Review

Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application

Pradeep Singh Negi ⁎

Human Resource Development Department, Council of Scientific and Industrial Research - Central Food Technological Research Institute, Mysore 570 020, India

Abstract

The microbial safety of foods continues to be a major concern to consumers, regulatory agencies and food industries throughout the world. Many food preservation strategies have been used traditionally for the control of microbial spoilage in foods but the contamination of food and spoilage by microorganisms is a problem yet to be controlled adequately. Although synthetic antimicrobials are approved in many countries, the recent trend has been for use of natural preservatives, which necessitates the exploration of alternative sources of safe, effective and acceptable natural preservatives. Plants contain innumerable constituents and are valuable sources of new and biologically active molecules possessing antimicrobial properties. Plants extracts either as standardized extracts or as a source of pure compounds provide unlimited opportunities for control of microbial growth owing to their chemical diversity. Many plant extracts possess antimicrobial activity against a range of bacteria, yeast and molds, but the variations in quality and quantity of their bioactive constituents is the major detriment in their food use. Further, phytochemicals added to foods may be lost by various processing techniques. Several plant extracts or purified compounds intended for food use have been consumed by humans for thousands of years, but typical toxicological information is not available for them. Although international guidelines exist for the safety evaluation of food additives, owing to problems in standardization of plant extracts, typical toxicological values have not been assigned to them. Development of cost effective isolation procedures that yield standardized extracts as well as safety and toxicology evaluation of these antimicrobials requires a deeper investigation.

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1. Introduction

Microorganisms are always associated with harvested plants and slaughtered animals, the basic raw material of the food industry. Except for the foods that are heat processed to the degree that makes them sterile, microorganisms are usually associated with all the food products. Although a few microorganisms may bring about desirable changes in food, others spoil the foods by deteriorating their organoleptic quality and by production of toxins. The microbial safety of foods continues to be a major concern to consumers, regulatory agencies and food industries throughout the world. Many food preservation strategies such as chilling, freezing, water activity...
reduction, nutrient restriction, acidification, fermentation, pasteuriza-
tion or synthetic antimicrobials have been used traditionally for the
control of microbial spoilage in foods (Davidson, 2001), but the con-
tamination of food and spoilage by microorganisms is a problem
that is yet to be controlled adequately. The safety and shelf life of
food ingredients can also be improved by application of novel tech-
nologies to avoid or delay microbial growth like packaging in con-
trolled atmosphere, activated films, non-thermal treatments,
irradiation, modified atmosphere packaging, and so on. However,
most of these procedures may cause loss of organoleptic properties
of foods and reduce consumer acceptability. Therefore, the consumer
demands are increasingly focusing on minimally processed food
products, with less use of synthetic additives and at the same time
without compromising food safety. Although synthetic antimicrobials
are approved in many countries, the recent trend has been for use of
natural preservatives due to the adverse health effect of synthetic
ones. Therefore, alternative sources of safe, effective and acceptable
natural preservatives need to be explored.

The use of natural antimicrobials such as organic acids, essential oils,
plant extracts, and bacteriocins could be a good alternative to ensure
food safety (Burt, 2004; Cleveland et al., 2001; Holley and Patel 2005;
Naidu, 2000; Raybaudi-Massilia et al., 2009; Serra et al., 2008; Tiwari
et al., 2009). Plant products have also been used since ancient time for
flavoring foods and beverages, and for medicinal purposes with varying
success to cure and prevent diseases. It is estimated that there are
250,000 to 500,000 plant species on the earth (Borrís, 1996) and only
two-thousand of these have been explored till date. In the last few years, a
number of studies have been conducted in different countries to
prove efficacy of plant products (Ahmad and Beg, 2001; Almagboul
et al., 1985; Ankari and David, 1999; Artizzu et al., 1995; Bhatt and
Negi, 2012; Burkhup et al., 2010; Chauhan et al., 2007; Ikram
and Inamul, 1984; Izzo et al., 1995; Jayaprakasha et al., 2000; Kubo et
al., 1993; Negi and Jayaprakasha, 2001, 2004; Negi et al., 1999, 2003a,
Shapoval et al., 1994; Shan et al., 2007; Silva et al., 1996; Suresh
Kumar et al., 2010; Tornuk et al., 2011; Zeng et al., 2012), and thousands
of compounds have been isolated from these plants, which are claimed
to have antimicrobial or medicinal properties. The use of plant extracts
with known antimicrobial properties can be of great significance in food
preservation. The value of plants lies in some chemical substances that
produce a definite action on the microbiological, chemical and sensory
quality of foods, and these phytochemicals have been grouped in
several categories including polyphenols, flavonoids, tannins, alkaloids,
terpenoids, isothiocyanates, lectins, polyphenolides or their oxygen-
substituted derivatives (Cowan, 1999; Edeoga et al., 2005; Geissman,
1963).

Natural products, such as plant extract, either as pure compounds
or as standardized extracts, provide unlimited opportunities for con-
trol of microbial growth owing to their chemical diversity. Besides an-
timicrobial, several plants are being used in different areas of human
health such as traditional medicine, functional foods, dietary supple-
mements and recombinant protein manufacturing. Phytochemicals, es-
pecially flavonoids, polyphenols, anthocyanins and carotenoids,
share the major market (Sloan, 2001), and worldwide functional
foods sales are projected to grow from $75 billion in 2007 to
$130 billion by 2015 (GBA, 2010). According to a recent study by
Price Waterhouse Coopers (Land, 2010), functional food sales reached
$30 billion in 2007 and now make up 5% of the food industry in US. As
healthcare trends move toward disease prevention there will be
higher market for these products, as consumers are choosing foods
over pharmaceuticals for their well being.

2. Food antimicrobials

Food antimicrobials are chemical compounds or substances that
may delay microbial growth or cause microbial death in a food
matrix. The major targets for such antimicrobials are food poisoning
microorganisms (infective agents and toxin producers) and spoilage
microorganisms whose metabolic end products or enzymes cause
off-odors, off-flavors, texture problems, and discoloration (Davidson,
2001). The food antimicrobials are usually classified into traditional
or natural and synthetic substances depending on their origin. Anti-
microbials are called traditional when they have been used for
many years and many countries approve them for inclusion in foods.
Although, many synthetic antimicrobials are found naturally
(benzoic acid in cranberries, sorbic acid in rowanberries, citric acid
in lemons, malic acid in apples, tartaric acid in grapes, etc.), the per-
cception of natural has become important for many consumers.

The efficiency of an antimicrobial compound depends on the type,
genus, species, and strain of the target microorganism, besides the en-
vironmental factors such as pH, water activity, temperature, atmo-
spheric composition and initial microbial load of the food substrate
(Gould, 1989). The antimicrobial nature of phytochemical is deter-
mined by its chemical properties, such as pKa value, hydrophobicity/
lipophilicity ratios, solubility, and volatility (Stratford and Eklund,
2003). The pH and polarity are the most prominent factors influenc-
ing the effectiveness of a food antimicrobial. Polarity is related to
both the ionization of the molecule and the contribution of any
alkyl side groups or hydrophobic parent molecules (Davidson,
2001). Therefore, it is very important to know the specific character-
istics of the food system that needs to be preserved since a high pro-
portion of lipids could limit the effectiveness of some antimicrobial
agents (Owen and Palombo, 2007). Further, hydrophobic properties
of some antimicrobial substances can make their dissolution difficult
in water limiting their use in foods. The concentration thresholds re-
quired for inhibition or inactivation of microorganisms will depend
on the specific targets of the antimicrobial substance, including cell
wall, cell membrane, metabolic enzymes, protein synthesis, and gen-
etic systems. The use of combinations of antimicrobials is usually
more effective than adding just one antimicrobial because some mi-
croorganisms are not inhibited or killed by the commonly used
doses of antimicrobials (Beuchat, 2001; Leistner, 1995).

3. Natural antimicrobials for food uses

As far as the use of natural antimicrobials in foods is concerned,
the lack of reproducibility of their activity is one of the major obsta-
cles, despite the great diversity of compounds they contain. Qualita-
tive and quantitative variations in the content of bioactive phytochemicals in plant extracts result in their variable effectiveness.
Further, the extrapolation of results obtained from in-vitro experi-
ments with laboratory media to food products is not straightforward
as foods are complex, multicomponent systems consisting of different
interconnecting microenvironments. Though there is vast potential
for natural antimicrobial agents in food preservation, most of the lit-
unature presents inactivation data from model foods or laboratory
media. The level of natural preservatives required for sufficient effica-
cy may be considerably higher in food products in comparison with
lab media, which may negatively impact the organoleptic
properties of food.

3.1. Essential oils

Essential oils (EOs) or volatile oils are aromatic oily liquids
obtained from plant materials (flower, bud, seeds, leaves, twig bark,
herbs, wood, fruit and roots) by distillation. Individual components
of essential oils used in foods are either extracted from plant mate-
rials or are synthetically manufactured. EOs contains a mixture of
compounds, which includes terpenes, alcohols, acetones, phenols,
acids, aldehydes, and esters and mainly used as food flavorings or
functional components in pharmaceuticals (Corbo et al., 2009;
Nychas et al., 2003). Individual components of EOs are also used as
food flavorings or antimicrobial compounds (Burt, 2004). Although the majority of the EOs are classified as GRAS (generally recognized as safe) substances, their use in food as preservatives is often limited due to flavor considerations (Lambert et al., 2001). The antimicrobial properties of EOs and the large number of different groups of chemical compounds present in EOs are not due to one specific mechanism but several targets in the cell may contribute for their effectiveness (Burt, 2004).

Different studies have demonstrated the effectiveness of EOs and their active compounds to control or inhibit the growth of pathogenic and spoilage microorganisms and reported its dependence on pH, chemical structure and concentration of EOs or active compound, besides the number and type of microorganisms. The bacterial susceptibility to EOs increases with a reduction in pH of the food, since at low pH the hydrophobicity of the oil increases, enabling it to more easily dissolve in the lipids of cell membrane of the target bacteria (Burt, 2004). Partition coefficients of the EOs might also have an effect on activity by influencing its diffusion rate through the cell membrane, as higher partition coefficient of citral as compared to cinnamaldehyde and eugenol resulted in the faster reduction of E. coli O157:H7 (Raybaudi-Massilia et al., 2009). Further, EOs showed antimicrobial effectiveness against microorganisms in concentration dependent manner (Ultee et al., 2000). Storage temperature also influences the antimicrobial effectiveness of EOs, as the bactericidal activity of different EOs or their active components against E. coli O157:H7 and Salmonella hadar in apple juice was higher at 37 °C than at 4 and 21 °C (Friedman et al., 2004).

3.2. Plant extracts

Plant extracts have shown a considerable promise in a range of applications in the food industry and several plant extracts enjoy GRAS status. The antimicrobial activities of plant extracts may reside in a variety of different components (Table 1), and several extracts owing to their phytochemical constituents have been shown to have antimicrobial activity. The antibacterial activity is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents (Izikai et al., 1993; Otake et al., 1991), and the generation of hydroperoxides from polyphenols (Akagawa et al., 2003). Plant extracts also showed antifungal activity against a wide range of fungi (Davidson and Parish, 1989; Grange and Ahmed, 1988; Jayaprakasha et al., 2001; Negi et al., 2002); antioxidant and antimutagenic activities (Boubaker et al., 2011; Cherdshewasart et al., 2009; Horn and Vargas, 2003; Jayaprakasha et al., 2002, 2006, 2007; Negi et al., 2003b, 2010) and inhibited lipid oxidation in foods (Shan et al., 2009). Although numerous studies have been done in-vitro to evaluate the antimicrobial activity of plant extracts, very few studies are available for food products, probably because plant extracts did not produce as marked inhibition as many of the pure compounds in foods. The reduced effectiveness may be attributed to the use of crude extracts in most studies. As the crude extracts generally contain flavonoids in glycosidic form, where the sugar present in them decreases effectiveness against some bacteria (Kapoor et al., 2007; Parvathy et al., 2009; Rhee et al., 1994).

Dietary herbs and spices have been traditionally used as food additives throughout the world not only to improve the sensory characteristics of foods but also to extend their shelf life by reducing or eliminating survival of pathogenic bacteria. Many herbs and spice extracts possess antimicrobial activity against a range of bacteria, yeast and molds (Beuchat 2001; Friedman et al., 2002, 2004; Raybaudi-Massilia et al., 2009; Tajkarimi et al., 2010). Herbs and spices are rich in phenolic compounds and besides exerting antimicrobial effect they may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity (Swarz et al., 2001; Shahidi et al., 1997; Shan et al., 2009; Tanabe et al., 2002; Yanishlieva et al., 2006). A wide variety of phenolic substances derived from herbs and spices possess potent biological activities, which contribute to their preservative potential (Surh, 1999). Careaga et al. (2003) reported that 1.5 ml/100 g of capiscum extract was sufficient to prevent the growth of Salmonella typhimurium in raw beef but that 3 ml/100 g was required for a bactericidal effect against P. aeruginosa. Treatment with hydrosol of thyme, black cumin, sage, rosemary and bay leaf was reported to reduce S. typhimurium and E. coli O157:H7 in apple and carrots (Tornuk et al., 2011). Black cumin ethanolic extract applied in a marinade base for raw trout was found to reduce aerobic plate count, yeast, and coliforms (Elgayyar and Draoughton, 1999). Lee et al. (2009) observed that the addition of green tea or rosemary (1 or 3%) to rice cakes significantly reduced the levels of B. cereus and S. aureus during 3 days storage at room temperature (22 °C). Ahn et al. (2007) reported that a range of plant extracts are useful for reduction of pathogens associated with cooked beef, however, Uhart et al. (2006) reported that spices inactivate S. typhimurium DT104 in in-vitro condition, but the activity decreased considerably when added to a complex food system such as ground beef. Kim et al. (2004) observed that ground beef samples did not show significant difference in L. monocytogenes, S. aureus and total bacterial counts after treatment with green and jasmine tea as compared to untreated samples, however, a slight reduction in viable count of Salmonella enterica Sero-type Enteritidis and Listeria monocytogenes in ground beef by water-soluble arrowroot tea extract (upto 6% w/w) was reported (Kim and Fung, 2004). Combination of different plant extracts showed better preservative effects on meat as rosemary extracts and dry powders of orange and lemon applied to beef meatballs were found to be effective in controlling bacterial spoilage during 12 days storage period at 8 °C (Fernandez-Lopez et al., 2005). Mixtures of Scutellaria, honeysuckle, Forsythia and cinnamon or cinnamon, rosemary and clove oil showed 1.81- to 2.32-log reductions in microbial counts as compared to control in vacuum-packaged fresh pork during 28 days storage (Kong et al., 2007).

The long history of the medicinal use of garlic is well-documented and much information on its antimicrobial properties is widely known (Ankari and David, 1999; Ichikawa et al., 2006; Kubec et al., 2000; Naganawa et al., 1996). Yin and Cheng (2003) reported that the antimicrobial properties of garlic are due to organosulfur compounds. Freshly ground garlic, when added to mayonnaise at a concentration of 1% reduced Salmonella count (Leuschner and Zamparini, 2002). Garlic also has been shown to reduce the levels of E. coli in ground meat (Ceylan et al., 1998). Sallam et al. (2004) observed that addition of fresh garlic and garlic powder controlled microbial contamination and preserved chicken sausages. Species of the genus Mentha (family Lamiaceae) are a rich source of polyphenolic compounds, flavonoids, terpenoids, and other volatile compounds, which imparts a strong antimicrobial property (Gulluce et al., 2007; Tassou et al., 1995, 2000). Nguyen and Mittal (2007) reported more than 8 log reductions in the artificially inoculated pasteurized tomato juice when mint was used as a preservative.

Turmeric, a tropical herb of Zingiberaceae family is used in Indian cuisine mainly for its coloring and flavoring characteristics, and curcumin is the active constituent of turmeric responsible for its preservative action (Majeed et al., 1995). Curcumin has attracted special attention due to its potent pharmacological activities such as remedy for hypercholesterolemia, arthritis, indigestion and liver problem, and its ability to protect cells from β-amyloid insult in Alzheimer’s disease, cancer preventive properties and antimicrobial, antioxidant and antimutagenic activities (Baik et al., 1993; Goud et al., 1993; Majeed et al., 1995; Miquel et al., 2002; Srima, 1997). Even, the byproducts of curcumin manufacture were found to have high biological activity (Jayaprakasha et al., 2001, 2002; Negi et al., 1999). Turmeric extract (1.5%, v/v) alone or in combination with shalot extract (1.5% each, v/v) were found to retain quality characteristics of vacuum-packaged rainbow trout (Oncorhynchus mykiss) during a refrigerated storage of over a period of 20 days (Pezeshk et al., 2011).
Table 1
Antimicrobial phytochemicals present in various plant extracts.

<table>
<thead>
<tr>
<th>Family</th>
<th>Latin Name</th>
<th>English/Local Name</th>
<th>Part(s) used</th>
<th>Phytochemicals</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araceae</td>
<td>Amorphophallus campanulatus</td>
<td>Oklachi</td>
<td>Root</td>
<td>Amblyone</td>
<td>Khan et al. (2008)</td>
</tr>
<tr>
<td>Aristolochiaceae</td>
<td>Aristolochia bracteata</td>
<td>Aristolochia</td>
<td>Flowers</td>
<td>Aristolochic acid</td>
<td>Neri et al. (2003a)</td>
</tr>
<tr>
<td>Bignoniaceae</td>
<td>Jacaranda mimosafoila</td>
<td>Jakarta</td>
<td>Leaves</td>
<td>Flavonoids, flavonoids, iridoids, triterpenes</td>
<td>Rojas et al. (2006)</td>
</tr>
<tr>
<td>Bixaceae</td>
<td>B. orellana</td>
<td>Achio</td>
<td>Leaves/ seeds</td>
<td>Alkaloids, tannins, flavonoids, terpenoids, phenolics, glycosides</td>
<td>Rojas et al. (2006)</td>
</tr>
<tr>
<td>B. pilosa</td>
<td>Hairy beggar ticks</td>
<td>Citrine</td>
<td>Flower</td>
<td>Tannins</td>
<td>Deba et al. (2008)</td>
</tr>
<tr>
<td>Combretaceae</td>
<td>Terminalia citrine</td>
<td>Ascorbic</td>
<td>Berries</td>
<td>Flavonoids, tannins, triterpenes</td>
<td>Burapadaja and Bunchoo (1995)</td>
</tr>
<tr>
<td>Elaeagnaceae</td>
<td>Hippophae rhamnoides L.</td>
<td>Sea buckthorn</td>
<td>Berries</td>
<td>Flavonoids, tannins, triterpenes</td>
<td>Gupta et al. (2011)</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td>Mentha spp., M. piprata, M. longifolia</td>
<td>Pudina</td>
<td>Leaves</td>
<td>Flavonoids, menthol, terpenoids</td>
<td>Chauhan et al. (2008)</td>
</tr>
<tr>
<td>Lecaniiaceae</td>
<td>Plectranthus amboicous (Lour) Spreng (syn Coleus amboicous Lour or Coleus aromaticus Benth)</td>
<td>Indian Borage</td>
<td>Whole plant</td>
<td>Caryophyllene oxime, trans-caryophyllene, mar-cububol, α-3-terosterol, vulgarol, luponol, marrubinol</td>
<td>Masoodi et al. (2008)</td>
</tr>
<tr>
<td>Lauraceae</td>
<td>Cinnamomum spp.</td>
<td>Cinnamomum</td>
<td>Bark/ Leaves/Bark/ Root bark/ Fruit</td>
<td>Cinnamaldehyde, flavon-3-ols, p-coumaric acid, Eugenol, Camphor α-pine, β-pine, β-caryophyllene, γ-cadinene</td>
<td>Jayaprakasha et al. (1997); Neri et al. (2007)</td>
</tr>
<tr>
<td>Liliaceae</td>
<td>Allium cepa L.</td>
<td>Onion</td>
<td>Bulb</td>
<td>Saponins, kampferol, furic acid, β-sitosterol, myrtic acid, prostaglandins, querertcin, alkenyl cysteine sulfoxide</td>
<td>Kubec et al. (2000); Tang and Cronin (2007)</td>
</tr>
<tr>
<td>Moraceae</td>
<td>Ficus religiosa L.</td>
<td>Pipal</td>
<td>Leaves</td>
<td>Bergaprol and bergapten</td>
<td>Shao et al. (2009)</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>Syzygium aromaticum L.</td>
<td>Clove</td>
<td>Bud</td>
<td>Eugenol, eugenin, acetyl eugenol, querertcin, acetyl cinnamaldehyde</td>
<td>Shapoval et al. (1994)</td>
</tr>
<tr>
<td>Poaceae</td>
<td>Allium sativum L.</td>
<td>Garlic</td>
<td>Bulb</td>
<td>Allicin, diallyl Koscinocyanate, allicin, diallyl disulfide, diallyl trisulfide, alkenyl cysteine sulfoxide, ajoene, allyl methy thiosulfinate</td>
<td>Naganawa et al. (1996); Hornickova et al. (2009); Yin and Cheng (2003); Kubec et al. (2000)</td>
</tr>
<tr>
<td>Polygineae</td>
<td>Polygonum hydropiper L.</td>
<td>Oat</td>
<td>Seed</td>
<td>Flavonoids, flavonoid glycosides, sesquiterpene acid, viscosumic acid</td>
<td>Rauha et al. (2000)</td>
</tr>
<tr>
<td>Punicaceae</td>
<td>Punica granatum L.</td>
<td>Pomegranate</td>
<td>Rind</td>
<td>Anthocyanins, ellagic acid, galloflavins, gallic acid, Punicalagin, ellagaltannins</td>
<td>Machado et al. (2002); Seeram et al. (2006)</td>
</tr>
<tr>
<td>Ranunculaceae</td>
<td>Nigella sativa L.</td>
<td>Black cumin</td>
<td>Seeds</td>
<td>Tannins, (gallo and ellagaltannins)</td>
<td>Suresh Kumar et al. (2010)</td>
</tr>
<tr>
<td>Rosaceae</td>
<td>Filipendula vulgaris Moench (syn. Filipendula hexapetala Gilib. ex Maxim.)</td>
<td>Dropwort</td>
<td>Leaves</td>
<td>Glucoside, melanthin, saponin, thymoquinone</td>
<td>Radulovic et al. (2007)</td>
</tr>
<tr>
<td>Rutaceae</td>
<td>Citrus reticulata</td>
<td>Claduous</td>
<td>Leaves</td>
<td>Flavonoids, ellagic acid, tannins, gallic acid and their derivatives</td>
<td>Thiem and Gusliniska (2004)</td>
</tr>
<tr>
<td>Sapindaceae</td>
<td>Citrus paradisi</td>
<td>Sapindus</td>
<td>Rind</td>
<td>Polymermalesitated flavones</td>
<td>Jayaprakasha et al. (2002)</td>
</tr>
<tr>
<td>Vitaceae</td>
<td>Vitis vinifera L.</td>
<td>Grape</td>
<td>Fruits</td>
<td>Coumarins, flavonones, methoxy flavones</td>
<td>Neri and Jayaprakasha (2001)</td>
</tr>
<tr>
<td>Zingiberaceae</td>
<td>Curcuma longa L.</td>
<td>Turmeric</td>
<td>Rhizome</td>
<td>Curcumin, turmune ar-turmerone, curtone, trans-β-farnesene, α-Zingiberene, β-isobalobline</td>
<td>Neri et al. (1999); Majeed et al. (1995); Srimal (1997)</td>
</tr>
</tbody>
</table>
Cinnamon is the source of cinnamon bark, fruit, leaf and their essential oils and many *Cinnamomum* species yield a volatile oil on distillation with different aroma characteristics and composition (Jayaprakash et al., 1997; Kaul et al., 2003). Extracts of the cinnamon bark and fruit and cinnamon oil have been reported to possess antimicrobial, antioxidant and antiinflammatory activities (Agnihotri and Vaidya, 1996; Dang et al., 2001; Kamath et al., 2003; Jayaprakash et al., 1997). Cinnamon was found to reduce the levels of E. coli in apple juice (Ceylan et al., 1999). Yuste and Fung (2002) reported up to 6 log cfu/ml reductions of artificially inoculated *L. monocytogenes* in pasteurized apple juice with 0.1–0.3% (w/v) of ground cinnamon after 1 h of incubation, and no further growth of the microorganism occurred during 7 days of storage. Ceylan et al. (1999) reported that the addition of 0.3% (w/v) cinnamon powder gradually decreased the counts of E. coli O157:H7 in pasteurized apple cider was reported even by addition of 2% (w/v) cinnamon powder (Lu et al., 2001).

*Punica granatum* L. has a rich history of traditional use of its bark, leaves, flowers, fruits and seeds to ameliorate diseases. The presence of phytoconstituents in the pomegranate extracts such as phenols, tannins and flavonoids as major active constituents may be responsible for these medicinal values (Jurenka, 2008; Seeram et al., 2006). Several studies have reported the efficacy of various extracts from the different parts of pomegranate plant against the growth of Gram positive and Gram negative bacteria (Ahmad and Beg, 2001; Aynchi et al., 1982; Burapadaja and Bunchoo, 1995; De et al., 1999; Machado et al., 2003; Navarro et al., 1996; Negi et al., 2003b; Prashanth et al., 2001; Rani and Khullar, 2004). Aqueous and ethanolic fruit shell extracts of *P. granatum* were found to have antibacterial activity against different strains of E. coli (Voravuthikunchai et al., 2004), *Salmonella* Typhi (Perez and Anesini, 1994), and it inhibited Staphylococcal enterotoxin A production (Braga et al., 2005). Various other solvent extracts from the rind of *P. granatum* also showed anti-bacterial activity against enterohaemorrhagic E. coli (Alkofahi et al., 1996; Nimri et al., 1999; Prashanth et al., 2001) and food spoilage bacteria (Seeram et al., 2006; Negi et al. 2003b). Pomegranate peel extracts were used to enhance the shelf life of chicken meat products by controlling oxidative rancidity and bacterial growth (Kanatt et al., 2010).

Various species of *Garcinia* contains several secondary metabolites which exhibit a wide range of biological and pharmacological activities such as antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities (Bakana et al., 1987; Mackeen et al., 2000; Minami et al., 1994, 1995, 1996). Likhitwitayawuid et al. (1998a, 1998b) reported antimalarial activity of xanthones isolated from the bark of *G. dulcis* and *G. cowa*. Crude extracts as well as partially purified compounds from different parts of some species of Garcinia have shown antibacterial potential (Negi and Jayaprakasha, 2004, Negi et al., 2010). A polyisoprenylated benzophenone (garcinol) isolated from stem bark of *G. huillensis* has been shown to be active against Gram positive and Gram negative cocci, mycobacteria and fungi but inactive against Gram negative enteric bacilli, yeast and viruses (Bakana et al., 1987). Alpha-mangostin, rubraxanthone, and xanthochymol isolated from *G. mangostana*, *G. diocia* and *G. subelliptica*, respectively, showed strong antibacterial activity against both methicillin-resistant and methicillin-sensitive *S. aureus* (Linuma et al., 1996a, 1996b). Crude extracts of leaves, fruits, root, stem and trunk bark of *G. atroviridis* exhibited antibacterial activity with the root extract showing the strongest inhibition, while the fruit and leaf extracts exhibited significant antifungal activity against *Cladosporium herbarum* (Mackeen et al., 2000). Crude extracts of *G. indica* also showed antiaflatoxic properties (Tamil Selvi et al., 2003).

Seabuckthorn has been widely used in traditional medicines, mainly of Tibetan, Mongolian, Chinese and Middle Asian cultures (Xu et al. 1994; Yang et al. 2000) for the treatment of asthma, skin diseases, gastric ulcers, lung disorders, cough, diarrhoea, and menstrual disorder (Ranjith et al., 2006). The health benefits of *Hippophae rhamnoides* oils, juice, leaves and bark are also well known and they have been used to treat several diseases (Li and Schroder, 1996; Li and Wang, 1998). All parts of the seabuckthorn plant are considered to be rich source of a large number of bioactive substances and are reported to have antimicrobial (Chauhan et al., 2005, 2007; Gupta et al., 2011; Negi et al., 2005; Shipulina, 2001), antioxidant (Negi et al., 2005; Rosch et al., 2003), and antimutagenic activities (Edenharder et al., 1995); and antitumoral, hepato-protective and immunomodulatory (Geetha et al., 2002; Xu et al., 1994), anti-platelet aggregating (Chen et al., 1995), anti-inflammatory (Ganju et al., 2005), and radio-protective properties (Chawla et al., 2007). The leaf extract was reported to have better immunomodulatory effect than fruit extracts (Geetha et al., 2002). Jelly prepared by using seabuckthorn berries showed microbiological stability at ambient temperature and 37°C for a period of 6 months (Selvamuthukumar et al., 2007).

Various other plant extracts were found to be effective against *L. monocytogenes* in refrigerated meat products (Hao et al., 1998; Ward et al., 1998). The effect of a mixture of oregano and cranberry (0.1 mg of phenolic/ml) on beef slices and cod fish filet was studied by Lin et al. (2004) and they observed that at pH 7, phytochemicals have no significant effect on cell numbers after 18 h of incubation, but at pH 6.0, differences in viable cell counts were observed in beef and fish slices. The oregano–cranberry extract mixture showed higher log reduction in viable counts than the slices treated with either oregano or cranberry extract (Lin et al., 2004). The effectiveness of rosemary and oregano extracts to reduce microbial growth in meat products has been reported (Cam0 et al., 2008; Djenane et al., 2002, 2003; Zinoviadou et al., 2009). Ruiz et al. (2009) also reported that although rosemary extract was not able to completely eliminate *L. monocytogenes* in ready-to-eat vacuum-packaged diced turkey and ham, it significantly decreased the counts when used along with nisin. Cranberry powder alone at 1%, 2% and 3% levels resulted in 2–4 log cfu/g reduction in growth of *L. monocytogenes* compared to the control (treated with nitrite, p ≤ 0.05), and similar effect on growth was seen when it was combined with cherry powder, lime powder and grape seed extracts in a cured cooked meat model system (Xi et al., 2011). Grape seed extract and pine bark extract were used to control the growth of artificially inoculated bacteria on the surface of raw ground beef during refrigerated storage (Ahn et al., 2004). The combination of grape seed extract and nisin gave the greatest inhibitory activity with reductions of *L. monocytogenes* populations to undetectable levels after 21 days indicating potential of natural antimicrobials to control the growth and recontamination of *L. monocytogenes* on meat products (Sivarooban et al., 2007).

Dried plum puree was found to reduce E. coli and *Salmonella* in ground meat (Pszczola, 2002). Karapinar and Sengun (2007) recommended use of unripe grape juice for enhancing the safety of salad vegetables. Grape pomace extract and olive extracts showed antimicrobial activity in apple juice (Serra et al., 2008). Grape seed extract (1%) and rosemary olearin (1%) reduced the populations of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* after 9 days in raw ground beef (Ahn et al., 2004). Owen and Palombo (2007) investigated the ability of *Eremophila duttonii* and *E. alternifolia* to control the growth of *L. monocytogenes* in full cream milk, skim milk, diluted homogenates of salami, pate and brie cheese, and reported that both the extracts inhibited the growth of *L. monocytogenes* in salami at 37°C, only *E. duttonii* extract was effective in pate at 4°C storage, and growth of *L. monocytogenes* was not affected by both the extracts in other products. Reduction in microbial load by water-soluble extract from pine needles of *Cedrus deodara* in fresh-squeezed tomato juice (Zeng et al., 2012) and by the extracts from cinnamon stick, oregano, clove, pomegranate peel and grape seeds in raw pork over 9 days storage at ambient temperature was reported by Shan et al. (2009).
4. Mechanism of action of natural antimicrobials

The mechanism of action for the antimicrobial activity of natural preservatives is not fully understood, however, membrane disruption by terpenoids and phenolics; metal chelation by phenols and flavonoids; and effect on genetic material by coumarin and alkaloids are thought to inhibit growth of microorganisms (Cowan, 1999). The exact target(s) for natural antimicrobials are often not known or well defined, as it is difficult to identify a specific action site where many interacting reactions take place simultaneously. It was observed that membrane-disrupting compounds can also cause leakage of cellular content, interference with active transport or metabolic enzymes, or dissipate cellular energy in ATP form (Davidson, 2001).

Different studies have demonstrated the effectiveness of antimicrobials and their effective compounds to control or inhibit the growth of pathogenic and spoilage microorganisms. Degradation of the cell wall, damage to cytoplasmic membrane and membrane proteins, leakage of intracellular contents, coagulation of cytoplasm and depletion of proton motive force can cause cell death (Burt, 2004; Nychas et al., 2003; Tiwari et al., 2009). The effectiveness of antimicrobial compound depends on pH of the food, type and number of contaminating microorganisms, and type and concentration of antimicrobial. Storage temperature may also influence the effectiveness of antimicrobial as diffusibility of compounds is related to the temperature (Friedman et al., 2004). Further, in case of tannins, mass and configuration of the ortho-phenolic hydroxyl groups have been reported to determine their biological activity (Schrofield et al., 2001).

Antibacterial compounds such as thymol, eugenol, and carvacrol have been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of microorganisms (Burt, 2004; Gill and Holley, 2006; Lambert et al., 2001; Oussalah et al., 2006; Raybaudi-Massilia et al., 2009). Oussalah et al. (2006) and Gill and Holley (2004, 2006) indicated that cinnamaldehyde produces a decrease in the intracellular ATP by ATPase activity without apparent changes on the cell membrane of E. coli, E. coli O157:H7 and L. monocytogenes. However, Kim et al. (1995) reported increased membrane permeability and leakage of cytoplasm by cinnamaldehyde, and it also interacts with enzymes necessary for amino acid biosynthesis (Parvathy et al., 2009) or amino acid metabolism (Hashimoto et al., 1999; Kitano et al., 1997; Terao et al., 1994). Catechins and epigallocatechin gallate interact in the outer polar zone of lipid bilayers in liposomes and cause membrane disruption (Hashimoto et al., 1999; Kitano et al., 1997; Terao et al., 1994). Vanillin showed antimicrobial affect by affecting membrane functions (Vaara, 1992) and through the inhibition of respiration in several bacteria (Fitzgerald et al., 2004). Terpenes accumulate in the membrane and cause a loss of membrane integrity and dissipation of the proton motive force (Sikkema et al., 1995) as well as disrupt the lipid structures (Kararli et al., 1995; Williams and Barry, 1991). Cell wall lysis also has been reported in the bacteria after treatment with phenolic compounds (Borneman et al., 1986).

5. Stability of phytochemicals during food processing

Given the recent trend of health promotion through diet, understanding processing effects is critical for conserving active phytochemicals as they not only preserve the foods, but also have beneficial effect on human health. Processing of foods containing phytochemicals is expected to result in some changes in their phytochemical contents. Phytochemicals present in many foodstuffs are lost by heat processing such as sterilization, pasteurization, and dehydration. Thermal processing caused marked losses in total anthocyanins in black raspberries (Hager et al., 2008) and blueberries (Brownmiller et al., 2008). Processing resulted in a significant decrease in biological activity of drumstick leave extract (Saeedeh Arabshahi et al., 2007); however, no difference in activity of carrot tubers extract was observed before and after heat treatment; while in some cases, processing induces the formation of the novel compounds, which either maintain or even increase the potential of various extracts (Manzocco et al., 1998; Nicoli et al., 1999).

The thermal stability of phytochemicals added to food depends on the matrix in which they are found. The presence of other polyphenolics and antioxidants in the matrix may help to stabilize the polyphenolic compounds. Talcott et al. (2003a) demonstrated that vitamin C fortification preserved more phytochemicals during processing compared to the nonfortified control yellow passion fruit. Isoflavonoid extracts from red clover leaves were found to enhance stability of anthocyanins in muscadine wines stored at 20 and 37 °C for 9 weeks (Talcott et al., 2003b). Significant changes in individual flavone levels were observed during storage of UHT processed chocolate flavored high protein beverage containing soy proteins isolates depending on storage temperatures (4, 23 and 38 °C), but total isoflavones remained the same irrespective of storage duration or temperature (Hayes et al., 2004).

Badei et al. (2002) reported the possible use of cinnamon in cookies as it was stable after baking. It has been reported that irradiation of cinnamon did not affect the antioxidant potential of the cinnamon compounds (Kitazuru et al., 2004). Nandita et al. (2009) reported that hexane extract from Garcinia is more suitable for biscuit making than turmeric powder as it retained higher antioxidant activity after baking followed by 2 months storage. Flower extracts of Peltophorum ferrugineum (Nandita et al., 2009); and marjoram, spearmint, peppermint and basil powders or their purified extracts (Bassiony et al., 1990) were also found to retain their antioxidant activity during baking. Biscuits treated with extracts of raisins, amla and drumstick leaves were stable during 6 weeks of storage (Reddy et al., 2005). Use of mango dietary fiber concentrate for cookies and bread are reported to give satisfactory shelf life (Vergara-Valencia and Granados-Perez, 2007). Microcapsule curcumin was found to have similar antibacterial and antifungal activities as curcumin after microencapsulation (Wang et al., 2009). Similarly, modifications of curcumin by glidoside synthesis (Parvathy et al., 2009) or amino acid conjugation (Parvathy et al., 2010) retained its antibacterial, antioxidant and antimutagenic activities, indicating that curcumin is stable to chemical modifications.

6. Toxicity evaluation of plant extracts

Most of the plant extracts, which might find application in foods, have been consumed by humans for thousands of years, however, typical toxicological information such as acceptable daily intake (ADI) or no observed adverse effect level (NOEL) are not available for them. Although International guidelines exist for the safety evaluation of food additives, due to problems in standardization of extracts...
or dried preparation owing to their batch-wise compositional variability, it becomes difficult to assign ADI or NOEL for the plant extracts. The marker compounds in extracts are affected by variety of plant, geographical origin, plant part used, age and growth condition of plants, method of extraction or drying, preparation, packaging and storage. According to Dietary Supplement Health and Education Act (DSHEA), 1994, botanicals are exempted from food additive category, and GRAS submission of safety evidence is not required as long as that ingredient was in market before October 1994.

Recently, there have been an increase in the number of botanical products as food ingredients or supplements and these are a commercially important part of the health food market. Botanicals may be derived from conventional primary food sources (soy extracts, tomato extracts) or from secondary sources such as herbs and spices (garlic oil, rosemary extracts, green tea extracts). Some botanicals may have no significant history of use as food ingredients but may be derived from sources that have been used in herbal medicinal products in various regions of the world and considered for food use (e.g. Ginkgo biloba, Ginseng extract, Hypericum perforatum (St. John’s Wort)). Further, materials with no history of human use (phytostanols derived as a by-product from wood, shikimic acid isolated from water-soluble extract of pine needles of C. deodara) may be considered for use in foods. Therefore, a simple check-list of tests that will be appropriate for establishing the safety of phytochemicals to be added to foods has not yet been available.

The International Life Sciences Institute-Europe has developed a comprehensive document on the use of plant materials in food products (Schilter et al., 2003), which stresses that the ingredient for use in food products must be well identified and characterized. The starting material must be accurately identified in order to ensure that the plant materials for food use are consistent with respect to quality and quantity of active ingredient and the method of preparation must meet good manufacturing practices. Risk assessment of natural products may require adequate specification of identity and composition as it may be the whole plant, extracts thereof or purified components, and the variability among plant source and the process used to obtain the constituents will be a limiting factor in adopting a generic approach to their risk assessment. The nature of the compound, prior knowledge of human consumption, likely exposure and nutritional impact will determine the approach for toxicological testing of such compounds. Generally, for herbs or complex extracts, it is not possible to make a risk assessment on the basis of a single active component as more than one component may be of toxicological significance and food matrix may affect their bioavailability. A decision tree has been suggested as an aid to the safety evaluation process for plant material intended for food use (Walker, 2004), and general framework for safety assessment of botanicals has been described (Speijers et al., 2010; van den Berg et al., 2011).

7. Perspectives and future

The identification and evaluation of natural products for the control of pathogens and to assure consumers a safe, wholesome and nutritious food supply is a challenge. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Even though pharmaceutical industries have produced a number of new antibiotics in the last few decades, resistance to these drugs by microorganisms has increased. Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial properties. The current focus in natural preservatives is on a small number of antimicrobial agents, which have been used for many years, and there is a need to expand this list for their food application to ensure safety and quality of the food products. There is no shortage of candidates to become the food preservatives of the future, but still many obstacles exist on the road to all-natural preservation. There are very few natural antimicrobials that can be used as direct replacements for existing preservatives owing to their lower effectiveness, higher cost and product organoleptic quality deterioration. Further, if a natural antimicrobial with potential as a food preservative can be shown to be sufficiently effective in foods, it will need regulatory approval before it can be used as a food additive. Once declared additive on the label, consumers will have different perspectives about these antimicrobials, but it is possible to classify them as processing aids, thus consumer perception of them being an additive can be avoided. Therefore, for the successful exploitation of the natural antimicrobials as food preservatives, probably it will not only require changes in legislation but also require better consumer education.

The information available to date demonstrates that different antimicrobials of plant origin can effectively reduce or inhibit pathogenic and spoilage microorganism, and thus have a potential to become a good alternative to synthetic antimicrobials. The development of cost effective isolation and purification procedures that avoid loss of functional properties of active compounds will aid in wider use and acceptance of plant extracts as natural preservatives. However, too much focus on the use of single compounds over mixtures may not be compatible with complex plant extracts in which valuable bioactive molecules are often present in mixed form and the biological activity of plant extracts mostly results from additive or synergistic effects of these components. Further, the use of natural antimicrobials in combination with another or with other technologies in a multi-hurdle preservation system can enhance the performance of natural antimicrobials. Studies have demonstrated that natural antimicrobial agents may offer unique advantages for food processing, and in addition to improving the shelf life and safety of foods; they may allow novel food products with enhanced quality and nutritional properties. The applications of natural antimicrobial agents are likely to grow steadily in the future because of consumer demand for minimal processing and food containing naturally derived preservatives is on rise. Further, it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. The impact of product formulation and storage parameters on the efficacy of natural antimicrobials as well as safety and toxicology evaluation of these natural antimicrobials require an in-depth study.

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References


Nguyen, P., Rast, P., 2003. Activation of naturally occurring microorganisms in tomato juice using pulsed electric field (PEF) with and without antimicrobials. Chemical Engineering and Processing 46, 360–365.


Oussalah, M., Caillet, S., Lacroix, M., 2006. Mechanism of action Spanish oregano, Chil-}


