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Herbal spices as alternative antimicrobial food preservatives: An update

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ABSTRACT
In recent years, there has been a constant search for alternative and efficient agents for food preservation aiming a partial or total replacement of antimicrobial chemical additives. A large number of herbal spices have been examined for their inhibitory action against the microorganisms responsible for food spoilage and foodborne illnesses. It has been reported by several researchers that natural antimicrobial and antioxidant properties of herbal spices reduce the risk of bacteria and fungi in foods and play a key role for enhancing shelf-life of foods and controlling food pathogens. Instead of these substantial findings, spices are still branded almost purely as flavoring agents. Recent research on antimicrobial and antioxidant activities of spices forms the basis for its application in raw and processed food preservation. It is now considered as a promising source of unique natural products to elucidate alternative food preservatives. This review gives a bird's eye view mainly on the recent research on antimicrobial potential of herbal spices and their derivatives against some pathogenic and spoilage microorganisms in foods, their antioxidant activities along with possible adverse effects and advocates for more research to elucidate their commercial utilization in food preservation.
KEY WORDS: Spices and derivatives; Antimicrobial activity; Antioxidant activity; Possible adverse effects; Alternative food preservatives

INTRODUCTION

Attention of the scientific community worldwide is shifting towards spices and herbs to harness their antimicrobial properties for use as natural food preservatives. Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods. The increasing antibiotic resistance of some pathogens that are associated with food borne illness is another concern (1-4). There has been increasing concern of the consumers about foods free or with lower level of chemical preservatives because this could be toxic for humans (5). Concomitantly, consumers have also demanded for foods with long shelf-life and absence of risk of causing foodborne diseases. This perspective has put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternative from other sources to obtain its goal concerning microbial safety (6) and one of the possible strategies towards this objective is the rational localization of bioactive phytochemicals (7).

Herbal Spices have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (8-10). In addition to imparting characteristic flavors, certain spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bacteriocidal activity (11). Herbal spices and their components are generally recognized as safe (GRAS), either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (7). The extracts of many spices and herbs have become popular in recent years for their antimicrobial and antioxidant properties and attempt to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. The antimicrobial activities of commonly used herbal spices (Table 1) form the basis for many applications including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies (12). Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods (13). Spices include leaves (bay, mint, rosemary, coriander, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander, cinnamon), rhizomes (ginger), and other plant parts (14). Although herbal spices have been well known for their medicinal, preservative and antioxidant properties, they have been currently used with primary purpose of enhancing the flavor of foods rather than extending shelf-life (15, 16).

Antimicrobial activity

Several scientific reports describe the inhibitory effect of herbal spices on a variety of microorganisms (Table 2), although considerable variation for resistance of different microorganisms to a given spice and herb and of the same organisms to different spices and herbs has been observed (17). Some of the important recent research on antimicrobial potential of herbal spices is cited here.

Jennifer Billing *et al* (18) found that 30 spices out of 43 seasoning exhibited antimicrobial property. Most effective seasoning inhibited 100 % to the bacterial species being tested. These were garlic, onion, allspice, and oregano. Next on the list, several spices inhibiting more than 75% of the microbial species. These were thyme, cinnamon, tarragon, cumin, cloves, lemongrass, bay leaf, capsicums and rosemary. Spices having inhibitory action less than 75% of the microbial species were marjoram, mustard, caraway, mint, sage, fennel, coriander, dill, nutmeg, basil, parsley, cardamom, pepper (white & black), ginger, anise seed celery seed, lemon/lime.

In a study garlic juice showed bacteriocidal activity against *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus mutants*, which are food pathogenic bacteria ; and *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus lactic* which are lactic acid bacteria in all concentrations (0.1-2.5% v/w) (19). In another study, an aqueous extract of garlic and onion were also reported to be effective against Gram-positive organisms, Gram-negative organisms, and fungi. A significant growth inhibition was shown by most of the organisms tested at random (20) .

It has been shown that Gram-positive bacteria were more sensitive than Gram-negative bacteria and sage had the highest antibacterial activity followed closely by rosemary. Allspice was the least effective. A combination of sage and rosemary enhanced the antibacterial effect (21).

Inhibitory effect of crude ethanolic extracts and essential oils of 14 spices including cardamom, cinnamon, cloves, coriander, cumin, garlic, ginger, holy basil, kaffir lime leaves and peels, lemongrass, mace, nutmeg, black and white pepper and turmeric against 20 serotypes of *Salmonella* and five species of other enterobacteria was also documented. The degree of antibacterial property of spices tested can be put in the following order. Cloves > Kaffir lime peels > cumin > cardamom > coriander > nutmeg > mace > ginger > garlic > holy basil > kaffir lime leaves (22).

Zuglal *et al* (23) studied the effectiveness of nine essential oils of spices to control mycotoxins producing moulds and noted that *Aspergillus paraciticus* and *Fusarium moniliforme* were inhibited by most of the spices used, while clove markedly reduced aflatoxin synthesis in infected grains. These findings could be useful for rural communities to prevent the synthesis of fungal toxins in contaminated grains by simple measures.

Inhibitory effect of various concentrations of mint, sage, bay leaf, anise and ground red pepper on the growth of *Aspergillus parasiticus* NRRL 2999 and its aflatoxin production was also analysed (24). In this study thyme presented significant delay in fungal growth up to 10 days at 2 % and up to 30 days at 4, 8 and 16 % .

Basilico *et al* (25) studied the inhibitory effect of oregano, mint, basil, sage and coriander on the mycellial growth of *Aspergillus ochracus* NRRL 3174 and its ochratoxin synthesis and they demonstrated that oregano completely inhibited the fungal activity of ochratoxin A-synthesis up to 14 days at 25ºC. Basil was effective to inhibit mycellial growth up to 7 days.

It has also been reported that basil, clove, garlic, marjoram, oregano, rosemary and thyme exhibited antibacterial activities against foodborne pathogen *Vibrio parahaemoliticus*. The sensitivity of these herbal spices was similar among different clinical serotypes including the emerging strain 03:K6 and suggested that the herbal spices can be practical for protecting seafood from the risk of contamination by *V. parahaemolyticus* (26).

In a study using 27 commonly used spices, garlic was shown to exert antimicrobial activity against *Bacillus subtilis*, *Clostridium botulinum*, *Escherichia coli*, *Salmonella typhosa*, and *Scigella parasysenteria*. Onion, clove and nutmeg were effective against all except *Bacillus subtilis*. Mace and Achiote (Annatto) have been shown to be especially effective against *Clostridium botulinum*. Also effective as antimicrobials are oregano, marjoram, pine, sage, rosemary, caraway, wasabi, allspice, pepper and ginger (27).

Inhibitory action of sage and rosemary is bacteriostatic at 0.03%, whereas at 0.05% it is bactericidal. This is attributed to terpene fraction, which is composed of borneole, anaeole, pinene, and camphor (28). Besides, antimicrobial activity of oregano and thyme has been attributed to their essential oils, which contain the terpenes, carvacrol, and thymol (29, 30).

Shan *et al* (1) reported that out of 46 spices and herb extracts, 12 exhibited high antibacterial activities against the five foodborne bacteria (*Bacillus cerelus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella anatum*). Many herb and spice extracts contained high levels of phenolics and exhibited antibacterial activity against foodborne pathogens.

It has also been shown that most of the foodborne bacterial pathogens examined were sensitive to extracts from plants such as cinnamon, cloves, garlic, mustard, onion and oregano and antimicrobial compounds in these spices are mostly in the essential oil fraction. The extent of sensitivity varied with the strain and environmental conditions imposed. Certain spices have a direct effect on the rate of fermentation by stimulating acid production in starter cultures (31).

It is well documented that the growth of both Gram-positive and Gram-negative foodborne bacteria, yeasts and moulds can be inhibited by garlic, onion, cinnamon, cloves, thyme and sage and other products. The fat, protein, water and salt contents of food influence microbial resistance. Thus, high levels of spices are necessary to inhibit growth of microorganisms in food than in culture media (14).

Several studies (32-34) showed that cinnamon, clove, pimento, thyme, oregano and rosemary had strong and consistent inhibitory effect against several pathogen and spoiling bacteria. In a study (35), it had been shown that cinnamon, cloves and mustard had strong; allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage and thyme had medium but black pepper, red pepper and ginger had weak antibacterial activity.

Grohs and Kunz (36) observed that spices mixtures were able to inhibit the growth of various meat-spoiling microorganisms (*Bacillus subtilis, Enterococcus sp.*, *Staphylococcus sp.*, *E. coli* K12 and *Pseudomonas fleuorescens*) providing stabilizing effect on colour and smell of fresh portioned pork meat.

It has also been reported that garlic and clove extracts were able to inhibit *Candida acutus*, *Candida albicans*, *Candida apicola*, *Candida catelumnata*, *Candida inconspicua*, *Candida tropicallis*, *Rhodotorula rubra*, *Sachromyces cerevisae* and *Trignopsis variabilis* and in some cases strong cidal effect was observed (37).

Cinnamon and clove oils inhibit the growth of moulds, yeasts and bacteria. Both cinnamon oil and clove oil added at 2% in potato dextrose agar (PDA) completely inhibited the growth of mycotoxigenic moulds (*A. flavus*, *A. parasiticus*, *A. ocraceus*, *Penicillium spp. M46, P. roqueforti, P. patulum* and *P. citrinum*) for various types up to 21 days (38) and could also inhibit the growth of yeasts (39,40). Similarly reported that cinnamon oil and clove oil could separately inhibit many other microbes including *Lactobacillus spp.*, *Bacillus thermoacidurans*, *Salmonella spp.*, *Corynebacterium michiganense*, *Pseudomonas striafaciens*, *Clostridium botulinum*, *Alternaria Spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Mucor spp.*, and *Penicillium spp.* (41).

Chemical compounds

The antimicrobial effectiveness of chemical compounds have also been investigated in order to improve the understanding about the cell targets of the molecules found in spices (42,43). Qualitative phytochemical analysis of crude spice extracts revealed the occurrence of alkaloids, cumarins,, flavonoids, saponins, terpenes and tannins. Turmeric, cloves and bay leaf showed the highest frequency of occurrence of these plant components. Terpenes were present in 94.12% of the samples evaluated (44).

Essential oil extracted from spices and herbs are generally recognized as containing the active antimicrobial compounds (45). The oils of cumin, cardamom and coriander were also highly inhibitory to the tested bacteria. The major constituents of these oils are: cuminaldehyde (20-72%) and monoterpene hydrocarbons (e.g. β-pinene, γ-terpinene, p-cymene) in cumin oil ; 1,8-cineole (20-60%) and α-terpinyl acetate (20-53%) in cardamom; linallol (74%) and other components (small amounts of α-pinene, γ-terpinene, geranyl acetate, camphor and geraniol) in coriander oil (46,47). Mace and nutmeg oils moderately inhibited the tested bacteria. Nutmeg oil contains monoterpene hydrocarbons (61-88%, e.g. α-pinene, , β-pinene, sabinene), oxygenated monoterpenes (5-15%), and aromatic ethers (2-18%), e.g. myristicin, elemicin, saffrole)(46). Whereas mace oil consists of monoterpenes (87.5%), monoterpene alcohols (5.5%), and other aromatics (7.0%). The major antimicrobial compound in garlic is allicin and has been found to possess antibacterial activity against both Gram-positive and Gram-negative bacteria (34). The major pungent components of ginger are gingerone and gingerol which have strong inhibitory activity against pathogenic bacteria (48). As most components of spice oils belong to the terpenoid family, there has been much speculation on the contribution of the terpene fraction of the oils to their antimicrobial activity (47). Some researchers have demonstrated the antimicrobial activity of the most common terpene compounds, such as thymol, carvacrol, linallol, eugenol, α -pinene, ans β-pinene in spices against several microbial strains (48-50). Eugenol, carvacrol, and thymol are phenol compounds and are found in cinnamon, cloves, sage and oregano. The essential oil fraction is particularly high in cloves, and eugenol comprises 95% of the fraction. The presence of these compounds in cinnamon and cloves, when added bakery items, function as mold inhibitors in addition to adding flavor and aroma to baked products . Paster *et al* (51) have shown that essential oils of oregano and thyme (which contain carvacrol and thymol) are effective as fumigants against fungi and stored grain. These investigators have proposed using them as an alternative to chemicals for preserving stored grains. The major antimicrobial components in cloves, cinnamon, and cassia have been reported to be eugenol and cinnamic acid (52). According to Bullerman *et al* (53), cinnamon contains 0.5-1.0% volatile oil of which 75% is cinnamic aldehyde and 8% eugenol. Clove contains 14-21% volatile oil, which is 95% eugenol. Some of the isolated compounds of herbal spices are cited in Table 3.

Antioxidant action

Spices and some of their compounds (Table 4) have achieved commercial importance as antioxidants. Beneficial influence of certain ground herbs and spices in fat stability has been known (54). Spice extractives, such as oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of "warmed-over" flavor in some products. Thus, some spices not only provide flavor and aroma to food and retard microbial growth, but are also beneficial in prevention of some off-flavor development of snacks food and meat products (55). Many spices are known to possess antioxidant compounds and are useful for preventing lipid oxidation in living organisms as well as in foods. Rosemary and sage were "remarkably effective" and by far the strongest antioxidants, with oregano, thyme, nutmeg, mace, and turmeric next in line. When the spices were tested in an oil-in-water emulsion, clove was found to exert the strongest antioxidant activity, followed by turmeric, allspice, mace, rosemary, nutmeg, ginger, cassia, cinnamon, oregano, savory, sage, anise, basil, cardamom, marjoram, and black & white pepper (28).

Ginger (*Zingiber officinale*) has been identified in several studies as a plant with a high antioxidant content (56,57). Extracts of several commonly Indian spices also have been shown to inhibit lipid peroxidation. In one study , relative antioxidant activities from highest to lowest were found in cloves, cinnamon, pepper, ginger and garlic (58) and the antioxidant activity of the extract was retained even after boiling for 30 minutes, suggesting that, unlike many antioxidants, the antioxidants in these spices were heat stable. In addition, synergistic antioxidant effects were

Table 1. List of commonly used herbal spices with their edible parts having antimicrobial activity

Herbal spices	Botanical Name	Family	Plant Parts Used
Allspice	*Pimenta diocia*	Myrtaceae	fruit
Basil	*Ocimum basilicum*	Labiatae	Leaf
Bay leaf	*Laurus nobilis L.*	Lauraceae	Leaf
Caraway	*Carum carvi L.*	Apiaceae	Fruit
Cardamom	*Eletataria cardamomum*	Zingiberaceae	Seed
Cinnamon	*Cinnamomum cassia Presl*	Lauraceae	Bark
Cloves	*Eugeniacaryophylata Thunb*	Myrtaceae	Bud
Coriander	*Coriandrum sativum L.*	Apiaceae	Whole plant
Cumin	*Cuminum cyminum L.*	Apiaceae	Seed
Dill	*Anethum graveolens*	Apiaceae	Seed
Fennel	*Foeniculum vulgare*	Apiaceae	Seed/leaf
Fenugreek	*Trigonella foenum-graecum*	Fabaceae	Seed
Garlic	*Allium sativum*	Alliaceae	Bulb
Ginger	*Zingiber officinale*	Zingiberaceae	Rhyzome
Kaffir lime	*Citrus hystrix DC*	Rutaceae	Leaf/peel
Lemongrass	*Cymbopogon citratus*	Poaceae	Rhyzome
Mace	*Myristica franrans*	Myristicaceae	Seed
Marjoram	*Origanum majorana*	Lamiaceae	Leaf
Mint	*Mentha Canadensis L.*	Lamiaceae	Leaf/branch
Mustard	*Brassila alba*	Cruciferae	Seed
Nutmeg	*Myristica fragrans Houtt*	Myristicaceae	Fruit
Onion	*Allium cepa*	Alliaaceae	Bulb
Oregano	*Oreganum vulgare L.*	Lamiaceae	Leaf
Parsley	*Petroselinum crispum L.*	Apiaceae	Leaf
Pepper (Black & white)	*Piper nigrum L.*	Piperaceae	Fruit
Rosemary	*Rosemarinus officinalis L.*	Lamiaceae	Leaf/branch
Sage	*Salvia officinalis L.*	Lamiaceae	Leaf/branch
Tarragon	*Artemisia dracunculus*	Asteraceae	Leaf
Thyme	*Thymus vulgaris L.*	Lamiaceae	Leaf/branch
Turmeric	*Curcuma longa L.*	Zingiberaceae	Rhyzome

Table 2. Herbal spices effective against some microorganisms

Herbal Spice	Microorganism
Allspice	*Micotoxigenic Aspergillus, Fusarium spp., Alternaria spp., Cladosporium spp.*
Basil	*Aeromonas hydrophylla, Pseudomonas flourescens, Ascophaera apis, Staphylococcus aureus, Escherichia coli, Aspergillus niger*
Bay leaf	*Clostridium botulinum*
Caraway	*Agrobacterium tumefaciens, Ralstonia salanacearum, Erwinia carotovora*
Cinnamon	*Mycotoxigenic Aspergillus, Aspergillus paraciticus*
Cloves	*Mycotoxigenic Aspergillus*
Coriander	*Ascophaera apis, Alternaria alternata, Fusarium solani*
Cumin	*Penicillium notatum, Aspergillus niger, Aspergillus funigatus, Microsporum canis*
Fennel	*Staphylococcus aureus, Bacillus subtilis*
Fenugreek	*Bordetella bronchiseptica, Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Micrococcus flavus, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Proteus vulgaris*
Garlic	*Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, Candida albicans, Bacillus*

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	cereus, Bacillus subtilis, Mycotoxigenic Aspergillus
Mint	*Aspergillus ochraceus*
Mustard	*Mycotoxigenic Aspergillus*
Onion	*Aspergillus flavis, Aspergillus paraciticus*
Oregano	*Mycotoxigenic Aspergillus, Salmonella spp., Listeria monocytogenes, Vibrio parahaemolyticus, Staphylococcus aureus, Aspergillus niger*
Parsley	*Staphylococcus aureus, Escherichia coli, Candida albicans, Aspergillus niger*
Rosemary	*Bacillus cereus, Staphylococcus aureus, Vibrioparahaelyticus*
Sage	*Bacillus cereus, Staphylococcus aureus, Vibrio parahaelyticus*
Thyme	*Vibrio parahaemolyticus, Streptococcus pneumoniae R36A*

Source : (i) Shelef, 1983 (14); (ii) www.indianspices.com/pdf/medi_prop.pdf

Table 3. Chemical compounds isolated from some herbal spices

Spice	Chemical Compounds
Allspice	Eugenol
Basil	Linallol, Eugenol
Bay leaf	Linalool
Caraway	Carvone, Limonene
Cardamom	Cineol, Limonene
Cinnamon	Cinnamaldehyde, ugenol
Cloves	Eugenol, β-caryophytine
Coriander	Linallol
Cumin	Phenolic acids, Flavonoids
Dill	Carvon, Linallol
Fennel	Phenolics, Flavonoids
Fenugreek	Steroids, Polyphenolic substances
Garlic	Allicin
Ginger	Sesquiterpenes, Di-allyl-di-sulphide
Kaffir lime	Citronellol
Lemongrass	Citronellol, Geraniol
Mace	Omega-chloroacetophenon, Phenacyl chloride
Marjoram	Monoterpenes
Mint	Methyl acetate, Menthol
Mustard	Allyl isothiocyanate
Nutmeg	Eugenol
Onion	Linolic acid, Palmitic acid
Oregano	Thymol, Carvacrol
Parsley	Ascorbic acid
Pepper	Lycopene, β-carotene, Ascorbic acid (Vit. C)
Rosemary	Carnasol, Rosanol, Carnosic acid
Sage	Thymol, Carvacrol
Tarragon	Z-anaethole, methyl-eugenol
Thyme	Carvacrol, Thymol
Turmeric	Curcum

Table 4. Antioxidants isolated from some herbal spices

Spice	Antioxidants
Rosemary	Carsonic acid, Carnosol, Rosemarinic acid, Rosemanol
Sage	Carnosol, Carnosic acid, Rosemanol, Rosemarinic acid
Oregano	Derivatives of phenolic acids, Tocopherols
Thyme	Carvacrol, Thymol, p-cymene, Caryophyllene, Carvone, Borneol
Marjoram	Flavonoids
Allspice	Pimentol

Source : www.indianspices.com/pdf/medi_prop.pdf

Table 5. Summary on the current research on antimicrobial potential of herbal spices

Sl. No.	Findings
1.	Microorganisms differ in their resistance to a given spice (17).
2.	A given microorganism differs in its resistance to various spices (17).
3.	The fat, protein, water and salt contents of food influence microbial resistance (32)
4.	Gram-negative bacteria are more resistant than Gram-positive bacteria (21)
5.	The effect of spice may be inhibitory or germicidal (28).
6.	Spices are less effective in foods than in cultured media (14).
7.	Amounts of spices added to foods are generally too low too prevent microbial contamination (14).
8.	Mixture of spices are more active antimicrobial agents than single one (21, 36, 65).
9.	Antimicrobial effectiveness of spices can be classified as strong, medium and weak (32-35).
10.	Spices harbour microbial contaminants (82-85).
11.	Essential oil extracted from spices are generally recognized as containing the active antimicrobial compounds (31, 45).
12.	Crude spice extracts revealed the occurance of alkaloids, flavonoids, saponins, terpenes and tannins (42).
13.	Most components of spice oil belongs to the terpenoid family (46).
14.	There is a high positive correlation between antimicrobial activity, total phenolics content and antioxidant capacity in spices (62,64).
15.	Antioxidant potential of spices are useful for preventing lipid oxidation in living organisms as well as in food (28, 56).
16.	Antioxidants present in spices are heat stable ((58).
17.	The mode by which the microorganisms are inhibited by essential oils and their chemical compounds seems to involve different mechanisms (6, 55, 70-76).
18.	Spices mostly used as antimicrobials and antioxidants do not exhibit toxicity at levels consumed and are considered as GRAS (generally recognized as safe) substance (91-93).

observed with combination of spices (59). Linallol, a terpene tertiary alcohol and major phytochemical in coriander seeds, is an antioxidant with high concentrations (60). Carcumin (diferuloyl methane), the active ingradient of the spice turmeric (*Curcuma longa*) is a strong antioxidant and reported several times more potent than Vitamin E as a free radical scavenger (61). Many studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant and pharmaceutical properties (62-64). Some studies claim that the antioxidant phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (65). Besides, it has also been reported that phenolic phytochemicals are excellent sources of antioxidants and antimicrobials in the diet, which contribute positively to the management of oxidation-linked and infectious diseases. Traditionally individual phenolics have been targated as antimicrobials with low efficacy. However, mixtures of phenolic phytochemicals in synergistic combination with lactic acid can strongly inhibit the growth of microorganisms. Such a strategy can be used for enhancing food safety in food industry (66). Numerous studies have also tested the antioxidant capacity of spices or their constituents *in vivo*. The antioxidant capacity of ginger has been reported in relation to LDL cholesterol oxidation in apolipoprotein E-defficient mice (67). Coriander, cinnamon and cardamom have also been shown to have antioxidant effects in rodents, in part through activating antioxidant enzymes in various tissues (68).

Relationship between antimicrobial activity, total phenolic content and antioxidant capacity

Shan *et al* (62) found high correlation, based on the analysis of a large number of spice samples and the wide ranges in total phenolic content and antioxidant capacity. They found a good linear relationship between antimicrobial activity and total phenolic content. The R^2 values were between 0.93 and 0.73 and decreased in the following order : *S. aureus* > *B. cereus* > *E. coli* > *S. anatum* > *L. monocytogenes*. Their results emphasized the importance of phenolic compounds in antibacterial activity of spice extracts and also indicated that the phenolic compound significantly contributed to their antibacterial activity. In addition, they reported that highly positive relationship also exits between antibacterial activity and antioxidant capacity of the extract. The R^2 values were between 0.84 and 0.70 and decreased in the following order : *E. coli* > *S. aureus* > *B. cereus* > *S. anatum* > *L. monocytogenes*. It has also been demonstrated that many of the spice extracts contained high levels of phenolics , possessed strong antibacterial activity and they could be a potential source for inhibitory substances against some foodborne pathogens as well as antioxidant agents (1).

Possible mechanism of antimicrobial action

(a) Against bacteria

Though exact mechanism of antibacterial action of spices and derivatives is not yet clear (69), some hypothesis have been proposed for the possible mechanism of antimicrobial action of spices. These are:

- Hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins followed by partition in a lipid bilayer (50).
- Purturbation of membrane permeability consequent to its expansion and increase fluidity causing the inhibition of membrane embedded enzymes (70).
- Membrane disruption (71).
- Destruction of electron transport systems (72).
- Cell wall purturbation (73).
- Gram-negative bacteria are more resistant than Gram-positive bacteria due to the presence of their cell wall lipopolysaccharide (74). This cell wall lipopolysaccharide

may prevent the essential oils active compounds reach the cytoplasmic membrane of Gram-negative bacteria (75).

(b) Against fungus

Mechanisms of antifungal action of spices and derivatives have not yet been fully established. Only a little information is available regarding fungstatic or fungicide effect of spices and derivatives. It has been reported that the inhibitory action of natural products on mould involves (i) cytoplasm granulation, (ii) cytoplasmic membrane rupture and (iii) inactivation and /or inhibition of intercellular and extra cellular enzymes. These biological events would take place separately or concomitantly culminating with mycelium germination inhibition (76). Also it is reported that plant lytic enzymes act in fungal cell wall causing breakage of ß-1,3 glycan, ß-1,6 glycan and chitin polymers (6).

Microbial contamination of spices

Presence of pathogenic and spoiling microorganisms in spices could act as vehicles for microorganisms to enter in foods. Mousuymi and Sarkat (77) reported the presence of various microorganisms including total heterotrophus *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella* and toxigenic moulds in spices. Flannigan and Hui (78) reported that 1 out of 20 spices contained *Aspergillus flavus* and 4 out of these spices supported the growth of these mould and production of aflatoxin. Spices may contain over 10^8 aerobic bacteria per gram (79). Mean standard plate count of over 10^6 per gram was obtained from black pepper, ginger and paprika (80). The International Commission of Microbiological Specifications for Foods (1974) has set up maximum limit of 10^6, 10^4 and 10^3 cfu of total aerobic mesophilic bacteria (TAMB), fungi, coliforms and *E. coli* respectively, per gram spice (81). In German legislation, standard limit value for TAMB, *Bacillus cereus*, and *S. aureus* is 10^5, 10^4 and 10^2 cfu per gram of spices, respectively (77). Thus, there is a strong need to evaluate and control the microbial quality of spices including bacterial and mycological analyses and presence of microbial toxic metabolites (82-85).

Possible adverse effects :

Some spices have inherent toxic substances and produce some allergic reactions which in large amounts are contraindicated as reported by Shibamoto and Bjedanes (86). Spices which are toxic to microbes and insects are also to some extent dangerous for people as well, but toxicity depends on dosage – the amount of toxic compounds per total body weight (87,58). Some scientists think that pregnant women tend to reject spicy foods during early pregnancy (as part of the nausea and vomiting called morning sickness) to shield the embryo from compounds that might cause deformities or miscarriage. Also there are a few data suggesting that some spices may increase cancer risk. Several care-control studies in India have observed that gastrointestinal cancer was higher with consumption of spicy food and chili (88, 89). The work of Jensen-Jarolim *et al* (90) suggests that spices may increase intestinal epithelial permeability through loosening cell contacts (e.g. paprika, chili pepper) or decrease permeability (e.g. black pepper, nutmeg) , possibly by self swelling. Generally, spices mostly used as antimicrobials and antioxidants do not exhibit toxicity at levels consumed and are considered as GRAS substance (7, 91-93).

Conclusions and Recommendations

The present review article demonstrated that many of the spice extracts contained high level of phenolics and possessed strong antimicrobial and antioxidant activities (Table 5) and could be a potential source for inhibitory substances against some foodborne pathogens as well as antioxidant agents. Still little information is available emphasizing the preservative and antimicrobial role of spices in the prevention of foods of the microbial action. Most of these studies have been carried out in laboratory culture media but a few attempts have also been made to assess their antimicrobial potential in food systems. However, in general, these studies use high doses of pure compounds or spice extracts. Only a handful of spice and their constituents have been tested and nothing is known about the interaction and contribution of spice mixtures. Furthermore, the capacity of spices to inhibit food spoilage microorganisms within the context of culinary use has not been evaluated. Therefore, before including spices and/or their derivatives in food preservative systems, some evaluations about microbiological quality, economic feasibility, antimicrobial effect for a long time, compatibility, toxicity, interactions and contribution of spice mixtures, as well as antagonistic or synergistic effects, if any, should be carried out. Presently, spices are cheap and easily available even in countries where they do not grow. It is evidently a group of plants, which has not been utilized in food technology despite its undisputed potential.

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REFERENCES

1. B.Shan, Y.Z. Cai, J.D. Brooks and H. Corke . The *in vitro* antibacterial activity of dietary spices and medicinal herbs extracts. *Int. J. Microbiol*., 117(1) : 112-19 (2007).
2. J.H.Meng , S.H. Zhao, M.P. Doyle, and S.W. Joseph . Antibiotic resistance of *Escherichia coli* 0157 : H7 and 0157 : NM isolated from animals, food and humans. *J. Food Prot*. 61 : 1511- 14 (1998).
3. V. Perrenten, N. Giampa , U. Schuler-Schmid, M. Teuber. Antibiotic resistance zenes in coagulase-negative *Staphylococci* isolated from food. *Systematic and Appl. Microbiol*. 21(1) : 113-20 (1998).
4. F.R. Stermitz , J. Tawara-Matsuda, P. Lovenz , P. Mueller, L. Zenewicz,K. Lewis. 5-methoxydnocarpin-D and pheophorbide A : berberis spices components that potentiate berberinee growth inhibition of resistant *Staphylococcus aureus*. *J. Nutr. Prod*., 63 : 1146-49 (2000).
5. C. Bedin, S.B. Gutkoski , J.M. Weist. Antividade antimicrobina das especiarias. *Hygiene Alimentar*, 13 : 26-29 (1999).
6. S. Brull , P. Coote . Preservative agents in foods : mode of action and microbial resistants mechanisms. *Int. J. Food Microbial*., 50 : 1-17 (1999).
7. E.J. Smid, and G.M. Gorris. Natural antimicrobials for food preservation. In : *Handbook of Food Preservation* (MS Rhman, Editor; Marceldekker, N.Y.,1999) pp. 285-08.
8. L.R. Buchart. Antimicrobial properties of spices and their essential oils.In : *Natural Atimicrobial Syastems and Food Preservation*(YM Dillon and RG Board (Eds), CAB International, Oxon ,1994), pp. 167-79.
9. H.G.Cutler. Natural flavor compounds as potential antimicrobials, insecticides, and medicinals, *Agro –Food-Industry Hi-Tech* 6 : 19-23 (1995).
10. N. Nakatani. Antioxidative and antimicrobial constituents of herbs and spices In : *Spices, Herbs, and Edible fungi*. (G. Charalambous (Ed), Elsevier-science, N.Y. (1994), pp. 251-71.
11. L.R. Buchart and D.A. Golden . Antimicrobials occurring naturally in foods. *Food Technology*, 43 : 134-42 (1989).
12. M. Lis-Balchin and S.G. Dens. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Microbiol*., 82 : 759-62 (1997).

13. P.M.L.Germano and M.I.S. Germano. Importantiae riscos das especiarias. *Higiene Alimentar*, 12 : 23-31 (1998).
14. L.A. Shelef . Antimicrobial effects of spices, *J. Food Safety*. 6 : 29-44 (1983).
15. S.E.Aktug and M. Karapinar . Sensitivity of some common food-poisoningbacteria to thyme, mint and bay leaves. *Int. J. food Microbiol*., 3 : 349-54 (1986).
16. C.A. Ristori , M.S. Pereira and D.S. Gelli. O efito da pimenta do reino moida frente a contaminacao *in vitro* can *Salmonella rubislaw*. *Rivista do Instituto Adolfo Lutz*., 61 : 131-33 (2002).
17. Akgul and M. Kivanc. Inhibitory effect of selected Turkish spices and oregano components on some foodborne fungi. *Int. J. Food Microbiol*., 6 : 263-68 (1988).
18. J. Billing, P.W. Sherman . Antimicrobial functions of spices : Why some like it hot. *The quarternary Review of Biology*, 73(1) : 3-49 (1998).
19. K.S. Chung, K. Seung Youn, J.I. Yeon Kim . The antibacterial activity of garlic juice against pathogenic bacteria and lactic acid bacteria. *Hangug misaengmul saengmyeong gomg haghoeji.* 31(1) : 32-35 (2003).
20. E.I. Elmina, S.A. Ahmed , A.G. Makkawi , J.S. Mossa . The antimicrobial activity of garlic and onion extracts. *Pharmazie*. 38(11) : 747-48 (1983).
21. L.A. Shelef , O.A. Naglik, D.W. Bogen. Sensitivity of some common foodborne bacteria to the spices sage, rosemary and allspice. *J. Food Sci.,* 45(4) : 1042-44 (1980).
22. S. Nanasombat and P. Lohasupthawee. Antibacterial activity of crude ethanolic extracts and essential oils of spices against *Salmonella* and other enterobacteria. *KMITL Sci. Tech. J.* 5(3) : 527-38 (2005).
23. S. Zuglal , R. Govinden , and B. Odhav B. Spices oils for the control of co-occurring micotoxin-producing fungi. *J. Food Prot*., 65 : 683-87 (2002).
24. M. Karapinar. The effect of citrus oil and some Turkish spices on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999. *Int. J. Food Microbiol*. 12 : 239-45 (1985).
25. M.Z. Basilico , and J.C. Basilico. Inhibitory effects of some spices essential oils on *Aspergillus ochracus* NRRL 3174 growth on ochratoxin-A production. *Int. J. Food microbial*. 29 : 238-41 (1999).
26. Y. Yani, M. Satomi and H. Oikawa. Antimicrobial effect of spices and herbs on *Vibrio parahaemolyticus*. *Int. J. Food Microbiol.,* 111(1) : 6-11 (2006).
27. Nakatani Nabuji . Antioxidative and antimicrobial constituents of herbs and spices. *Herbs and Edible Fungi*.(Elsevier Science, B.V.,1994). pp. 251-71.
28. M.J. Jay. *Modern Food Microbiology*. (Indian 1st Edition, CBS Publishers and Distributors, Delhi, India, 1987).
29. L.R. Beuchart. Sensitivity of *Vibrio parahaemoliticus* on spices and organic acids., *Food Res*., 11 : 273 (1976).
30. L.L. Zaika and J.M. Kissinger. Effect of some spices on acid production on starter cultures. *J. food Prot.* 42 : 576 (1976).
31. E. Ceylan and Y.C. Fung Daniel. Antimicrobial activity of spices.*J Rapid Methods and Automation in Microbiol,* 12(1) : 1-55 (2004).
32. B. Outara, R.E. Simard, R.A. Holley, G.J.P. Piette and A. Begin. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms.*Int. J Food Microbiol*. 37 : 155-62 (1997).
33. P. Aureli, A. Constatini, and S. Zoles. Antibacterial activity of some plants essential oils against *Listeria monocytogenes*. *J Food prot*. 55 : 344-48 (1992).
34. D.E. Conner. Naturally occurring compounds. In : *Antimicrobials in foods* (Davidson P.M. & Branen A.L. (Eds), N.Y. Marceldekker, 1993). pp. 441-68.
35. L.L. Zaika . Spices and Herbs : Their antimicrobial activity and its determination. *J Food safety.* 9 : 97-18 (1988).
36. B.M. Grohs and B. Kunz . Use of spices for the stabilization of fresh portioned pork. *Food Control.* 11: 433-36 (2000).
37. D. Arora and J. Kaur. Antimicriobial activity of spices. *Int. J. Antimicrobiol Agents.* 12 : 257-62 (1999).
38. M.A. Azzouz, and L.B. Bullerman . Comparative antimicotic effects of selected herbs, spices, plant components, and commercial antifungal agents. *J Food Prot.,* 45 : 1298-01 (1982).
39. D.E. Conner and C.R. Beuchart . Effect of essential oils of plants on growth of foods spoilage yeasts. *J. Food Sci.,* 49 : 429-34 (1984).
40. B. Suksrikram . *Herbs and spices*.(Amorn Ptrinting, Thiland, 1987).
41. K.M. Soliman and R.I. Badea . Effect of oil extracted from some medicinal plants on different microtoxigenic fungi. *Food and Chemical Toxicology*, 10 : 1669-75 (2002).
42. A.K. Karatzas, M.H.J. Bennit , E.J. Smid and E.P.W. Kets. Combined action of S-carvone and mild heat treatment on *Listeria monocytogenes Scot A*. *J. Appl. Bacteriology*, 89 : 296-301 (2000).
43. B.L. Vasquez , C. Fente , C.M. Franco, M.J. Vasquez and A. Cepeda. Inhibitory effect of eugenol and thymol on *penicillium citrium* strains in cultured media and cheese. *Int. J Food Microbiol*., 67 : 157-63 (2001).
44. Hassan , Q. Mandeel , Z. Esa. Antibacterial activity of certain spice extracts. *Spices and Aromatic Crops*. 12(1) : 146-52 (2003).
45. Antimicrobial effects of spices and herbs : Available In : *http://www.hi-tm.com/Documents/spices.html*
46. C.C. d Guzman , and J.S. Siemonsma . *Plant resoursces of south-east Asia no. 13 : Spices*. (Bogor, Indonesia, Prosea Foundation, 1999).
47. R.R. Ashurst (1991) *Food flavorings*. (Scotland, Blackie and Sons Ltd., 1991)
48. K. Hirasa and M. Takemasa. *Spice Science and Technology*.(N.Y., Marceldekker Inc., 1998)
49. J. Kim , M.R. Marshall and C. Wei. Antibacterial activity of some essential oil components against five foodborne pathogens. *J. Agri. Food Chem*. 43(11) : 2839-44 (1995).
50. B.J. Juven , J. Kanner , F. Schved and H. Weisslouicz . Factors that interact with the antimicrobial action of thyme essential oil and its active constituents. *Appl. Bacteriol.*, 76 : 626-31 (1994).
51. N. Paster N, M. Menasherov, U. Ravidu and B. Juven . Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grains. *J. Food Prot.* 58 : 31-85 (1994).
52. P.M. Davidson, S.L. Post, A.L. Brannenal *et al*. Naturally occurring and miscellaneous food antimicrobials. In : *Antimicrobials in Food* (Brannenal, AL and Davidson PM Eds., Marceldekker Inc., 1983) pp. 371-19.
53. L.B. Bullerman, F.Y. Lieu and S.A. Seier Inhibition of growth and aflatoxin production of cinnamon and clove oils : cinnamic aldehyde and eugenol. *Food sci.,* 42 : 1107 (1977).
54. P. Schuler . Natural antioxidants exploited commercially. *Food Antioxidants*.(Hadson B.J.F. Eds., Elsevier Sci. Pub., Essex, England, 1990).
55. J. Giese. Spices and seasoning blends : A taste for all seasons. *Food Technol*., 48(4) : 87-98 (1994).
56. S. Shobna , K.A. Naidu. Antioxidant activity of selected Indian spices. *Prostaglandins Leucot Essent Fatty Acids*. 62 : 107-10 (2000).
57. B.L. Halvorsen, K. Holte, M.C.W. Myhrstad *et al*. A systematic screening of total antioxidants in dietary plants. *J. Nutr*., 132 : 461-71 (2002).
58. J.W. Lampe. Spicing up a vegetarian diet : Chemopreventive effects of phytochemicals. *Am. J. Clin. Nutr*., 78(3) : 579S-583S (2003).
59. T.P. Krishnakanta, B.R. Lokesh . Scavenging of superoxide anions by spice principles. *Indian J Biochem Biophys.,* 30 : 133- 34 (1993).
60. B.L. Zhao, X.J. Li , R.G. He , S.J. Cheng , W.J. Xin . Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys.,* 14 : 175-85 (1989).
61. Y.Z. Cai , Q. Luo, M. Sun , and H. Corke . Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Science*., 74 : 2157-84 (2004).
62. B. Shan , Y.Z. Cai , M. Sun , H. Corke. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agricult. Food Chem*. 53 : 7749-59 (2005).
63. C.Q. Wu , F. Chen , X. Wang , H.J. Kim, G.Q. He , V. Haley- Zitlin, G. Huang. Antioxidant constituents of feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. *Food Chem*., 96 : 220-27 (2006).
64. Y. Hara-Kudo, A. Kobayashi, Y. Sugita-Konishi and K. Kondo. Antibacterial activity of plants used in cooking for aroma and taste. *J Food Prot*. 67 : 2820-24 (2004).
65. Y.T. Lin , R.G. Lobbe and K. Shethy . Inhibition of *Vibrio parahaemolyticus* in seafood systems using oregano and cranberry phytochemical synergies and lactic acid. *Innovative Food Sci. & Eng. Technol*., 6(4) : 453-58 (2005).
66. B. Fuhram , M. Rosenblat, T. Hayek , R. Coleman , M. Aviram.Ginger extract consumption reduce plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic apolipoprotein E-deficient mice. *J. Nutr.,* 130 : 1124-31 (2000).
67. J.N. Dhuley. Antioxidant effects of cinnamon (cinnamonum) bark and greater cardamom seeds in rats fed with high fat diet. *Indian J. Exp. Biol.,* 37 : 238-42 (1999).
68. K.R. Anilkumar, N.S. Nagaraj , K. Santhanam. Effect of coriander seeds on hexachlorocyclohexane -induced lipid peroxidation in rat liver. *Nutr. Res*., 21(11) :1455-62 (2001).
69. R. Lanciotti , A. Gianotti , N. Patrignani , N. Belleti , M.E. Guerzoni and F. Gardini. Use of natural aroma compounds to improve shelf-life of minimally processed fruits. *Trends in Food Sci. & Technol*., 15 : 201-08 (2004).
70. S.D. Cox , C.M. Mann and J.L. Markham. The mode of antimicrobial action of the essential oil of *Malaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol.* 88 : 170-75 (2000).
71. D.L.R. Caccioni , M. Guizzardi , D.M. Biondi , A. Renda , and G. Ruberto Relationship between volatile components of citrus fruit essential oil and antimicrobial action on *Penicillium digitatum* and *Panicillium italicum*. *Int. J. Food microbiol.,* 88 : 170-75 (2000).
72. C.C. Tassou , K. Koutsoumanis and G.J. E. Nychas. Inhibition of *Salmonellaenteridis* and *Staphylococcus aureus* on nutrient broth by mint essential oil. *Food Res. Int.,* 48 : 273-280 (2000).
73. B. Odhav , S. Juglal , and R. Govinden. Spices oliz for the control of co-occurring mycotoxins producing fungi. *European Food Res. & Technol*.,65 : 683-87 (2002).
74. A.D. Russel. Mechanisms of bacterial resistance to non-antibiotics : food additives and pharmaceutical preservatives. *J. Appl. Bacteriology*, 71 : 191-01 (1991).
75. N. Chanegriha , N. Sabaou, A. Baaliouamer and B.Y. Meklati . Activite anti-bacterienne of antifongique de I' huile essentielle du cypress d Ageric.*Rivista Italiana Epposs*, 12 : 5-12 (1994).

76. M.M. Cowan. Plant products as antimicrobial agents. *Clin. Microbiol. Rev*., 12 : 564-82 (1999).

77. B. Mousuymi , and P.K. Sarkat. Microbiological quality of some retail spices in India. *Food Res. Int*., 36 : 469-74 (2003).

78. B. Flannigan , and S.I. Hui . Occurance of aflatoxin producing strains of *Aspergillus flavus* in the mould flora of ground spices.*Appl. Bacteriol*., 41 : 411-18 (1976).

79. W.J. Banswart . *Basic Food Microbiology*. (The AVI publishing Company, Inc, West Port Connecticut., 1981).

80. R.M. Julseth and R.H. Diebel. Microbial profile of selected spices and herbs at import. *J. Milk Food Technol.,* 37 : 441-49 (1974).

81. C.D. Zamboni , H.I. Alves , R.M.M.S. Rodrigues, N. Spiteri, M.C. Atui and MC Santos. Frudes e Sujdades em condimentos comercializadors na cidade de sao Paulo. *Revista do Instituto Adolfo Lutz.,* 51 : 19-22 (1991).

82. J.E. Fernandez , D.D. Kriviruchco and O.J. Mitschele. Estudio microbiologico de especiarias. *Rivista Argentina de Microbiologia,* 16 : 111-18 (1984).

83. S.I.I. Abdel-Hafez and A.H.M. El-said. Effect of garlic, onion and sodium benzoate on the microflora of pepper, cinnamon, and rosemary in Egypt.*Int. Bacterioration & Biodegradation*. 39 : 67-77 (1997).

84. F.C.O. Freire , and L. Offord. Bacterial and Yeasts counts in Brazilian commodities and spices. *Braz. J. Microbiol.,* 2 : 145 – 48 (2003).

85. A.Benezet ., J.M. Osa , E. Pedregal , M. Botas , N. Olmo , and F. Perez-Flores.Microbiological quality of spices used in meat products. *Alimentaria*, 349 : 65-71(2003).

86. T. Shibamoto , and L.F. Bjeldanes. *Introduction to Food Technology*.(Academic Press Inc., San Diego, California, USA., 1993).

87. Plant Scents : *American Scientist Online*, 92(6) : 514 (2004) Available In : www.americanscientist.org/template/AssetDetail/assetid/37190

88. A.Mathew , P. Gangadharan , C. Varghese , M.K. Nair. Diet and Stomach cancer :A cancer-control study in South Indian. *Eur. J. Cancer Prev.* 9 : 89-97 (2001).

89. R.K. Phukan , C.K. Chetia, M.S. Ali , J. Mahatma. Role of dietary habits in the development of esophageal cancer in Assam, the north-eastern region in India.*Nutr. Cancer*. 39 : 204-09 (2001).

90. E. Jensen-Jarolim , L. Gajdzik , I. Haberl , D. Kraft , O. scheiner , J. Graf. Hot spices influence permeability of human intestinal epithelial monolayers.*Nutr*. 128 : 577-81 (1998).

91. S.O.P. Oiye , N.M. Muroki. Use of spices in foods. *The J. Food Techno. in Africa*. 7(2) : 39-44 (2002).

92. E.L. de Souza, T.L.M. Stamford E. de Oliveria Lima, V.N. Tarjano, J.M.B. Filho. Antimicrobial effectiveness of spices : an approach for use in food conservation systems. *Braz. Arch. Biol. Technol.* 48(4) : 1-13 (2005).

93. V.A. Pandit and L. A. Shelf . Sensitivity of *Listeria monocytogenes* to rosemary (*Rosimarinus officilalis L.*) . *Food Microbiol*. 11 : 57-63 (1994).