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Evaluation of Chemical Composition, Antioxidant Property and Antimicrobial Activity of Plant Based Essential Oils

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Abstract:

Essential oils derived from plant extracts are precious gift by nature. These essential oils are odorous and volatile in nature and have tendency to undergo evaporation when exposed to the air. Essential oils have multiple active components which make them biologically active against several microorganism and fungi such as: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, and antifungal activity against phytopathogenic strains such as Alternaria sp, Pencillium expansum, Rhizopus stolonifer, Botrytis cinereasuch. These essential oils possess potential antioxidant properties that are highly required for human health against several diseases. The evaluation and assessment of the antioxidant and antibacterial activity performance of essential oils are highly recommended, because many commonly and traditional used "tests" are inappropriate to establishment of active principle. The future research based on isolation of compounds and their mechanism will explain antioxidant, antimicrobial and cytotoxic activity of essential oils. In this review mechanism of antioxidant activity and antimicrobial activity performance of essential oils are critically reviewed.

Key words: chemical composition, antioxidant property, antimicrobial activity, plant based essential oils

Introduction

In recent years there is a dramatic upsurge in the areas related to newer developments in prophylaxis and treatment of diseases, particularly the role of free radicals and antioxidants. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Pala and Tabakcioglu 2006). They are primarily derived from oxygen (reactive oxygen species; ROS) and nitrogen (reactive nitrogen species; RNS), and are generated in the body milieu by a number of endogenous physiological and pathological processes concerning redox enzymes and bioenergetic electron transfer (Halliwell and Gutteridge 2006). Free radicals can adversely alter macromolecules such as lipids, proteins and DNA and have been implicated in cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Braca *et al*. 2002; Maxwell 1995). Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations (Devasagayam and Kesavan 1996). Free radical damage to protein can result in loss of enzymatic activity, whereas, damage caused to DNA can result in mutagenesis and carcinogenesis (Stadtman 1992). Apart from many dietary components, nature has endowed living organisms with protective antioxidant mechanisms, e.g., antioxidant enzymes including superoxide dismutase (SOD), catalase, glutathione, glutathione peroxidases, glutathione reductase, and antioxidant molecules including α-tocopherol and ascorbic acid (Halliwell and Gutteridge 2006). There are epidemiological evidences correlating higher intake of components/foods with antioxidant

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ability to lower the incidences of various human morbidities or mortalities (Devasagayam *et al*. 2004).

Food-borne disease also referred to as food poisoning, is any illness resulting from the consumption of food contaminated with food-borne pathogenic bacteria, has been of vital concern to public health (Bajpai, Baek, & Kang, 2012). Controlling pathogens could reduce food-borne epidemics and assure consumers a safe, healthy, and nutritious food supply. Several attempts have been made to control microbial growth and to reduce the incidences of food poisoning and spoilage using synthetic antimicrobials (Burt, 2004). Increase in microbial resistance and adverse effects including hypersensitivity, allergic reaction and immunity suppression associated with synthetic antimicrobials motivate researchers towards the development of natural, safe and effective antimicrobial agents (Cakir, Kordali, Kilic, & Kaya, 2005). The food industry requires the design of food preservation treatments capable of ensuring microbial inactivation to assure food safety, extended shelf-life and objectionable changes in nutritional and physicochemical properties of foods (Pflug & Gould, 2000). Therefore, researchers are proposing the use of natural antimicrobial substances for controlling food-borne pathogenic bacteria (Burt, 2004).

Nowadays, different strategies are applied in order to control food-borne pathogens in food commodities, and particular interest has been focused on the application of natural antimicrobial agents, including the essential oils from various species of edible and medicinal plants, herbs, and spices that are relatively much safer to human being (Yoon, Bajpai, & Kang, 2011).

Recently much attention has been directed towards the development of less toxic ethnomedicines and their potential applications in prophylaxis and treatment of various diseases (Bajpai *et al*. 2011; Lee *et al*. 2011; Lee *et al*. 2012). The plant-based volatile essential oils and non-volatile secondary

metabolites have wide applications in dietary supplements, food flavoring and preservation, folk medicine and fragrance industry (Huang *et al*. 2005; Kalemba and Kunicka 2003). Several reports have confirmed the antioxidant efficacy of plant-based essential oils *in vitro* and *in vivo* (Bajpai *et al*. 2009; Hsu 2012). Application of plant materials as dietary supplements and preservatives is mainly due to their antioxidant, antimicrobial and other biological as well as pharmaceutical potentials. Nowadays, essential oils and their components are gaining increasing attention because of their relatively safe status, their wide acceptance by consumers, and the possibility of their exploitation for potential multi-purpose functional uses. Hence, it will be relevant to examine the possible roles of free radicals on various diseases and antioxidants on disease prevention (Ormancey *et al*. 2001; Devasagayam *et al*. 2004).

Essential oils are complex mixtures of compounds, mainly monoterpenes, sesquiterpenes and their corresponding oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides) from plants, which are widely known for their scents and flavors (Bajpai et al., 2012). Plant-derived essential oils have long been used as flavoring agents or preservatives in foods, beverages and confectionary products. The chemical composition and antimicrobial properties of plant-based essential oils have been demonstrated using a variety of experimental models (Yoon et al., 2011; Bajpai et al., 2012; Although antimicrobial mode of action of synthetic antimicrobial agents has been reviewed to some extent, there is still lack on determining the mode of action of plant-based natural compounds including essential oils against pathogenic microorganisms.

The present review covers chemical composition, protocols for the determination of antimicrobial and antioxidant activity, future prospects and practical applications of plant based essential oils.

1. Antioxidant Screening Models

1.1 *Determination of DPPH Radical Scavenging Activity*

The scavenging activity of stable DPPH free radical was determined by the previous method with a slight modification (Braca *et al*. 2001). Different concentrations of essential oil were added to desired volume of methanolic DPPH solution (0.004%) as 1:1 ratio. The mixture was incubated at 37ºC in dark for 30 min with shaking. Absorbance was recorded at 517 nm using spectrophotometer against a blank sample. The percent inhibition activity was calculated using the following formula:

$$\text{Percent inhibition (\%)} = (\text{A control} - \text{A test}) / (\text{A control}) \times 100$$

Where, A control is the absorbance of the control reaction and A test represents the absorbance of a test reaction.

1.2 *Determination of nitric oxide radical scavenging activity*

In aqueous solution at physiological pH, SNP automatically generates nitric oxide, which intermingles with oxygen to generate nitrite ions that can be anticipated by the Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) (Green *et al*. 1982). Scavengers of free radicals result in the reduced production of nitric oxide. The solution of SNP (10 mM) in phosphate buffer saline (PBS pH 7.4) was mixed with different concentrations of essential oil. The mixture was incubated at 37ºC for 60 min in light. The half quantity of aliquots was taken and mixed with equal quantity of the Griess reagent, and the mixture was incubated at 25ºC for 30 min in dark. The absorbance of pink chromophore generated during diazotization of nitric ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm against a

blank (Green *et al.* 1982). The percent inhibition activity was calculated by the formula:

$$\text{Percent inhibition (\%)} = (\text{A control} - \text{A test}) / (\text{A control}) \times 100$$

Where A control is the absorbance of control reaction and A test is the absorbance of test reaction.

1.3 *Determination of Superoxide Radical Scavenging Activity*

Superoxide radical scavenging activity is measured by the reduction of nitro blue tetrazolium (NBT) according to a previously reported method (Fontana *et al.* 2001) with slight modification. The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple color formazan. In this assay, the reaction mixture (150 µL) contained phosphate buffer (0.2 M, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations of the essential oil. After incubation for 60 min at room temperature, the absorbance of the reaction mixture was measured at 560 nm against an appropriate blank to determine the quantity of formazan generated. The percent inhibition activity was calculated by the formula:

$$\text{Percent inhibition (\%)} = (\text{A control} - \text{A test}) / (\text{A control}) \times 100$$

Where A control is the absorbance of control reaction and A test is the absorbance of test reaction.

1.4 *Determination of Hydroxyl Radical Scavenging Activity*

A previously described method was adopted for determining the hydroxyl radical scavenging activity of essential oil (Elizabeth and Rao 1990). The assay is based on quantification of the

degradation product of 2-deoxy-2-ribose sugar by condensation with 2-thiobarbituric acid (TBA). Hydroxyl radical was generated by the Fenton reaction using Fe^{3+}-ascorbate-EDTA-H_2O_2 system. The reaction mixture contained 2-deoxy-2-ribose (3 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (2 mM), ascorbic acid (0.1 mM) and various concentrations of essential oil or standard compounds. After incubation for 45 min at 37C, 40 µL of 2.8% TCA, and 40 µL of TBA (0.5% in 0.025M NaOH solution containing 0.02% BHA) were added in the reaction mixture, and the mixture was incubated at 95ºC for 15 min to develop the pink color. The absorbance was measured at 532 nm against an appropriate blank solution. The percent inhibition activity was calculated by the formula:

Percent inhibition (%) = (A control - A test) / (A control) × 100

Where A control is the absorbance of control reaction and A test is the absorbance of test reaction.

1.5 *Lipid Peroxidation Assay*

The Fe^{3+}/ascorbic acid dependent non-enzymatic lipid peroxidation in bovine brain extract was performed according to the method (Houghton *et al*. 1995) with slight modification. The reaction mixture, in the absence and presence of CTEO or reference compounds, containing 50 µL of bovine brain phospholipids (5 mg/mL), 1 mM $FeCl_3$ and 1 mM ascorbic acid in 20 mM phosphate buffer with a final volume of 330 µL, was incubated at 37ºC for 1 h. The hydroxyl radicals generated in the reaction initiated the lipid peroxidation, resulting in malondialdehyde (MDA) production that was measured by TBA reaction. The percent inhibition activity was calculated by the formula:

Percent inhibition (%) = (A control - A test) / (A control) × 100

Where A cont. is the absorbance of control reaction and A test is the absorbance of test reaction.

1.6 *Reducing Power Assay*

The Fe^{3+} reducing power of the CTEO was determined by the method of Oyaizu (1986) with slight modification. Aliquots (50 µL) of different concentrations of essential oil is mixed with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1% w/v in H_2O), followed by incubation at 50ºC in dark. After incubation, 50 µL of TCA (10% w/v in H_2O) was added to terminate the reaction and the mixture was subjected to centrifugation at 3000 rpm for 10 min. For final reaction mixture, the supernatant (50 µL) was mixed with 50 µL distilled water and 10 µL $FeCl_3$ solution (0.1% w/v in H_2O). The reaction mixture was incubated for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. A higher absorbance of the reaction mixture indicated greater reducing power ability.

1.7 *Determination of Total Phenolic Content*

Total phenolic content was determined using Folin-Ciocalteu reaction according to the method as described previously (Kujala *et al.* 2000) with slight modification. An aliquot of 50 µL CTEO (100 µg/mL) was mixed with 50 µL of 5% Folin-Ciocalteu reagent and the reaction mixture was incubated 25ºC for 5 min in dark followed by addition of 100 µL of 20% (w/v in H_2O) Na_2CO_3 solution. After incubation at room temperature for 20 min, the absorbance was measured at 730 nm against the appropriate blank solution.

2. Antimicrobial Screening Models

2.1 *Determination of antibacterial activity by disc diffusion assay*

Standard agar diffusion method was used for the determination of antibacterial efficacy of essential oil (Bajpai et al., 2009). Petri-plates were prepared by pouring nutrient agar medium and allowed to solidify. Plates were dried and standardized inoculum containing 10^7 CFU/mL of bacterial suspension was poured and the inoculum was allowed to dry. A sterile filter paper disc was impregnated with essential oil. Standard reference antibiotic is used as positive control against the tested food-borne pathogenic bacteria. The plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameters of inhibition zones against the tested bacteria.

2.2 *Determination of minimum inhibitory and minimum bactericidal concentrations*

The minimum inhibitory concentration (MIC) of the essential oil is tested by two-fold serial dilution method (Bajpai et al. 2009). The essential oil incorporated into NB medium for bacterial pathogens to obtain a concentration of 2000 µg/mL, and serially diluted to achieve 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/mL, respectively. Standardized suspension of tested organism (10^7 CFU/mL) is transferred to each tube. The control tubes containing only bacterial suspensions were incubated at 37°C for 24 h. The lowest concentration of essential oil, which did not show any visible growth of test organisms after macroscopic evaluation, was determined as MIC, which was expressed in µg/mL. Further, the concentrations showing complete inhibition of visual growth of bacterial pathogens is identified. The complete absence of growth of bacterial colonies on the agar surface is the lowest concentration of sample and was defined as the minimum bactericidal concentration (MBC).

2.3 *Effect of essential oilon viable counts of bacterial pathogens*

For each strain, active stock solution was transferred to NB broth. All treated cultures were kept at 37°C for 2 h. The cultures were centrifuged at 10,000 rpm for 10 min. The pellets were suspended with 1 mL of phosphate-buffered saline (PBS). For viable counts, each of the tubes containing re-suspended bacterial suspension (approximately 10^7 CFU/mL) of microbes inoculated with essential oil at MIC concentration and kept at 37°C. Samples for viable cell counts were taken out at different time intervals. A sample of each treatment was diluted and spread on the surface of NB agar. The colonies were counted after 24 h of incubation at 37°C (Bajpai et al. 2009).

2.4 *Scanning electron microscopic analysis*

To determine the efficacy of CTEO on the morphology of *microbes*, a scanning electron microscopic (SEM) study was performed using the MIC of essential oil. Further, to observe the morphological changes, the SEM was modified from the Kockro method (Bajpai et al., 2009). The bacterial sample is washed gently with phosphate buffer solution (pH 7.2), fixed with 2.5%, glutaraldehyde and osmic acid solution (1%). The specimen was dehydrated using sequential exposure per ethanol concentrations ranging from 50-100%. The ethanol was replaced by tertiary butyl alcohol. After dehydration, the specimen was dried with carbon dioxide (CO_2). Finally, the specimen was sputter-coated with gold in an ion coater, followed by microscopic examinations.

2.5 *Measurement of extracellular adenosine 5'-triphophate (ATP) concentration*

To determine the efficacy of essential oil on membrane integrity, the extracellular ATP concentrations were measured according to the previously described method (Lee, Kim, & Shin, 2002). The working cultures of microbes containing

approximately 10^7 CFU/mL is centrifuged for 10 min at 1,000g and the supernatants were removed. The cell pellets were washed with sodium phosphate buffer (pH 7.0) and then cells were collected by centrifugation under the same conditions. A cell suspension was prepared with sodium phosphate buffer and cell solution was taken tube. Then, the different concentrations (control and MIC) of essential oil are added to the cell solution. Samples were maintained at room temperature, centrifuged, and incubated in ice immediately to prevent ATP loss until measurement. The extracellular ATP concentrations were measured using an ATP bioluminescent assay kit which comprised ATP assay mix containing luciferase, luciferin, $MgSO_4$, dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA) and tricine buffer salts. The ATP concentration of the supernatants, which represented the extracellular concentration, was determined using a Luminometer.

2.6 *Measurement of release of 260-nm absorbing cellular materials*

The measurement of the release of 260-nm-absorbing *materials from microbial cells was* carried out in aliquots of 2 mL of the bacterial inocula in sterile peptone water added of essential oil (MIC) at 37°C. At different time interval of treatment, cells were centrifuged, and the absorbance of the obtained supernatant was measured at 260 nm using spectrophotometer (Carson, Mee, & Riley, 2002).

2.7 *Assay of potassium ions efflux*

A previously described method was used to determine the amount of the potassium ions (Lee et al., 2002). The concentration of free potassium ions in bacterial suspensions is measured after the exposure of bacterial cells to essential oil at MIC concentration. At each pre-established interval, the

extracellular potassium concentration was measured by a photometric procedure using the Kalium/Potassium.

3. Mechanism of Antioxidant Activity

In recent years, several researchers have reported that phenolics, hydrocarbons and oxygenated phytoconstituents of plant-derived essential oils have enormous antioxidant activity and free radical scavenging potential (Devasagavan *et al*. 2004; Bajpai *et al*. 2009). Previously it has been reported that essential oils containing phenolic compounds e.g., butylated hydroxyl toluene (BHT), phenol and antiarol, aromatic acid (e.g., benzoic acid), oxygenated sesquiterpene (e.g., palustrol) and polyhydric alcohol (e.g., scyllitol) which were also present in CTEO, have been shown to exert potent antioxidant and scavenging activities in various antioxidant models (Panchon *et al*. 2008; Natella *et al*. 1999; Faraji and Lindsay 2004; Lazarevi *et al*. 2011). In general, the antioxidant compounds of essential oils are terpenes, which are phenolic in nature, and it would seem rational that their antioxidant mode of action might be related to that of other compounds (Hocman 1988). These findings were also confirmed in the present study with the presence of phenolics, and most importantly the presence of BHT in CTEO, one of the promising antioxidant molecules (Hocman 1988). The CTEO also contained dopamine, a powerful inhibitor of iron-catalyzed lipid peroxidation due to its iron chelating ability (Miura *et al*. 1998).

DPPH radical scavenging assay is the most popular method for the determination of antioxidant capacity of antioxidant compounds due to its simplicity, rapidity, sensitivity, and better reproducibility (Nakayama *et al*. 1993). Radical scavengers may protect cells, tissues from free radicals, thereby preventing diseases such as cancer and atherosclerosis (Nakayama *et al*. 1993; Ozcelik *et al*. 2003).Unlike other free radicals such as hydroxide and superoxide, DPPH has the

advantage of being unaffected by certain side reactions such as metal ion chelation and enzyme inhibition (Amarowicz *et al*. 2004). The antioxidant molecule quenches DPPH molecule by donating a hydrogen atom or electron and converts it to a colorless hydrazine analogue, resulting in a decreased absorbance. The antioxidant is able to scavenge the radicals effectively, particularly peroxy radicals which are the propagators of lipid peroxidation and thereby breaking the free radical chain reaction (Frankel 1991).

Nitric oxide scavenging effect of CTEO was determined using the Griess reagent (Tylor *et al*. 1997). Nitric oxide is an abundant reactive radical generated endogenously that acts as an important oxidative biological signaling molecule in a variety of physiological process, such as inflammation, vasodilatation, neurotransmission, smooth muscle relaxation and immune regulation (Tylor *et al*. 1997). Sustained levels of nitric oxide radical are cytotoxic and contribute to the vascular collapse coupled with septic shock, furthermore chronic expression of nitric oxide radical is associated with various cancers and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Tylor *et al*. 1997). When superoxide reacts with nitric oxide, it produces more oxidatively active molecules, peroxy nitrite ions ($ONOO^-$), which cause DNA fragmentation, cell damage, neurotoxicity and lipid peroxidation (Reddy *et al*. 2008; Valko *et al*. 2007). The Antioxidant may inhibit nitrite formation by directly competing with oxygen in the reaction with nitric oxide. Superoxide anion, reduced form of molecular oxygen, is associated with aging by initiating oxidation reactions (Wickens 2001). Superoxide anion is also implicated in several pathophysiological processes, due to its transformation into more reactive species such as hydroxyl radical that initiates lipid peroxidation (Yen and Duh 1994). The antioxidant properties of some of the secondary metabolites have been reported highly effective mainly via scavenging of superoxide

anion radical that plays an important role in the formation of other ROS such as hydrogen peroxide, hydroxyl radical and singlet oxygen (O_2), inducing oxidative damage in lipids, proteins and DNA (Gulcin *et al*. 2006). Superoxide anion, derived from the oxidation coupled with PMS-NADH reaction system, can reduce NBT in the detection system. The decrease of absorbance at 560 nm in the presence of antioxidant indicates the consumption of superoxide anion in the reaction mixture (Gulcin *et al*. 2006). The essential oil is analyzed for hydroxyl radical scavenging activity in order to determine its efficacy in another antioxidant model. Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions, such as copper or iron. When a hydroxyl radical reacts with an aromatic compound, it can add on across a double bond, resulting in hydroxycyclohexadienyl radical. The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxyl radical, or decompose to phenoxyl type radical by water elimination (Lee *et al*. 2004). Among all biological macromolecules, unsaturated membrane lipids are predominantly prone to oxidative damage. Therefore, the lipid peroxidation, as a well-established mechanism of cellular injury, can be used as an indicator of oxidative stress in cells and tissues (Niki 2008, Houghton *et al*. 1995). Electron donation is an important means by which antioxidants promote the formation of less reactive species, and may be assessed using the reducing power assay. The reducing properties are generally associated with the presence of reductones (Duh *et al*. 1999), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The essential oil has marked ferric ions (Fe^{3+}) reducing ability and

had electron donor properties for neutralizing free radicals by forming stable products. The phenolic content could be used as an important indicator of the antioxidant capacity, which may be used as preliminary screening tool for utilizing the essential oils in dietary supplements (Liu *et al*. 2008). Many research indicated polyphenols as potent antioxidants (Hernandez *et al*. 2009; Estaca *et al*. 2009). These compounds act as antioxidants by donation of a hydrogen atom, as an acceptor of free radicals, by interrupting chain oxidation reactions or by chelating metals (Gramza *et al*. 2004).

Future Prospects and Practical Applications

Undesirable problems and detrimental effects arisen from the consumption of synthetic antimicrobials, promoted the development of bioactive secondary metabolites as natural antimicrobial agents and their utilization in food and pharmaceutical industries. Hence, natural food preservatives targeted to food and food products that are easily contaminated by microorganisms are highly desirable. The plant based essential oil exerts its inhibitory effect against microorganisms through permeabilization and disruption of cell membrane, loss of 260-nm absorbing materials, and leakage of vital ions with decreased pool of extracellular ATP being an indication of loss of membrane integrity. The biologically active essential oil are useful in the food industry to control food-borne pathogens, where these pathogens cause severe destruction by slow down the quality of foods and consumer demand. Thus, present review strengthen the suggestions that essential oil derived antimicrobials might act against broad spectrum of microbes for their applications in the food and pharmaceutical industry, because plant-derived natural antimicrobials have been consumed by mankind for centuries with lesser or no signs of any toxicity. However, further studies regarding safety,

toxicology, combined use with traditional medicines and legislation are warranted.

Food-borne pathogens are the leading causes of illness in the developed countries. These pathogens are responsible for millions of cases of infectious diseases each year, costing billions of dollars in medical care. New food-borne pathogens and food-borne diseases are likely to emerge driven by factors such as pathogen evolution, changes in food manufacturing practices, and changes to the human host status. Fuelled by these concerns, research on biologically active plant-based natural compounds to control the food-borne pathogens has escalated to unprecedented levels in recent years. Plant-based antimicrobials such as essential oils have been found to suppress the growth of food-borne pathogens in various models. The present review clearly indicates that the plant based essential oil could be used as an affective candidate to control the growth of certain important food-borne pathogens in practical applications.

Moreover, Free radicals play a very important role in food deterioration and the disease progression. Numerous study indicates that plant-based essential oil have wide application in dietary health supplements, preservatives, cosmetics and fragrance, mainly due to their wide margin of safety and broad range of antioxidant and other biological properties. Antioxidant, lipid peroxidation and free radical scavenging activities of the several plants based essential oil were studied using *in vitro* radical scavenging models. The main compounds from the essential oil such as oxygenated sesquiterpenes, sulphur compounds, aliphatic and aromatic hydrocarbons, phenolics as well as amine derivatives contribute to its antioxidant and radical scavenging potential. The results of previously reported *in vitro* assays indicate that essential oil can be a significant source of natural antioxidant, which might be applicable for preventing the progress of various oxidative stress induced diseases. In addition, the use of essential oil as a

natural antioxidant agent will be a suitable strategy for its applications in the food industries with lesser or no signs of toxicity as a potent food stabilizer against oxidative deterioration. Plant-based essential oils have wide applications in food preservation, cosmetics and fragrance industry, mainly due to their wide margin of safety and broad range of antioxidant and other biological properties.

Conclusions

The review showed that essential oil exhibits high antioxidant, free radical scavenging, and reducing power activities. Moreover, the essential oil exerted a potent inhibitory effect on ferric ion-induced lipid peroxidation. These *in vitro* assays indicate that essential oil can be a significant source of natural antioxidant, which might be applicable for preventing the progress of various oxidative stress induced diseases. In addition, the use of essential oil as a natural antioxidant agent will be a suitable strategy for its applications in the food industries with lesser or no signs of toxicity as a potent food stabilizer against oxidative deterioration.

Moreover, essential oil exerts its inhibitory effect through permeabilization of the cell membrane associated with generalized membrane-disrupting effects, and this corresponds to a simultaneous reduction in the number of viable bacteria, loss of 260-nm absorbing materials, and leakage of potassium ions with decreased pool of extracellular ATP being indicative of loss of membrane integrity. Moreover, the SEM observation supports the above hypothesis, and strongly indicates the membrane activity of essential oil. This should lead to effective application of the essential oil as natural antimicrobial agent to control food-borne pathogens in food industries. The heterogeneous composition of essential oil and the antimicrobial activities of many of its components, it seems unlikely that there is only one mechanism of action or that only one

component is responsible for the antimicrobial action. Further work is required to understand fully the mechanisms involved in order to justify the real applications of essential oil in food practices as a natural antibacterial agent.

RST and PS contributed equally in this paper.

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