alter the active ingredients total content, functionality, or natural characteristics, or could produce unsafe compounds [126, 127]. Extracts from fruits and vegetables could be obtained using the direct extraction method. Direct extraction is a simple and safe procedure that prevents any possible alteration or destruction to the native structure of the active ingredients. For example, direct extract from guava and xoconostle pears were found to be effective against *Salmonella* spp. and *E. coli* O157:H7 respectively [43, 44]. Direct extract from fruits and vegetable can be obtained mechanically without any chemical, heating, or concentration processing. In addition, direct extracts can be applied to food products in a safe manner.

Water extraction is another simple method that could be used to extract water soluble phenolic compounds from seeds and leaves [61]. Heat treatment of water-soluble muscadine seed extracts was found to enhance the antimicrobial activity and to increase acidity, total phenolics, and individual phenolic compounds [61]. Alternative treatments such as ultrahigh-temperature processing, far-infrared radiation, and/or enzyme treatments could maximize the release of low-molecular-weight compounds from the polymeric form and thus reduce the antimicrobial efficiency [61, 128, 129]. Thus direct extract or juice extraction seems to be the most promising methods to avoid possible alteration or modification for the nature of natural antimicrobial compounds.

8. Summary

Natural antimicrobials are secondary metabolites that can be produced by living organisms including plants, animals, and microorganisms. The antimicrobial activity of these metabolites has only been scientifically confirmed in the last 30 years. Herbs and spices, generally contain EOs, are the most common plants used by the food industry as natural antimicrobials to inhibit foodborne pathogens and to extend food shelf life. Antimicrobial agents inherent to animals are mainly in the form of peptides (polypeptides). Microorganisms produce different compounds that could active against different pathogenic and spoilage microbes. Most antimicrobial compounds from microbial origin are produced during food fermentation as end products metabolites. The antimicrobial effect of natural products could be affected by different factors including botanical source, time of harvesting, stage of development, and method of extraction. In food applications, these natural antimicrobial compounds could be also influenced by food components, processing, and storage and thus could require higher concentrations than that used in laboratory media. On the other hand, the addition of natural antimicrobials to the food products may affect the sensory characteristics of the final product. Thus the challenge for practical application of natural antimicrobials is to develop an optimized combination of low doses of antimicrobial agents that could maintain product safety and extend the shelf life but minimize undesirable flavor and sensory changes associated with the addition of high concentrations of natural antimicrobials. However, only a limited number of natural antimicrobials are used in commercial food products.

Even though a huge number of natural antimicrobial are nowadays known, only a limited number of natural antimicrobial compounds are currently used in commercial applications due to the higher cost compared to chemical preservatives. Thus, more research on possible low cost production of natural products is required in order to be used in food systems. In most cases natural antimicrobials are extracted and purified to be tested or applied to food products. An extraction method with minimal processing such as direct extraction seems to be a promising method to avoid possible alteration or destruction of active ingredients. Natural antimicrobial appears to be the most promising solution for many food safety and food quality concerns. Thus, the future will anticipate more investigation of naturally antimicrobials to food products, especially in the areas of synergistic effectiveness and optimum concentrations.

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