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# Essential Oil as Antimicrobial Agents: Efficacy, Stability, and Safety Issues for Food Application

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## Abstract

The use of natural antimicrobial compounds in food has gained much attention by the consumers and the food industry. This is primarily due to two major factors. First, the misuse and mishandling of antibiotics has resulted in the dramatic rise of a group of microorganisms including foodborne pathogens that are not only antibiotic resistant but also more tolerant to several food processing and preservation methods. In addition, increasing consumers' awareness of the potential negative impact of synthetic preservatives on health versus the benefits of natural additives has generated interest among researchers in the development and use of natural products in foods. Essential oils are volatile, natural, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. The bioactivity properties of essential oils are generally determined by the major compounds present in them. They have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal, and antioxidant applications. The biological activity of the oils can be compared with the activity of synthetically produced pharmacological preparations. Thus, essential oils are promising natural extracts that need further evaluation for possible application as supplement, preservatives, or antioxidants in food or pharmaceutical industries.

**Keywords:** essential oil, antimicrobial activities, natural preservatives, pathogenic bacteria, microorganisms

## 1. Introduction

Aromatic plants have been used since ancient times for their preservative and medicinal properties, and to impart aroma and flavor to food. Hippocrates, sometimes referred to as the “father of medicine,” prescribed perfume fumigations. The pharmaceutical properties of aromatic plants are partially attributed to essential oils. The term “essential oil” was used for the first time in the sixteenth century by Paracelsus von Hohenheim, who named the effective component of a drug, “Quinta essential” [1]. By the middle of the twentieth century, the role of essential oils had been reduced almost entirely to use in perfumes, cosmetics and food flavorings, while their use in pharmaceutical preparations had declined.

The natural mixtures of volatile and aromatic compounds (Essential oils) are secondary aromatic plant metabolites. Essential oils are complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components. Several techniques can be used to extract essential oils from different parts of the aromatic plant, including hydrodistillation (HD), solvent extraction and supercritical fluid extraction (SFE) [2]. Essential oils are derived from various parts of the plant, including leaves, flowers, fruits, seeds, rhizomes, roots, and bark. In the plant, these constituents serve several physiological purposes for the plant protection from pests and microorganisms, attraction of pollinating insects or birds, providing photoprotection to the plant, and allelopathy.

Essential oils are usually obtained by steam or hydro-distillation first developed in the Middle Ages by Arabs. Known for their antiseptic, i.e., bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies. Up to the present day, these characteristics have not changed much except that more is now known about some of their mechanisms of action, particularly at the antimicrobial level. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides, and also against herbivores by reducing their appetite for such plants. They also may attract some insects to favor the dispersion of pollens and seeds, or repel undesirable others. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopeia. They are liquid, volatile, limpid and rarely colored, lipid soluble and soluble in organic solvents. Essential oils can be synthesized by all plant organs, i.e., buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in storage cells like cavities, canals, epidermic cells or glandular trichomes [3, 4]. Most of the commercialized essential oils are chemotyped by gas chromatography and mass spectrometry analysis. Analytical monographs have been published (European Pharmacopoeia, ISO, WHO, Council of Europe; [5]) to ensure good quality of essential oils.

Essential oils have been largely employed for their properties already observed in nature, i.e., for their antibacterial, antifungal and insecticidal activities. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. Essential oils or some of their components are used in perfumes and make-up products, in sanitary products, in dentistry, in agriculture, as food preservers and additives, and as natural remedies. For example, d-limonene, geranyl acetate or d-carvone are employed in perfumes, creams, soaps, as fragrant components and in food, as natural flavoring agents fragrances for household cleaning products and as industrial solvents. Moreover, essential oils are used in massages as mixtures with vegetal oil or in baths but most frequently in aromatherapy. Some essential oils appear to exhibit particular medicinal properties that have been claimed to cure one or another organ dysfunction or systemic disorder [6–8].

Essential oils have traditionally been used to impart flavoring or preservative effects to foods, or to instill fragrances in cosmetics and aromatherapy. Since ancient times, numerous civilizations have also valued essential oils for their therapeutic qualities in disease prevention and treatment. Later, the Greeks and Romans absorbed Egyptian practices of using essential oils in aromatherapy and expanded it to their baths for promotion of well-being. For instance, baths infused

with the oils of jasmine, lavender, or ylang-ylang stimulated mental relaxation. Similarly, current interest in essential oils arises from the various bioactive effects they display, including antioxidant [9, 10], anti-inflammatory [11, 12], antimicrobial [13, 14], antiviral [15, 16], and anticarcinogenic [17]. In developed countries, the benefits derived from using essential oils appear optimistic. Demand for plant essential oils has risen as a consequence of consumers searching for cheaper, more 'natural' alternatives to disease-fighting medications. In food and cosmetic applications, essential oils are considered to be biodegradable, readily available, and 'less toxic' than synthetic preservative agents. As such, this optimism has raised concerns and stimulated studies to evaluate the safety and efficacy of essential oils in various systems in order to better understand their pharmacological properties and roles in health.

Today there is significant consumer demand for foods that are minimally processed and free from synthetic chemical preservatives with the perception of being "natural" [18, 19]. As a result the food industry is facing great challenges to produce naturally occurring food antimicrobials and antioxidants to reduce the use of synthetic chemical preservatives and still produce safe foods that are also regarded as healthy. Spices and herbs are well known for their antimicrobial and antioxidant properties and have the ability to produce multidimensional flavors in food [20]. The clove, cinnamon, oregano and rosemary are considered as the most common spices and herbs with strong antimicrobial activity. Their essential oils containing chemical compounds such as carvacrol, cinnamaldhyde, eugenol and camphor are identified as the major chemical components responsible for exerting antimicrobial activity [21–24]. Some studies reported that there is a highly positive linear relationship between antioxidant activity, antibacterial activity and total phenolic content in some spices and herbs [25, 26].

Antimicrobials are used in food for two main reasons: (1) to control natural spoilage processes (food preservation) and (2) to prevent/control growth of micro-organisms, including pathogenic micro-organisms (food safety). Natural antimicrobials are derived from animal, plant and microbial sources. There is considerable potential for utilization of natural antimicrobials in food, especially in fresh fruits and vegetables. However, methods and mechanisms of action, as well as the toxicological and sensory effects of natural antimicrobials, are not completely understood [18, 27–30].

There are more than 1340 plants with defined antimicrobial compounds, and over 30,000 components have been isolated from phenol group-containing plant-oil compounds and used in the food industry. However, commercially useful characterizations of preservative properties are available for only a few EOs. There is a need for more evaluation of EOs in field and food systems. Food-preservative utilization of spices and their EOs as natural agents has recently been focused on extending the shelf life of foods, reducing or eliminating pathogenic bacteria, and increasing overall quality of food products [27, 31–35].

## 2. Composition of essential oils

Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. For example, carvacrol (30%) and thymol (27%) are the major components of the *Origanum compactum* essential oil, linalool (68%) of the *Coriandrum sativum* essential oil,  $\alpha$ - and  $\beta$ -thujone (57%)



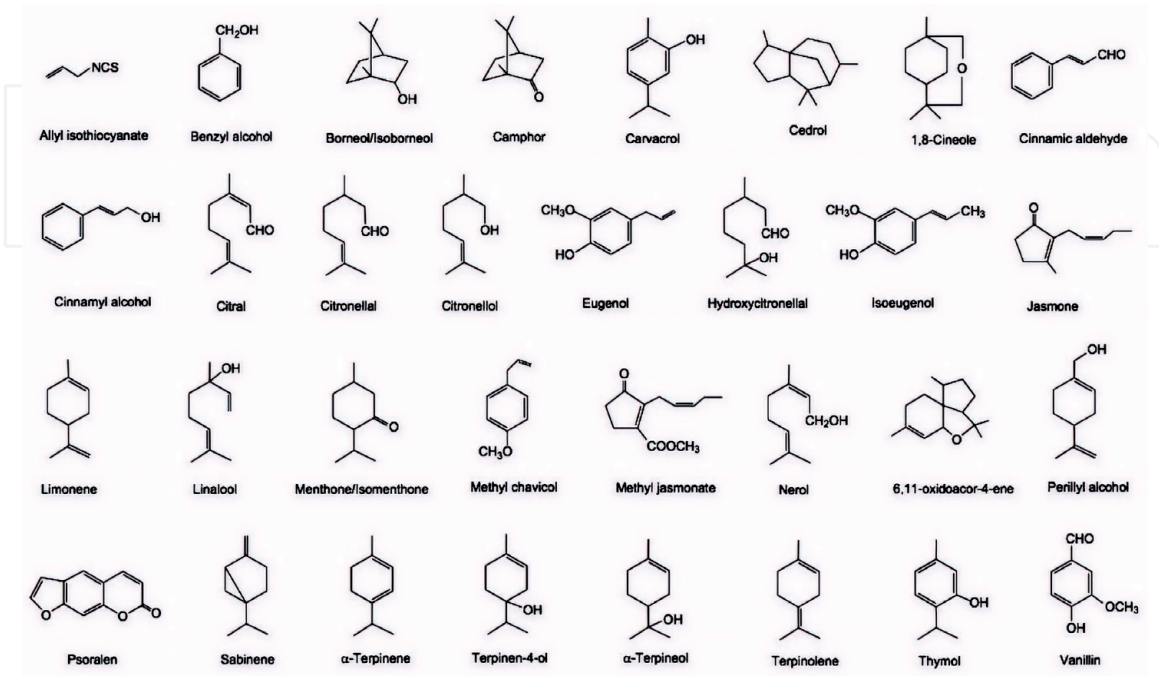
and camphor (24%) of the *Artemisia herba-alba* essential oil, 1,8-cineole (50%) of the *Cinnamomum camphora* essential oil,  $\alpha$ -phellandrene (36%) and limonene (31%) of leaf (of what)? and carvone (58%) and limonene (37%) of seed *Anethum graveolens* essential oil, menthol (59%) and menthone (19%) of *Mentha piperita* essential oil. Generally, these major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetic origin [36–39]. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular (see **Figure 1**).

The essential oil of juniper berry (*Juniperus drupacea* L.) was analyzed by chromatographic analysis and it was found that  $\alpha$ -pinene (44.2%), thymol methyl ether (22.2%) and camphor (10.2%) present in higher concentration [40]. The major volatile compounds found in caper (*Capparis ovata* desf. Var. *caescens*) bud oil were benzyl alcohol (20.4%), furfural (7.4%), ethanol methyl pentyl acetal (5.9%), thymol (5.1%) as well as the major volatile compound found in capers leaves were methyl isocyanate (20.0%), thymol (15.5%) [41].

The following paragraphs will describe some features that characterize each chemical group constituent of essential oils.

## 2.1 Terpenes

Terpenes form structurally and functionally different classes. They are made from combinations of several 5-carbon-base (C5) units called isoprene. The biosynthesis of the terpenes consists of synthesis of the isopentenyl diphosphate (IPP) precursor, repetitive addition of IPPs to form the prenyldiphosphate precursor of the various classes of terpenes, modification of the allylic prenyldiphosphate by terpene specific synthetases to form the terpene skeleton and finally, secondary enzymatic modification (redox reaction) of the skeleton to attribute functional properties to the different terpenes. The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes



**Figure 1.**

The structure of the chemicals discussed in this chapter with respect to their biological activity in alphabetical order.

(C30) and tetraterpenes (C40) also exist. A terpene containing oxygen is called a terpenoid.

The monoterpenes are formed from the coupling of two isoprene units (C10). They are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures. They consist of several functions:

#### 2.1.1 Carbures

- Acyclic: e.g., myrcene and ocimene, etc.
- Monocyclic: e.g., terpinenes, p-cimene and phellandrenes, etc.
- Bicyclic: e.g., pinenes,  $\alpha$ -3-carene, camphene, sabinene, etc.

#### 2.1.2 Alcohols

- Acyclic: e.g., geraniol, linalol, citronellol, lavandulol, and nerol, etc.
- Monocyclic: e.g., menthol,  $\alpha$ -terpineol and carveol.
- Bicyclic: e.g., borneol, fenchol, chrysanthenol, thuyan-3-ol, etc.

#### 2.1.3 Aldehydes

- Acyclic: e.g., geranial, neral, citronellal, etc.

#### 2.1.4 Ketone

- Acyclic: e.g., tegetone, etc.
- Monocyclic: e.g., menthones, carvone, pulegone and piperitone, etc.
- Bicyclic: e.g., camphor, fenchone, thujone, ombellulone, pinocamphone and pinocarvone, etc.

#### 2.1.5 Esters

- Acyclic: e.g., linalyl acetate or propionate, citronellyl acetate, etc.
- Monocyclic: e.g., menthyl or  $\alpha$ -terpinyl acetate, etc.
- Bicyclic: e.g., isobornyl acetate, etc.

#### 2.1.6 Ethers

- 1,8-cineole, menthofurane, etc.
- Peroxydes: e.g., ascaridole, etc.
- Phenols (aromatic ethers): e.g., thymol, carvacrol, etc.

When the molecule is optically active, the two enantiomers are very often present in different plants. For example, (+) $\alpha$ -pinene from *Pinus palustris*, (–)

$\beta$ -pinene from *Pinus caribaea* and from *Pinus pinaster*. Another example is linalool from coriander is (+); however, linalool from lavender oil is (–). In some cases, it is the racemic form which is the most frequently encountered. ( $\pm$ )citronellol is widespread, the form (+) is characteristic of *Eucalyptus citriodor*, the form (–) is common to the rose and geranium essential oils.

The sesquiterpenes are formed from the assembly of three isoprene units (C<sub>15</sub>). The extension of the chain increases the number of cyclisations which allows a great variety of structures. The structure and function of the sesquiterpenes are similar to those of the monoterpenes. Examples of plants containing these compounds are angelica, bergamot, caraway, celery, citronella, coriander, eucalyptus, geranium, juniper, lavandin, lavender, lemon, lemongrass, mandarin, mint, orange, pepper-mint, petitgrain, pine, rosemary, sage, thyme.

## 2.2 Aromatic compounds

Derived from phenylpropane, the aromatic compounds occur less frequently than the terpenes. The biosynthetic pathways concerning terpenes and phenylpropanic derivatives generally are separated in plants but may coexist in some, with one major pathway taking over (e.g., cinnamon oil with cinnamaldehyde as major and eugenol as minor constituents, also clove oil, fennel, etc.).

Aromatic compounds comprise:

1. **Aldehyde:** e.g., cinnamaldehyde
2. **Alcohol:** e.g., cinnamic alcohol
3. **Phenols:** e.g., chavicol and eugenol
4. **Methoxyderivatives:** e.g., anethole, elemicine, estragole and methyleugenols
5. **Methylene dioxy compounds:** e.g., apiole, myristicine and safrole

The principal plant sources for these compounds are anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, tarragon, and some botanical families (Apiaceae, Lamiaceae, Myrtaceae, Rutaceae).

Nitrogenous or sulfured components such as glucosinolates or isothiocyanate derivatives (garlic and mustard oils) are also characteristic as secondary metabolites of diverse plants or of torrefied, grilled or roasted products.

## 3. Effects of essential oils as antibacterial agents

Various studies showed that essential oils also have antibacterial properties against a wide range of bacterial strain such as *Listeria monocytogenes*, *L. innocua*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteria*, *Bacillus cereus*, *Staphylococcus aureus*, and *Salmonella typhimurium* [42]. Direct inhibition correlation due to presence of thymol and carvacrol in the essential oils of thyme and oregano can inhibit some pathogenic bacterial strains such as *E. coli*, *Salmonella enteritidis*, *Salmonella choleraesuis*, and *Salmonella typhimurium* [43]. The same correlation was also confirmed for oils rich in carvacrol alone. Eugenol and carvacrol showed an inhibitory effect against the growth of four strains of *Escherichia coli* O157:H7 and *Listeria monocytogenes* [44]. The carvacrol showed strong antibacterial activity due to presence of phenolic hydroxyl group. Some essential

oils demonstrated antibacterial activity against zoonotic enteropathogens including *Salmonella spp.*, *Escherichia coli* O157, *Campylobacter jejuni*, and *Clostridium perfringens*. Thus, these oils could possibly be used as an alternative to antibiotics in animal feed [45]. Essential oils with high concentrations of thymol and carvacrol e.g., oregano, savory and thyme, usually inhibit Gram-positive more than Gram-negative pathogenic bacteria. However the essential oil of *Achillea clavennae* exhibited strong antibacterial activity against the Gram-negative *Haemophilus influenzae* and *Pseudomonas aeruginosa* respiratory pathogens, while Gram-positive *Streptococcus pyogenes* was the most resistant to the oil. Most antiseptic agents can damage the skin, leading to a change in microbial flora, and an increased shedding of the original protective bacterial flora of the hand leads to an increased risk of transmission of pathogenic microorganisms [46]. Reports suggest that repeated use of formulations containing tea tree essential oil (TTO) does not lead to dermatological problems, nor affect the original protective bacterial flora of the skin [47], so the antibacterial activity of some skin-wash formulas containing TTO as well as pure TTO was evaluated against *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The antibacterial activity of tea tree essential oil has recently been reviewed. It was found that antibacterial property of TTO is mainly due to presence of terpinen-4-ol. The essential oil of oregano is found to be effective against *Pseudomonas aeruginosa* and *Escherichia coli* [48]. *Ocimum gratissimum* essential oil can also inhibit extracellular protease and the expression of O-lipopolysaccharide rhamnose in virulence and multidrug-resistant strains of 22 *Shigellae*. Thus, the oil may find a use as a therapeutic measure against shigellosis. Methicillin-resistant *Staphylococcus aureus* can also be inhibited by the application of peppermint and spearmint essential oils. Essential oils could be used as antibacterial agents against some respiratory tract pathogens. The oil of *Achillea clavennae* showed its maximum activity against *Klebsiella pneumoniae* and penicillin-susceptible and penicillin resistant *Streptococcus pneumoniae*. The oil also exhibited strong activity against *Haemophilus influenzae* and *Pseudomonas aeruginosa*. An increased density of *Helicobacter pylori* in the gastric mucosa is associated with severe gastritis and an increased incidence of peptic ulcers [49]. The activities of 60 essential oils against *H. pylori* P1 were evaluated: 30 oils were able to affect the growth in vitro, and 15 showed strong activity. Among the individual constituents of these oils, carvacrol, isoeugenol, nerol, citral and sabinene exhibited the strongest anti-*H. pylori* effects. Further investigations are underway regarding the ability of essential oils to control *H. pylori* infections [50]. Croton cajucara Benth essential oil was found to be toxic for some pathogenic bacteria and fungi associated with oral cavity disease and may be useful for controlling the microbial population in patients with fixed orthodontic appliances. A 6-month controlled clinical study demonstrated that a mouth rinse containing essential oils showed a comparable antiplaque and anti-gingivitis activity to that containing the synthetic antibacterial agent, chlorhexidine [51]. Mouth rinses containing essential oils (specially phenolic rich types) with chlorhexidine gluconate are commonly used as preprocedural preparations to prevent possible disease transmission, decrease chances of postoperative infection, decrease oral bacterial load and decrease aerosolization of bacteria. Mouth washes containing essential oils could also be used as a part of plaque control routine since they can penetrate the plaque biofilm, kill pathogenic plaque-forming microorganisms by disrupting their cell walls and inhibiting their enzymatic activity. In addition, essential oils in mouth washes prevent bacterial aggregation, slow the multiplication and extract bacterial endotoxins. The mechanisms by which essential oils can inhibit microorganisms involve different modes of action, and in part may be due to their hydrophobicity. As a result, they get partitioned into the lipid bilayer of the cell membrane, rendering it more permeable, leading to leakage



of vital cell contents [52]. Impairment of bacterial enzyme systems may also be a potential mechanism of action. Essential oils show bactericidal activity against oral and dental pathogenic microorganisms and can be incorporated into rinses or mouth washes for pre-procedural infection control, general improvement of oral health, inter-dental hygiene and to control oral malodor [53].

#### 4. Effects of essential oils as antifungal agents

Some EOs demonstrate a broad range of natural fungicidal effects against post-harvest pathogens, especially because of their bioactivity in the vapor phase for storage applications [54]. However, more time is needed for vapor-phase bioactivity effect, possible absorption into the food material needed to be considered [55]. Antifungal activity might be affected by the targeted fungal physiological activity [56].

The reported effective compounds against food-borne fungi, including *Aspergillus niger*; *A. flavus*; and *A. parasiticus* is carvacrol and thymol [57]. Water-distilled EO from leaves and flowers of *Micromeria nubigena* H.B.K. (Lamiaceae) also showed anti-fungal properties [58]. Essential oils from thymol at 500 µg/ml concentration and at 1.0% and 100 µg/ml concentrations; cinnamic aldehyde and eugenol extracted from cinnamon and clove at 1.0% and 100 µg/ml concentrations also showed anti-fungal properties [59]. *Aspergillus parasiticus* growth and Aflatoxins production has been inhibited by *Thymus vulgaris* and *Citrus aurantifolia*. On the other hand *Mentha spicata* L., *Foeniculum miller*, *Azadirachta indica* A. Juss, *Conium maculatum*, and *Artemisia dracunculus* has only inhibited the fungal growth. While *Carum carvi* L. has controlled aflatoxin production without effect on the fungal growth [34]. Linalool and methyl chavicol and vanillin extracted from sweet basil and vanilla at 2000 µg/ml concentration has the same effect on aflatoxin production [59].

Hydro-distilled EOs of stems, leaves (at vegetative and flowering stages) and flowers of *Eugenia chlorophylla* O. Berg. (Myrtaceae) and various extracts of thyme, were active against molds and yeasts [59–61], respectively. Oleoresin extracted from cinnamon and clove inhibited mycotoxin-producing *Aspergillus* and *Penicillium* species at 2.0% w/v, 300 µg/ml concentrations [59].

Higher levels of phenolic compounds in thyme and clove showed antifungal effects. Phenolic compounds, such as allyl isothiocyanate and citralon in mustard and lemongrass, have been more effective as volatiles [62]; however, in EOs such as marjoram oil, they have been effectively antifungal in a matrix with mainly hydrocarbons [56].

#### 5. Mechanism of action

It has been demonstrated that the antimicrobial effects of the Eos acts by causing structural and functional damages to the bacterial cell membrane. It is also indicated that the optimum range of hydrophobicity is involved in the toxicity of the EOs.

Application of antimicrobials by different exposure methods, such as vapor phase compared to direct contact method, of mustard and clove EOs showed noteworthy differences [63]. The stereochemistry, lipophilicity and other factors affected the biological activity of these compounds which might be altered positively or negatively by slight modifications [64]. It has been shown that plant substances affect microbial cells by various antimicrobial mechanisms, including

attacking the phospholipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material of bacteria, and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids [65–70]. Allyl isothiocyanate derived from mustard seems to have multi-targeted mechanisms of action in metabolic pathways, membrane integrity, cellular structure and statistically significant higher release of the cell compounds of *Escherichia coli* O157:H7 [71].

Carvacrol increases the heat shock protein 60 HSP 60 (GroEL) protein and inhibited the synthesis of flagellin highly significantly in *E. coli* O157:H7 [66]. There are concerns regarding the enhanced aroma and taste of oregano at the higher levels of application in food items, especially at 1% [72]. The apparent antimicrobial efficacy of plant origin antimicrobials depends on factors such as the method of extracting EOs from plant material, the volume of inoculum, growth phase, culture medium used, and intrinsic or extrinsic properties of the food such as pH, fat, protein, water content, antioxidants, preservatives, incubation time/temperature, packaging procedure, and physical structure of food [73, 74]. Another important parameter regarding effects of food preservatives is ability to reduce the pH level inside the bacterial cell (pHin). It has been shown that pHin of both *E. coli* and *Salmonella* has been reduced by the effect of mustard's EOs [71].

Generally, Gram-negative bacteria are less sensitive to the antimicrobials because of the lipopolysaccharide outer membrane of this group, which restricts diffusion of hydrophobic compounds. However, this does not mean that Gram-positive bacteria are always more susceptible [27]. Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria [61, 75].

## 6. Synergistic and antagonistic effects of components

When the combined effect of substances is higher than the sum of the individual effects, this is synergy; antagonism happens when a combination shows less effect compared to the individual applications [27]. Synergistic effects of some compounds, in addition to the major components in the EOs, have been shown in some studies [76–78]. Application of a certain combination of carvacrol-thymol can improve the efficacy of Eos against pathogenic micro-organisms [79].

Synergism between carvacrol and p-cymene, a very weak antimicrobial, might facilitate carvacrol's transportation into the cell by better swelling the *B. cereus* cell wall [27]. Antimicrobial activity of combination of cinnamon and clove EOs in vapor phase showed better antimicrobial with less active concentration in the vapor phase compare to liquid phase [63]. Thymol and carvacrol showed synergistic and antagonistic effects, in different combinations of cilantro, coriander, dill and eucalyptus EOs (each containing several components) and mixtures of cinnamaldehyde and eugenol, against *Staphylococcus sp.*, *Micrococcus sp.*, *Bacillus sp.* and *Enterobacter sp.* [27]. An antagonistic effect on *Bacillus cereus* was seen in rice when carvacrol and p-cymene were used with salt; high-hydrostatic pressure showed a synergistic effect in combination with thymol and carvacrol against *L. monocytogenes*. Vacuum packing in combination with oregano EOs showed a synergistic effect against *L. monocytogenes* with 2–3 log<sub>10</sub> reduction. Similar results have been recorded when clove and coriander EOs have been used against *Aeromonas hydrophila* on vacuum-packed pork. Application of oregano EO has a synergistic effect in modified-atmosphere packaging (MAP) including 40% CO<sub>2</sub>, 30% N<sub>2</sub> and 30% O<sub>2</sub>. The available oxygen is another factor antagonistic on EO activities; by decreasing the oxygen level, the sensitivity of micro-organisms to the EOs has been increased [27].

Residual hydrosols after distillation of EOs from plant materials can be used as economical sources of antimicrobial components [74].

Application of nisin with carvacrol or thymol has been positively effective against *Bacillus cereus* with temperatures increasing from 8 to 30°C [27]. Application of nisin with rosemary extract enhanced the bacteriostatic and bactericidal activity of the nisin [80]. Oregano EOs, in combination with modified-atmosphere packaging, have effectively increased the shelf life of fresh chicken [72]. Antimicrobial resistance did not develop in *Yersinia enterocolitica* and *Salmonella choleraesuis* after sub-inhibitory passes with cinnamon, by direct contact or vapor phase [63]. Combination of linalool and 1, 8-cineole (1:1) created more resistance in *E. coli*, compared to application of pure linalool. Either synergism or antagonism of 1, 8-cineole and linalool derived from *Cinnamosma fragrans* could happen against Gram-negative bacteria and *Fusarium oxysporum* [81]. The synergistic effect of different components could offer a way to prevent possible off flavor caused by clove and tea tree when used to protect against *Escherichia coli* O157:H7 and minimize off flavor effects in meat products [32]. Combinations of EOs of oregano and thyme, oregano with marjoram and thyme with sage had the most effects against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* O157:H7 and *L. monocytogenes* [82].

## 7. Effects of essential oils as antiviral agents

Synthetic antiviral drugs have been used for the curing of Herpes simplex virus (type I, II) that causes some of the most common viral infections in humans, and can be fatal but not all are efficacious in treating genital herpes infections. HSV-1 and HSV-2 have also developed resistance to one of these (acyclovir) mainly in immuno-compromised hosts. Essential oils are considered to cure these viral diseases because plant extracts have low toxicity as compared to synthetic antiviral drugs. Natural material is considered as potential alternative. The activity of multilamellar liposome showed better improvement by introduction of essential oils with it. Due to presence of citral and citronellal, the essential oil of *Melissa officinalis* L. can inhibit the replication of HSV-2 and the ability to replicate of HSV-1 can be suppressed by incubation with different essential oils in vitro [83]. Of these, lemongrass essential oil possessed the most potent anti-HSV-1 activity and completely inhibited viral replication after incubation for 24 h, even at a concentration of 0.1%. The virucidal activity was found at high levels in peppermint essential oil against HSV-1 and HSV-2. When the viruses were pretreated with essential oil before adsorption than its antiviral activity was confirmed. Junin virus was successfully inhibited by the essential oil of *Lippia junelliana* and *Lippia turbinata* [84]. The essential oils of eucalyptus, *Santolina insularis* and Australian tea tree showed the antiviral effects against HSV-1. These oils gave good result both before and after adsorption. The oil directly inactivated virus particles, thus preventing adsorption of virion to host cells. Isoborneol, a common monoterpene alcohol, showed dual virucidal activity against HSV-1 [85] and specifically inhibited glycosylation of viral polypeptides. The essential oils have shown good results as antiviral but unfortunately, according to our information till now there is no study about the antiviral properties against the major viruses of era such as HIV and hepatitis C viruses [86].

## 8. Effects of essential oils as preservatives

In-food studies depend on several additional factors, which have not been tested in similar in vitro studies [87]. Spices and herbs can be used as an alternative



preservative and pathogen-control method in food materials. Application of both extracts and EOs of plant-origin antimicrobials such as floral parts of *Nandina domestica* Thunb could be a potential alternative to synthetic preservatives [88]. Generally, effective EOs in decreasing order of antimicrobial activities are oregano > clove > coriander > cinnamon > thyme > mint > rosemary > mustard > cilantro/sage [27]. However, in another study, mint showed less antimicrobial effect compared to mustard [89]. There are differences between in vitro and in-food trials of plant-origin antimicrobials, mainly because only small percentages of EOs are tolerable in food materials. Finding the most inhibitory spices and herbs depends on a number of factors such as type, effects on organoleptic properties, composition and concentration and biological properties of the antimicrobial and the target micro-organism and processing and storage conditions of the targeted food product [82, 90, 91]. In a study on blanched spinach and minced cooked beef, using clove and tea tree EOs, three and four times the MIC in in vitro studies were needed to restrict *E. coli* O157:H7 populations in the food materials [32]. Despite some positive reports in regard to application of plant-origin natural antimicrobials, two major issues are faced regarding application of plant-origin antimicrobials in food: odors created mostly by the high concentrations, and the costs of these materials [68, 69].

## 9. Antimutagenic properties of essential oils

Many studies showed that the mutations can be prevented by the inhibition the penetration of mutagens into cells, by adding antioxidants which inactivate the free radicals produced by mutagens, also by activation of cell antioxidant enzymes and by detoxification of mutagens by activation of enzymes by using plant extracts [92]. Some antimutagenic compounds works in two ways those are by promotion of error-free DNA repair or by inhibition of error-prone DNA repair [93]. During recent years the role and reaction of reactive oxygen species (ROS) scavengers, such as glutathione, superoxide dismutase, catalase, N-acetylcystein, provitamins like retinoid, carotenoids and tocopherols, flavonoids and other polyphenols. Also the biochemistry of antimutagens has been published in various documents [94]. However, since the work of [93] on *Escherichia coli*, nobody has examined in more detail this type of antimutagenicity possibly involving interference with DNA repair via intracellular pro-oxidant reactions of the latter compounds or terpenic and phenolic compounds from aromatic plants. Natural compounds, tannic acid and apigenin, reduced the frequency induced by nitropyrenes in CHO cells [95]. *Matricaria chamomilla* essential oil inhibits SCEs (sister chromatid exchanges) induced by daunorubicin and methyl methane sulfonate in mouse bone marrow cells. The aromatic plant *Salvia officinalis* and major components thujone, 1,8-cineole, camphor and limonene inhibit UV-C-induced mutagenesis in *Salmonella typhimurium*, *Escherichia coli* and *Saccharomyces cerevisiae*. The chemical compounds extracted from plants such as  $\alpha$ -terpinene,  $\alpha$ -terpineol, 1,8-cineole, d-limonene, camphor, citronellal and citral modulate hepatic mono-oxygenase activity by interacting with promutagen or procarcinogen xenobiotic biotransformation [96]. In a more recent study, they showed in the same system that *Origanum compactum* essential oil and some of its sub-fractions and constituents are antimutagenic against the indirect-acting mutagen urethane and also against the direct-acting mutagen methyl methanesulfonate. It is now accepted that pro-oxidant activities can induce late apoptosis and necrosis. Pro-oxidant activities may damage cellular membranes, in particular those of mitochondria, and thus promote the release of  $Ca^{++}$ , cytochrome C and ROS (reactive oxygen species). This leads to cell death, at least in mammalian cells, whereas yeast cells are able to



survive in spite of mitochondrial damage. It has been recently demonstrated in the yeast *Saccharomyces cerevisiae* that induction of mitochondrial damage transforming Rho<sup>+</sup> cells into Rho<sup>0</sup> cells and the induction of apoptosis/necrosis by a combined exposure to essential oils and nuclear mutagens caused a striking reduction of the frequency of nuclear genetic events [86]. Typical mutagenic agents were used such as ultraviolet C (UV-C) radiation which forms pyrimidine dimers and 6-4 photoproducts, 8-methoxypsoralen (8-MOP) activated by ultraviolet A (UVA) radiation which forms DNA mono- and biadducts, or methyl methanesulfonate (MMS) which methylates DNA bases. The reduction in mutant frequency in the presence of essential oils was accompanied by a strong synergistic induction of cytoplasmic “petite” mutants. The anti-mutagenic effect was independent of the type of mutations, i.e., reversion, intra- or intergenic recombination. The extent of this anti-mutagenic effect depended on the mutagen and oil concentrations. However, unexpectedly, the mechanism of the decrease of mutagenicity did not depend on the type of essential oil but on the type of mutagen, thus on the type of lesions and consequently on the DNA repair or lesion avoidance system involved. In fact, after combined treatment by UVC or 8-MOP/UVA plus essential oils, the transformation of Rho<sup>+</sup> cells into rho<sup>0</sup> cells resulted in a decrease of the frequency of mutants accompanied by a slight resistance of the survival [97]. After UVC or 8-MOP/UVA alone, less mutants were also found in a rho<sup>0</sup> mutant, i.e., a complete BET-induced rho<sup>0</sup> selected by the alkaloid lycorine, than in the wild type Rho<sup>+</sup>. In that case, the reduction in mutation frequency was the same as that after the combined treatments confirming the importance of mitochondrial dysfunction for these effects [98]. The same decrease of mutant frequencies was also found in a nucleotide excision repair (NER) defective rad3 mutant after UVC/essential oil combined treatment. Thus the error-free NER repair system does not play any role in this decrease and probably not the error-free homologous recombination in the case of 8-MOP/UVA. However, as a function of survival, this additional cytotoxicity caused a notable reduction of the mutant frequencies for a same survival level. The decrease of cell survival was also accompanied by a synergistic increase of cytoplasmic petite mutants. Thus, in this case, the essential oils contributed to the elimination of the cells already affected by MMS, leading potential mutants to death by late apoptosis and necrosis [97]. The reduction by essential oils of the frequency of mutations induced by the mutagens was always accompanied by a synergistic induction of complete petite mutants. Moreover, essential oils alone or in combined treatments mainly induced necrosis rather than apoptosis. This corroborates with the fact that petite mutants were true rho<sup>0</sup> mutants unable to perform apoptosis but only able to passively undergo necrosis, since functional mitochondria are necessary to induce apoptosis [99].

## 10. Role of essential oils as antioxidants

From the safety point of view, one of the important sources for the search of essential oils is herbs and spices. Since from prehistoric era, these had been used for flavoring and medicinal properties. However, recent reports showed that the radical scavenging activity of various essential oil is very high. The lag time in conjugated diene formation was dose-dependently prolonged by addition of the essential oils of some aromatic plants such as black cumin, cinnamon bark, ginger [100]. At a level of 200 µg/ml DPPH activity ranged from 39 to 90% in different plants. Phenolic compounds such as thymol, eugenol, linalool etc. are considered to be responsible for scavenging activity of essential oils. Biomolecules such as protein, amino acids, unsaturated lipids are mostly oxidized by free radicals and

reactive oxygen species. The human body is equipped with an inherent defense system which can destroy free radicals present in almost all cells [101]. Oxidative stress is the cause of imbalance between free radical production and their removal from the body. So to overcome oxidative stress antioxidants should be taken externally. Nowadays, there is more interest on natural antioxidants from plants sources to replace those of synthetic origin. Various studies have shown that essential oils are natural source of antioxidants. Aromatic plants are rich in natural antioxidants. The essential oils of coriander, Eucalyptus, juniper, cumin, basil, cinnamon, clove and thyme have proven radical-scavenging and antioxidant properties in the DPPH radical assay at room temperature [2, 40, 102]. The main constituents of the volatile extract of Egyptian corn silk were *cis*-R-terpineol (24.22%), 6,11-oxidoacor-4-ene (18.06%), citronellol (16.18%), *trans*-pinocampnone (5.86%), eugenol (4.37%), neo-iso-3-thujanol (2.59%), and *cis*-sabinene hydrate (2.28%). The water extract inhibited DPPH activity by 81.00 (6.00% at a level of 200 µg/ml). These results suggest that corn silk is a flavor ingredient source and a natural antioxidant supplement for various food products [41]. The antioxidant activity was attributed to the high content of the phenolics thymol and carvacrol (20.5 and 58.1%, respectively). *Thymus spathulifolius* essential oil also possessed an antioxidant activity due to the high thymol and carvacrol content 36.5 and 29.8%, respectively [103]. The activity is again attributed to the content of thymol and carvacrol (35.0 and 32.0%, respectively). Although dietary supplementation of oregano oil to rabbits delayed lipid oxidation, this effect was less than that of supplementation with the same concentration of tocopheryl acetate [104]. However, when tested on turkeys it showed an equivalent performance to the same concentration of  $\alpha$ -tocopheryl acetate in delaying iron-induced, lipid oxidation [105]. The essential oils of *Salvia cryptantha* and *Salvia multicaulis* have the capacity to scavenge free radicals. The activity of these oils was higher than that of curcumin, ascorbic acid or BHT [106]. The essential oil of *Achillea millefolium subsp. millefolium* (Asteraceae) exhibited a hydroxyl radical scavenging effect in the Fe<sup>3+</sup>  $\alpha$ -EDTA-H<sub>2</sub>O<sub>2</sub> deoxyribose system and inhibited the non-enzymatic lipid peroxidation of rat liver homogenate [107]. In addition, *Curcuma zedoaria* essential oil was found to be an excellent scavenger for DPPH radical [108]. The antioxidant activity of essential oils cannot be attributed only to the presence of phenolic constituents; monoterpene alcohols, ketones, aldehydes, hydrocarbons and ethers also contribute to the free radical scavenging activity of some essential oils. For instance, the essential oil of *Thymus caespitius*, *Thymus camphoratus*, and *Thymus mastichina* showed antioxidant activity which in some cases was equal to that of  $\alpha$ -tocopherol. Surprisingly, the three species are characterized by high contents of linalool and 1,8-cineole, while thymol or carvacrol are almost absent. The essential oil of lemon balm (*Melissa officinalis* L.) shows an antioxidant and free radical scavenging activity [109] with the most powerful scavenging constituents comprising neral/geranial, citronellal, isomenthone and menthone. Tea tree (*Melaleuca alternifolia*) oil has been suggested as a natural antioxidant alternative for BHT with the inherent antioxidant activity attributed mainly to the  $\alpha$ -terpinene,  $\beta$ -terpinene and  $\beta$ -terpinolene content. Essential oils isolated from *Mentha aquatica* L., *Mentha longifolia* L. and *Mentha piperita* L., were able to reduce DPPH radicals into the neutral DPPH form [110]. The most powerful scavenging constituents were found to be 1,8-cineole for the oil of *M. aquatica* while menthone and isomenthone were the active principles of *M. longifolia* and *M. piperita*. From the above studies, we may conclude that essential oils are rich sources of natural antioxidants. So we can use essential oils as natural sources of antioxidants instead of synthetic antioxidants to prevent from various degenerative diseases as well as to preserve food safely [111].

## 11. Photo toxicity of essential oils

The essential oils of some aromatic plants contain some photoactive compounds in their composition. Psoralens present in essential oil of citrus bergamia were found to be effective for binding of mono and biadducts produced under UV-light. These were found to be mutagenic and cytotoxic [112]. However, in the dark, this oil is not cytotoxic or mutagenic by itself. It has been noted that *Fusanus spicatus* wood essential oil was not phototoxic but was very cytotoxic. In other words, cytotoxicity seems rather antagonistic to phototoxicity. In the case of cytotoxicity, essential oils damage the cellular and organelle membranes and can act as pro-oxidants on proteins and DNA with production of reactive oxygen species (ROS), and light exposures do not add much to the overall reaction. In the case of phototoxicity, essential oils penetrate the cell without damaging the membranes or proteins and DNA. Radical reactions by excitation of certain molecules and energy transfer with production of oxygen singlet occur when cells are exposed to activating light. This may cause damage to cellular macromolecules and in some cases the formation of covalent adducts to DNA, proteins and membrane lipids. Obviously, cytotoxicity or phototoxicity depends on the type of molecules present in the essential oils and their compartmentation in the cell, producing different types of radicals with or without light exposure. However, such an antagonism is not quite a strict rule [86]. It was also found that *Citrus aurantium* dulcis (*Citrus gracilis* subf. dulcis) and *Cymbopogon citratus* essential oils were phototoxic and cytotoxic. Therefore, the potential toxicity of essential oil should be considered before use as antibacterial for human as well as for animals [113].

## 12. Other activities

EOs and their monoterpenes affected bone metabolism when added to the food of rats. It was demonstrated that these lipophilic compounds inhibited bone resorption [114]. It was reported that (2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoic acid, a novel monoterpene, from *Cistanche salsa* had antiosteoporotic properties [115].

Pine EOs prevented bone loss in an osteoporosis model (ovariectomized rats). The monoterpenes borneol, thymol and camphor directly inhibited osteoclast resorption [114]. It was observed that inactive monoterpenes can be metabolized to their active forms *in vivo*; thus, *cis*-verbenol, a metabolite of  $\alpha$ -pinene, inhibited osteoclastic resorption activity, in contrast to the parent compound  $\alpha$ -pinene.

Potential activities for the treatment of Alzheimer's disease were demonstrated in a pilot open-label study involving oral administration of the EO of *Salvia lavandulaefolia* Vahl. known as Spanish sage [7].

Chinese angelica (*Angelica sinensis*) is the most important female tonic remedy in Chinese medicine. The effects of angelica EO in three assays in mice (elevated plus maze, light/dark and stress-induced hyperthermia test) suggested that angelica EO exhibited an anxiolytic-like effect [116]. A link to emotion and cognitive performance with the olfactory system was reported [117]. Moreover, the EOs could affect mood, concentration and sleep [118], while other studies had shown that EOs were potentially important to boost the immune system [119, 120].

EOs from different *Lippia alba* chemotypes showed behavioral effects. Greater effects were presented by chemotype 2 (with citral and limonene), while chemotype 1, containing citral, myrcene and limonene, decreased only the number of rearings in the open-field test [121]. The EO of lemon was found to modulate the behavioral and neuronal responses related to nociception, pain and



anxiety [122, 123]. Thus, there is widespread and increasing interest in complementary and alternative medicines using EOs [124].

*Aloe vera* gel enhanced the antiacne properties of *Ocimum gratissimum* L.oil; the oil or its combination with *Aloe vera* gel was more effective than 1% clindamycin in the treatment of *Acne vulgaris* [124]. Linalool-rich EO was potent against promastigotes and amastigotes of *Leishmania amazonensis* [125].

### 13. 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 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 [126, 127], and the generation of hydroperoxides from polyphenols [128]. Plant extracts also showed antifungal activity against a wide range of fungi, antioxidant and antimutagenic activities [129] and inhibited lipid oxidation in foods [130]. 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 [131, 132].

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. 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 [133]. A wide variety of phenolic substances derived from herbs and spices possess potent biological activities, which contribute to their preservative potential [134]. Careaga et al. [135] reported that 1.5 ml/100 g of capsicum 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 [136]. Black cumin ethanolic extract applied in a marinade base for raw trout was found to reduce aerobic plate count, yeast, and coliforms [137]. Lee et al. [138] 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. [139] reported that a range of plant extracts are useful for reduction of pathogens associated with cooked beef, however, Uhart et al. [140] 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. [141] 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* Serotype Enteritidis and *Listeria monocytogenes* in ground beef by water-soluble arrowroot tea extract (upto 6% w/w) was reported [142]. 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 [143]. Mixtures of Scutellaria, honeysuckle, Forsythia and cinnamon or cinnamon, rosemary and clove oil showed 1.81–2.32-log reductions in microbial counts as compare to control in vacuum-packaged fresh pork during 28 days storage [144]. Yin and Cheng [145] 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 [146]. Garlic also has been shown to reduce the levels of *E. coli* in ground meat [147]. Sallam et al. [148] 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 it a strong antimicrobial property [149]. Nguyen and Mittal [150] 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 [151]. Even, the byproducts of curcumin manufacture were found to have high biological activity [152, 153]. Turmeric extract (1.5%, v/v) alone or in combination with shallot 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 [154].

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 [155, 156]. Extracts of the cinnamon bark and fruit and cinnamon oil have been reported to possess antimicrobial, antioxidant and antimutagenic activities [157]. Cinnamon was found to reduce the levels of *E. coli* in apple juice [158]. Yuste and Fung [159] 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. [158] reported that the addition of 0.3% (w/v) cinnamon powder gradually decreased the counts of *E. coli* O157:H7 in pasteurized apple juice, whereas only 2 log cfu/ml reduction of *E. coli* O157:H7 in unpasteurized apple cider was reported even by addition of 2% (w/v) cinnamon powder [160].

*Punica granatum* L. has a rich history of traditional use of its bark, leaves, flowers, fruits and seeds to ameliorate diseases. The presence of phytochemicals in the pomegranate extracts such as phenols, tannins and flavonoids as major active constituents may be responsible for these medicinal values [161, 162]. 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 [163]. Aqueous and ethanolic fruit shell extracts of *P. granatum* were found to have antibacterial activity against different strains of *E. coli* [164], *Salmonella* Typhi [165], and it inhibited Staphylococcal enterotoxin A production [166]. Various other solvent extracts from the rind of *P. granatum* also showed antibacterial activity against enterohaemorrhagic *E. coli* and food spoilage bacteria [162]. Pomegranate peel extracts were used to enhance the shelf life of chicken meat products by controlling oxidative rancidity and bacterial growth [167].

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 [168].

Likhitwitayawuid et al. [169, 170] reported antimalarial activity of xanthonoids 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 [171]. 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 [172]. 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* [173, 174]. 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* [168]. Crude extracts of *G. indica* also showed antiaflatoxigenic properties [175].

Seabuckthorn has been widely used in traditional medicines, mainly of Tibetan, Mongolian, Chinese and Middle Asian cultures [176, 177] for the treatment of asthma, skin diseases, gastric ulcers, lung disorders, cough, diarrhea, and menstrual disorder [178]. The health benefits of *Hippophae rhamnoides* oils, juice, leaves and bark are also well known and they have been used to treat several diseases [179]. 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 [180], antioxidant [181], and antimutagenic activities [182]; and antitumoral, hepato-protective and immunomodulatory [183], anti-platelet aggregating [184], anti-inflammatory [185], and radio-protective properties [186]. The leaf extract was reported to have better immunomodulatory effect than fruit extracts [183]. Jelly prepared by using seabuckthorn berries showed microbiological stability at ambient temperature and 37°C for a period of 6 months [187]. Various other plant extracts were found to be effective against *L. monocytogenes* in refrigerated meat products [188, 189]. 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. [190] 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 [190]. Ruiz et al. [191] 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,  $p \leq 0.05$ ), 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 [192]. 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 [193]. 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 [194].

Dried plum puree was found to reduce *E. coli* and *Salmonella* in ground meat [195]. Karapinar and Sengun [196] 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 [197]. Grape seed extract (1%) and rosemary oleoresin (1%) reduced the populations of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* after 9 days in raw ground beef [193]. Owen and Palombo

[198] 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 [199] 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. [130].

## 14. Future potential

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 pharmacological 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.

Antimicrobial and antioxidative packaging systems containing natural biological active substances may have high potential for commercial food packaging applications. Consumers would prefer to obtain better food safety of fresh produce and minimally processed foods using this type of packaging system. However, it is necessary that new active packaging systems must satisfy food safety regulations, which are different in each country. Greater food safety and quality assurance may be improved by the use of both antimicrobial and antioxidative packaging systems incorporating natural active agents. Some commercial products, such as films coated with wasabi extract, are already on the market and satisfy the regulations and consumer needs of particular countries. Most materials containing natural active agents are more effective when there is direct contact of the packaging materials with the food product. For new packaging systems to be introduced into the market effectively, careful design is required. Food contact layers with the appropriate concentrations of the active agents may be laminated or coated onto the barrier layer of the package structure. There are some suggestions that before long many countries would adopt the new active packaging concepts into their packaging regulations. Therefore, new applications of antimicrobial and antioxidative packaging are likely to be available in the market sooner or later.

Also, the products which produced by nanotechnology as (Aquanova product) the product description include "Aquanova's recent nanotech antioxidant system for essential oils and flavours is a clear signpost of where food ingredient technology in the twenty-first century is headed." It is designed to help manufacturers introduce antioxidants into food and beverage products easily and effectively. The product micelle allows to create crystal clear solutions ("solubilisates") of lipophilic or waterinsoluble substances not just from a visual standpoint. The product micelle is stable with respect to pH and temperature and has a diameter of approximately 30 nm. Here, where microemulsions and liposomes prove to be problematical and unsuitable, the product micelle represents the optimum solution in the fields of foodstuffs (functional food), cosmetics, pharmaceuticals and biotechnology (nutritional solutions for cell and bacterial cultures). The 100% water-soluble micelle can be integrated directly and independently of recipe characteristics into final products in the quoted fields. The product micelle is proving to be an optimum carrier system of hydrophobic substances for a higher and faster intestinal and dermal resorption and penetration of active ingredients.

On the other hand, it is important we must realize that further investigations into the deleterious or adverse biological effects of essential oils in *in vivo* models need to be performed. By doing so, we can better understand their mechanisms of action in combating disease, and better evaluate the quantities at which they best exert their beneficial actions to improving human health.

## 15. Conclusion

Natural materials should be considered as potential alternative. Owing to the new attraction for natural products like essential oils and plant extracts, despite their wide use, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the



environment. To preserve food safely and to prevent human beings from various degenerative diseases we can use essential oils as natural antioxidants and anti-mutagenic preferably over synthetic. Essential oils can be incorporated into rinses or mouth washes as antibacterial to protect from infection and for general improvement of oral health. The essential oils have also shown good antiviral activity but antiviral activity against the major viruses of twenty-first century such as HIV and hepatitis C viruses should also be studied. As the essential oils and plant extracts have useful biological properties, so their uses in food and pharmaceutical industry should be more as natural ingredients instead of synthetic chemicals, to save and protect the ecological equilibrium.

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