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Review Article

ESSENTIAL OILS AND ITS ANTIBACTERIAL PROPERTIES – A REVIEW

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Abstract

A large portion of the world population, especially in developing countries depends upon the traditional system of medicine for a variety of diseases. Several hundred genera of plants are used as vital sources for potent and powerful drugs. In herbal medicine, crude plant extracts in the form of herbal extracts are used by the population for the treatment of diseases, including infectious diseases. Although, their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents. Essential oils are volatile, natural, complex compounds that are produced by plants as secondary metabolites for protection against bacteria, viruses, fungi and pests. They also have an important role in dispersion of pollens and seeds by attracting some insects. In Middle Ages essential oils were used for preservation of foods and as flavoring, antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies. But, characterizing these properties in laboratory dated back to the early 1900s. At present, about 3000 essential oils are known and 300 of them are used commercially in different industries such as pharmaceutical, agronomic, food, sanitary, cosmetic and perfume. Today, antioxidant, antitumor and antiviral, antifungal and antibacterial activity of essential oils and their constituents were widely studied. The present review was focused on the antibacterial activity of various essential oils. In this present review, we discussed about the Essential oils, Historical use of Essential oils, Antimicrobial components present in Essential oils, Mechanism of action of Essential oils, Antibacterial activity of Essential oils, Factors affecting antibacterial activity of Essential oils, Synergic effect of Essential oils and Limitations of Essential oils.

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1. Introduction

Nature is a source of medicinal agents and an impressive number of modern drugs have been isolated from natural sources. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infectious diseases. Traditional medicine has long been accepted as an alternative

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to western medicinal practice in many countries. Traditional medicine was once regarded as the sole source of treatment, making it a focus in the search for solution to increasing drug resistance among pathogenic microorganisms. The World Health Organization (WHO) has reported that over 80 % of the world's population relies on traditional medicine which is largely plant based for their primary healthcare needs (Kamazeri, 2012).

Infectious diseases caused by infective microorganisms like bacteria, fungi, viruses or parasites represent a vital pathological state and one in every of the most causes of morbidity and mortality are included in the list of the ten leading causes of death worldwide (Menchaaca Md *et al*., 2016; Nunkoo and Mahomoodally, 2016). In the recent years, the emergence of resistant Gram negative and Gram positive bacteria presents a serious challenge for the antimicrobial medical aid and drastically narrows the treatment choices of human infections (Marzougui *et al*., 2016). So, there is an imperative ought to resolve newer, safer and more effective natural or artificial antibacterial drug molecules so as to fight the emergence of those new resistant.

Medicinal and aromatic plants have been used by mankind since ancient time to treat various ailments. Medicinal plants are rich source of bioactive compounds and have played an important role in drug discovery (Hossain *et al*., 2014). Recently, there has been a growing interest in the medicinal properties of essential oils which are concentrated essences of the plant materials such as fruits, buds, flowers, leaves, etc. Plant essential oils are primarily secondary plant metabolites which have been reported to possess various biological activities such as antimicrobial, anticancer, anti–inflammatory, analgesic, as well as antifungal activities, etc. (Rana *et al*., 2011; Srivastava *et al*., 2014). Over the years, many pathogenic bacterial strains have developed high antimicrobial resistance, this necessitates the discovery of new effective antibiotic treatment. Essential oils are considered as the important source of bioactive compounds especially antibacterial agents, as they have shown promising antimicrobial action against a wide range of microorganisms (Sharma *et al*., 2014).

One of the challenges in controlling infectious diseases is antimicrobial drug resistance. Formation of multiple drug resistance in bacteria, against common antibiotics, over the past several decades has emerged many problems in the treatment of bacterial diseases. This causes increasing attention to natural materials as new antimicrobial substances. Plants have known as the cheapest and safer substitute sources of antimicrobials (Kumar *et al*., 2012). Since the first documented scientific research about antimicrobial activity and chemical composition of plant, in the second half of the 19^{th} century, research have continued in this area and plants were investigated for better recognizing of their properties, safety and efficiency (Gobalakrishnan *et al*., 2013).

The emergence of microbial resistance to multiple antimicrobial agents has become a significant global concern. Microorganisms resistant to more than two groups of antibiotics are regarded as multi-drug resistant (MDR). The global emergence of MDR bacteria is gradually elevated morbidity and mortality rates as well as in the increased treatment costs which limit the effectiveness of existing drugs and significantly cause treatment failure (Levy and Marshall, 2007; Lin *et al*., 2015). It often stops to respond to conventional antimicrobial agents and treatment, resulting in adverse effects on the patients, greater threat of death and higher costs (Jindal *et al*., 2015). Hospital acquired infections caused by MDR bacteria is major challenge for clinicians and it creates problems in cancer and AIDS patients. Most widespread multidrug resistant bacteria include Gram positive Methicillin - resistant, *Staphylococcus aureus*, *Enterococcus* and Gram negative bacteria i.e. members of Enterobacteriaceae and others like *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* (Walsh and Amyes, 2004). The most wide spread multidrug resistant fungal pathogens which cause the nosocomial infections belonging to the genera *Candida*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium*, *Cryptococcus* and *Mucormycoses* show high resistance to antifungal agents (Singh, 2003; Mota *et al*., 2012).

The indiscriminate exposure of antibiotics caused resistance and this microorganisms to have a superior ability to stay alive even the strongest antibiotics (Fagon *et al*., 2000). Therefore, it becomes necessary to enforce and implement some measures to reduce this problem of MDR; it can be achieved by some actions like to control the use of antibiotics, understand the genetic mechanisms of resistance, continue search for drugs with novel mechanisms of action, either synthetic or natural and take accuracy of diagnostic procedures or reduce the length of treatment (Paul *et al*., 2011). In current scenario, number and types of infectious diseases are increasing at an alarming rate. This knowledge of risk associated with the use of antimicrobial agents or antibiotics has prompted research to explore medicinal properties of plants and their extracts which can serve as herbal sources of antimicrobial agents for protection against a wide range of bacteria (Gram negative and Gram positive) including antibiotic resistant species and fungal species (Arellanes *et al*., 2003). Plants contain active metabolites which may serve as alternative source of folk medicines and useful in treating various infectious diseases. Plant secondary metabolites and essential oils can be used as an alternative remedies for the treatment of many infectious diseases (Cowan, 1999).

2. Essential Oils

Essential oils are the volatile liquids of the secondary metabolism of aromatic plants. They are termed "essential" because they represent the most important part of the plant. They are synthesized by all plant organs such as flowers, leaves, stems, seeds, barks, fruits, roots, peels and are stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes. Essential oils are not limited to a particular class or family of plants but they are widely distributed in all plant kingdom. The essential oils are found in plants belonging to the families like, Asteraceae, Aristolochiaceae, Cupressaceae, Fabaceae, Lamiaceae, Lauraceae, Meliaceae, Myrtaceae, Rutaceae, etc (Shah *et al*., 2014; Raut *et al*., 2014). Essential oils are extracted from Aromatic plants are frequently used in folk medicine for prevention and treatment of different human diseases. The urge to develop alternative treatment strategies follows three different directions; being one of them the treatment with natural antibacterial substances like essential oils of various plants (Genersch, 2009). Plant essential oils have been studied for their antimicrobial activity against microorganisms, including many pathogens (Dorman and Deans, 2000; Delaquis *et al*., 2002). *In vitro* antibacterial, antifungal and miticide activity of some essential oils have shown effective results in the control of bee pests (Albo *et al*., 2003; Dellacasa *et al*., 2003; Eguaras *et al*., 2005; Ruffinengo *et al*., 2006; Fuselli *et al*., 2006; Fuselli *et al*., 2007) offering a natural desirable alternative to antibiotics and other synthetic chemical substances.

Essential oils are aromatic oily liquids which are obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market. Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects (Sylvestre *et al*., 2006).

Essential oils are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils. Therefore, the intimate knowledge of essential oil composition allows for a better and specially directed application (Dorman and Deans, 2000). Many essential oils have been identified as antimicrobial agents due to present of vast number of bioactive chemicals. This activity of essential oils is variable from one microbial strain to another due to the nature of the microbial strains (Kalemba and Kunicka, 2003). Essential oils and their components are becoming increasingly popular as natural antimicrobial agents to be used for a wide variety of purpose, including food preservation, complementary medicine and natural therapeutics. The potential use of these essential oils as natural antimicrobial agents has been less explored (Cosentino *et al*., 2003). Essential oils are used in the pharmaceutical industry as active ingredients or constituents of drugs, soaps and shampoos. Interest in essential oils and other extracts of plants as sources of natural products has increased during the best year a major problem in antibiotics and chemotherapeutics which leads to the insufficiency of antimicrobial treatment. It has been recognized that some essential oils have different antimicrobial activities against individual strains of microorganisms. Apart from the antimicrobial activity, essential oils also have insecticidal, antiparasitic, antiviral and antifungal activities.

Essential oils are the volatile products of a plant's secondary metabolism that are normally formed in special cells or groups of cell are well known antimicrobial properties that could be used to control food spoilage and food - borne pathogenic bacteria for a long time (Burt, 2004; Bajpai *et al*., 2008). Essential oils are used in many industrial fields for the perfuming and flouring of various products because of their various components of antimicrobial compounds (Conner, 1993). Chemical analysis of essential oils has revealed their antimicrobial properties against bacteria (Exarchou *et al*., 2002; Botsoglou *et al*., 2003). The essential oils are used in perfumes, flavoring, cosmetics and pharmaceutical preparation. The oils from some of the species are used in treatment of various human ailments such as cough, fever, gout, leprosy, stomach disorders and have sedative properties. The essential oils that are derived from cymbopogon plants are of medicinal importance. Essential oils produced by plants have been traditionally used for respiratory tract infection and are used nowadays as ethical medicines for colds. In the medicinal field, essential oils are playing the vital role for treating the Bronchitis and Acute sinusitis. Inhalation of vapours of essential oils augmented the output of respiratory tract fluid, maintained the ventilation and drainage of the sinuses had an anti-inflammatory effect on the trachea and reduced asthma.

Essential oils are made from a very complex mixture of volatile molecules that are produced by the secondary metabolism of aromatic and medicinal plants and can be obtained by different methods, including the use of low or high pressure distillation of different parts of plants or the employment of liquid carbon dioxide or microwaves. Several factors influence the quality and quantity of the extracted product, in particular the soil composition, plant organ, vegetative cycle phase and climate (Miguel *et al*., 2005; Angioni *et al*., 2006). The essential oils are complex mixers comprising of many single compounds. Chemically they are derived from terpenes and terpenoids (isoprenoids) and aromatic and aliphatic aldehydes and phenols, all characterized by low molecular weight (Freires *et al*., 2015). Each of these constituents contributes to the beneficial or adverse effects. There are many methods of extraction of essential oils. They can be obtained by steam distillation, mechanical expression, hydro distillation, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. During distillation, water condensate and is separated by gravity leaving a very small amount of volatile liquid that is the essential oil. Due to their extraction procedure, they contain a variety of volatile molecules such as terpenoids, terpenes, aromatic compounds and aliphatic components (Yap *et al*., 2014).

The use of essential oils as function of ingredients in foods, drinks and cosmetics is gaining force, both for the rising interest of consumers in use of natural ingredients and also increasing concern about potentially unsafe of synthetic additives (Cavaleiro *et al*., 2015; Venturi *et al*., 2015). Essential oils are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (Thusoo *et al*., 2014). Essential oils exhibit various biological activities like antibacterial, antifungal, antiviral, insecticidal, antioxidant, anticancer activity, anti-inflammatory, anti-staphylococcal activity, Antimycotic activities and antidiabetic, etc. Some oils are also used in food preservation, aromatherapy and fragrance industries (Baptista *et al*., 2015).

3. Historical use of Essential oils

Although spices have been used for their perfume, flavour and preservative properties since antiquity (Bauer *et al*., 2001), of the known EOs, only oil of turpentine was mentioned by Greek and Roman historians. Distillation as a method of producing EOs was first used in the East (Egypt, India and Persia) more than 2000 years ago and was improved in the 9th century by the Arabs. The first authentic written account of distillation of essential oil is ascribed to Villanova, a Catalan physician. By the 13th century EOs were being made by pharmacies and their pharmacological effects were described in pharmacopoeias but their use does not appear to have been widespread in Europe until the 16th century, from which time they were traded in the City of London (Cutter, 2000). Publishing separately in that century on the distillation and use of EOs, two Strassburg physicians, Brunschwig and Reiff, mention only a relatively small number of oils between them; turpentine, juniper wood, rosemary, spike (lavender), clove, mace, nutmeg, anise and cinnamon. According to the French physician, Du Chesne (Quercetanus), in the 17th century the preparation of EOs was well known and pharmacies generally stocked 15 - 20 different oils. The use of tea tree oil for medicinal purposes has been documented since the colonization of Australia at the end of the 18th century, although it is likely to have been used by the native Australians before that (Carson and Riley, 2001). The first experimental measurement of the bactericidal properties of the vapours of EO is said to have been carried out by De la Croix in 1881 (Boyle, 1955). However, in the course of the 19th and 20th centuries the use of EOs in medicine gradually became secondary to their use for flavour and aroma (Gill *et al*., 2002).

The use of (EO) dates back to the earliest civilizations: first in the East and the Middle East and later in North Africa and Europe. The Hydrosols (aromatic) were used in India over than 7000 years. Between 3000 and 2000 B.C., the Egyptians made used extensively aromatic plants and other plants to treat the sick. The Persians seem to be the first ones who used the hydrodistillation in 1000 B.C (Romane *et al.*, 2012).

4. Antimicrobial components present in Essential Oils

Essential oils can be liquid at room temperature though a few of them are solid or resinous, and showing different colors ranging from pale yellow to emerald green and from blue to dark brownish red. They are synthesized by all plant organs, *i.e.*, buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidermiccells or glandular trichomes (Bassole and Juliani, 2012). The composition of the Essential oils can vary among different parts of the same plant. Chemically, Essential oils are variable mixtures of principally terpenoids, mainly monoterpenes (C10) and sesquiterpenes (C15), although diterpenes (C20) may also be present, and a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally N- and S-containing compounds, coumarins and homologues of phenylpropanoids (Benchaar *et al.*, 2008). They can be obtained by expression, fermentation, effleurage or extraction but the method of steam distillation is most commonly used for commercial production of Essential oils (Burt, 2004).

Terpenes are hydrocarbons made up of several isoprene units and terpenes containing oxygen are called terpenoids. Examples of terpenes present in EOs are p-cymene, terpinene, limonene, sabinene, and pinene. Terpenoids can be subdivided into alcohols, esters, aldehydes, ketones, ethers, and phenols. Geraniol, menthol, linalool, citronellol, carvone, thymol, carvacrol, geranyl acetate, eugenyl acetate, geranial, neral and 1,8-cineole are the well-known terpenoids found in EOs. Cinnamaldehyde, cinnamyl alcohol, chavicol, eugenol, estragole, methyl eugenols and methyl cinnamate are phenyl propanoids (Jayasena and Jo, 2013). It has been found that oxygenated monoterpenes, exhibit strong antimicrobial activity, especially pronounced on whole cells, while hydrocarbon derivatives possess lower antimicrobial properties, as their low water solubility limits their diffusion through the medium. Hydrocarbons tend to be relatively bound capacity and water solubility. Ketones, aldehydes and alcohols are active, but with differing specificity and levels of activity which was related to the present functional group but also associated with hydrogen bounding parameters in all cases (Sokovic *et al*., 2010). Essential oils or their active compounds containing hydroxyl group (-OH) are highly antimicrobial. The presence of aromatic nucleus with a polar functional group determines the inhibitory properties of the essential oils. A hydroxyl group is much more effective compared to a carbonyl group. The hydroxyl group can easily bind the active site of enzymes and alter their metabolism. Besides the presence of the hydroxyl group in the phenolic structure, its position in the phenolic ring strongly influences the microbial effectiveness of components. The alkyl substitution into phenolic compound increases the antimicrobial activity. The type of alkyl substituent in a nonphenolic ring structure affects the antimicrobial activity. An alkenyl substituent (-CH =CH-) as in limonene resulted in increased antimicrobial activity compared to an alkyl substituent (-C =C-) as in p-cymene (Ceylan and Fung, 2004).

Lezcano *et al*. (2000) analyzed the antimicrobial effect of the ozonizal sunflower oil and showed the activity against all strains analyzed with an MIC ranging from 1-18 to 9.5 mg. Oleozon showed a valuable antimicrobial tested. Results suggested that the *Mycobacterium* is more susceptible to olezon than the other bacteria tested. Shigeharu *et al*. (2001) evaluated the antibacterial activity of 14 essential oils and their major constituents in the gaseous state were evaluated against *Haemophillus influenza* and *Streptococcus aureus.* For most essential oils examined, *Haemophillus influenza* was most susceptible, followed by *Streptococcus pneumoniae* were comparable in susceptibility. *Escherichia coli* which was used as control and showed test susceptibility. A minimal inhibitory does (MID) was introduced as a measure of the vapour activity. Among the 14 essential oils, cinnamon bark, lemon grass and the oils showed the lowest MID followed by essential oils containing terpene alcohols as major constituents the essential oils containing tempene ketone and ether. In particular, hydrocarbon had high MIDS. The vapor activity of short exposure was comparable to that following overnight exposure, and rapid evaporation was more effective than slow evaporation of essential oils. The vapours concentration and absorption into agar of essential oils reached a maximum 1 or be halter rapid evaporation. These results indicate that the antibacterial action of essential oils was most effective when at high vapour concentration for a short time. Rosangela *et al*. (2005) determined the antimicrobial activity of essential oils by an Agar diffusion method against food borne pathogens and spoilage bacteria. The essential oils could be used for the development of novel system for food preservation.

Seenivasan *et al*. (2006) screened the selected essential oils against four Gram negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and two Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* at four different concentrations (1:1, 1:5, 1:10 and 1:20) using Disc diffusion method. The MIC of the active essential oils were tested using two fold Agar dilution method at concentrations ranging from 0.2 to 25 mg/m. Out of 21 essential oils tested, 19 oils showed antibacterial activity against one or more strains cinnamon, clove, geranium, lemon, lime, organe and resemary oils exhibited significant inhibitory effect. Cinnamon oils showed promising inhibitory activity even at low concentration, whereas aniseed *Eucalyptus* and camphor oil were least active against the tested bacteria. In general, *B. subtilis* was the most susceptible. On the other hand, *Klebsiella pneumoniae* exhibited low degree of densitivity. Majority of the oils showed antibacterial activity against the tested strains. However, cinnamon, clove and lime oils were found to be inhibiting both Gram positive and Gram negative bacteria cinnamon oil can be a good source of antibacterial agents.

Heather Cavanagh *et al*. (2007) assessed the antimicrobial activity of volatile oil of *Syzygium anomaticum*, *Thymus serphyllum*, *Lavandula angustifolia* and *Lavandula intervnedia* using the micro atmosphere method an essay was also performed to determine how different exposure time to essential oils effected the inhibition of microbes the oils were tested organisms. Methicillin - resistant *Staphylococcus aureus* (MRSA), vancomycin - resistant *Enterococci*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Candida albicans.* The results showed considerable variability in the size of zone of inhibition depending on which oil was used and no essential oil was observed to be the best against all organisms.

Ahmetalim *et al*. (2009) examined the *in vitro* antimicrobial and antifungal activities of the essential oil of *Cyclotrichium niveum*. The antimicrobial activity of the oil was also tested against Gram positive and negative bacteria and fungus using a disc diffusion method and the Minimal inhibitory concentration (MIC) values. The oils showed remarkable antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*. The essential oil exhibited also strong antifungal activity against *Candid albicans*.

Volatile chemicals from certain soil microbes are sufficient to elicit growth and defense responses in Arabidopsis, such volatile signals can induce essential oil accumulation and chemical emissions has yet to be report the plant growth promoting soil bacterium *Bacillus subtilis* GB03 releases volatile chemicals that elevate fresh weight essential oil accumulation and emissions along with plant size in the terpene rich herb sweet basil the two major essential oil components from sweet basil, terpicol and eugenol increased. These results elicitors can coreomitanity increase essential oil production and biomass in an herbaceous species rich in commercially valued essential oils (Erika Banchio *et al*., 2009).

Roseboro *et al*. (2009) tested twelve essential oils for their inhibitory activity against some microorganisms of veterinary interest using disc diffusion procedure and the most active were selected for further study in Agar dilution method. Disc diffusion technique showed variation in the antimicrobial activity of selected essential oils. According to agar dilution method, the most potential essential oils were cinnamon, oregano, lemongrass and the MICS were tested at concentration ranging from 2.0 to 0.0008 % (r/r). These inhibitory effects are interesting in relation to treatment of bacterial and yeast infections in animals.

The antibacterial activity of refined sunflower oil, between 20, water micro emulsion system. Pseudo-ternary phase diagram was constructed to obtain the concentration range of oil, surfactant and water. The concentration of refined sunflower oil varied from 5 % to 15 % the surfactant concentration varied from 10 % to 30 % and water consecration varied from 55 % to 85 % the oil and surfactant concentration was increased pH of the microemulsion system decreases. Bacterial growth was enhanced in the case of oil and surfactant alone (Aanjali *et al*., 2010). Ranzia Mothanaa *et al*. (2010) investigated the chemical composition of 3 essential oils obtained from the barks of three endemic *Boswellia* sp. namely *Boswellia discorides*, *Boswellia elongate* and *Boswellias ocotrana* which were collected from the soqotrais land. All essential oils possessed antimicrobial activity especially against Gram positive bacteria with MIC values between 1.8 and 17.2 mg/m/. Furthermore, the DPPH – radical scavenging assay exhibited only weak antioxidant activities (28 %) at 1.0 mg/m.

Sarath Chandrabose *et al*. (2012) extracted the essential oil from *Cymbopogon caesius* and *Cymbopogon* colorants and checked the susceptibility of *Citrobacter*, *Klebsiella pneumoniae* and *Aspergillus niger*. The essential oil was obtained by steam distillation of leaves *Cymbopogon caesius* and *Cymbopogon coloratus*. The agar diffusion method using filter paper disks was employed to assess the antibacterial activity and antifungal activity. The antimicrobial activity of twelve essential oils against Gram positive and Gram negative bacteria for a potential use in food industry.

Sivakami *et al*. (2012) analyzed the antibacterial and antifungal activities of the essential oils against the common infective bacteria present in the wounds for the purpose, the essential oil such as clove oil, olive oil, mustard oil, eucalptus oil, neem oil, anis oil, cumin oil, cinnamon oil and mint oil. The infective bacteria were collected from the pus found on the wounds. The isolates were tested against the standard antibiotics by Kirby Bauer Disc diffusion method. The antibacterial activity of essential oils was tested against the isolated bacteria such *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* from the result obtained, the cinnamon oil is found to be having biocontrol activity towards all pathogens and can be used for isolation of effective antibiotic compounds.

Oyeboade *et al*. (2013) investigated the antibacterial activity of the essential oil of *Origanum ulgare* and of a commercial formulation of thyme oil was investigated against *Bacillus cinerea* and compared with control. The essential oils and commercial formulations were found to inhibit the growth of *Bacillus cinerea* in a dose dependant manner. Light microscopic observation revealed that the essential oil caused morphological degeneration such as lytoplasmic loagulation hyphal shriveling hyphae. The essential oils of *O. vulgare* L. and *M. didyma* L. are promising antifungal agent against *B. cinerea* similar to the commercial formulation.

Amala Rajoo *et al*. (2013) examined the antimicrobial activity of the methods leaf extract of the plant against *Staphylococcus aureus* by Disc diffusion and Broth dilution methods. The extract showed a good antimicrobial activity against *Staphylococcus aureus* with a minimum inhibition concentration of 6.25 mg/ml and for chloramphenicol was 30.00 µg/ml. The main changes observed under SEM and TEM were structural disorganization of cell membrane which occurred after 12 hrs and total lollapse of the cell 36 hrs after exposure to the extract. They concluded that the methanolic extract of *E. guinessis* leaf exhibited good antimicrobial activity against *Staphylococcus aureus* and this was supported by SEM and TEM.

5. Mechanism of action of Essential Oils

The antimicrobial activity of Essential oils, similar to all natural extracts is dependent on their chemical composition and the amount of the single components. These molecules can be naturally present in their active form in the plant or can be activated by specific enzymes when the vegetal organism is subjected to particular biotic or abiotic stress. Their antimicrobial activity was not attributable to a unique mechanism but is instead a cascade of reactions involving the entire bacterial cell together these properties are referred to as the essential oils versatility. The mechanisms of action of the Essential oils include the degradation of the cell wall, damaging the cytoplasmic membrane, cytoplasm coagulation, damaging the membrane proteins, increased permeability leading to leakage of the cell contents, reducing the proton motive force, reducing the intracellular ATP pool *via* decreased ATP synthesis and augmented hydrolysis that was separated from the increased membrane permeability and reducing the membrane potential *via* increased membrane permeability (Nazzaro *et al.*, 2013). An important characteristic of Essential oils and their components was their hydrophobicity which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Leakage of ions and other cell contents can then occur (Burt, 2004).

The cell wall of Gram negative bacteria was more resistant to the activity of Essential oils and their components. The Gram negative cell wall does not allow for the entrance of hydrophobic molecules as readily as Gram positive bacteria. Thus, Essential oil is less able to affect the cell growth of the Gram negative bacteria (Nazzaro *et al.*, 2013). It was apparent that the generally greater resistance of Gram negative bacteria to essential oils is likely to be due in part to the greater complexity of the double membrane containing cell envelope of these organisms in contrast with the single membrane glycoprotein or techoic acid or membrane – glycoprotein or β-glucan based structures of Gram positive bacteria and yeast, respectively. Resistance also seems to be related to the rate and extent of antimicrobial dissolution or ability to partition in the lipid phase of the membrane. It was more likely that the effects of differences in hydrophobicity between these two bacterial groups, with Gram negative cells having more hydrophobic surfaces can be offset by the presence of porin proteins in the outer membrane of Gram negative cells. These can create channels large enough to allow restricted passage of small molecular mass compounds, like the substituted phenolics in essential oils, allowing their access to the periplasmic space, the glycoprotein layer and the cytoplasmic membrane (Holley and Patel, 2005).

6. Antibacterial activity of Essential Oils

The inhibitory effect of thyme, mint, bay leaves and their alcohol extracts on the growth of *Salmonella typhimurium, Vibrio parahaemolyticus* and *Staphylococcus aureus* in a medium was evaluated by Ceylan and Fung (2004). *Salmonella typhimurium* was found to be the least sensitive to these spices. Thyme was the inhibitoriest against the bacteria tested. Antimicrobial effect of the essential oils of sage, rosemary, cumin, caraway, clove and thyme against *Pseudomonas fluorescens, Serratia marcescens, Escherichia coli, Surcina* sp., *Micrococcus* sp., *Staphylococcus aureus, Bacillus subtilis* and *Mycobacterium phlei* was evaluated. Clove and thyme essential oils and their active compounds were highly inhibitory against the cultures tested while others exhibited moderate or no antimicrobial activity. The essential oil of bay leaf, pimento berry, pimento leaf and cloves having eugenol as a major component exhibited the minimum inhibitory concentration from 10 to 100 pg against Gram positive, Gram negative and yeast and fungi.

Inhibitory action of thyme (*Thymus vulgaris*), mint (*Mentha piperita*) and laurel (*Laurus nobilis*) ground leaves and their extracts on *Staphylococcus aureus, Salmonella typhimurium* and *Vibrio parahaemolyticus* was studied by Souza *et al*. (2005). Thyme was most prominent antibacterial product being active upto concentration 0.5 % (w/v) for ground and 5000 ppm (v/v) for extracts. Testing of antilisterial effect of 18 spices observed significant inhibitory effect of rosemary (*Rosmarinus officinalis*) and clove (*Eugenia cariophylata*) on *Listeria monocytogenes*. Spices mixtures were able to inhibit the growth of various meat spoiling microorganisms (*Bacillus subtilis, Enterococcus* spp., *Staphylococcus* spp., *Escherichia coli* and *Pseudomonas fluorescens*) providing stabilizing effect on colour and smell of fresh portioned pork meat.

In 2007, 46 spice and herb extracts tested by Shan and co-workers, amongst them twelve exhibited high antibacterial activities against the five foodborne bacteria. Methanolic and ethanolic extracts of *Punica granatum* were effective against *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus*. The extracts and essential oils of *E. caryophylata, O. vulgare*, and *C. burmannii* had significant inhibitory properties against *Staphylococcus aureus, Escherichia coli* and *Listeria monocytogenes.* The ethanolic extracts of *S. officinalis* had strong antimicrobial activity against *Bacillus cereus, Escherichia coli*, and *Staphylococcus aureus*. The extracts of *P. cuspidatum* strongly inhibited the growth of *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli*. *Cassia auriculata* exhibited significant activity against *Escherichia coli* and *Staphylococcus aureus*.

The antibacterial properties of 14 Essential oils (clove, oregano, rosemary, pepper, nutmeg, liquorice, turmeric, aniseed, cassia bark, fennel, prickly ash, round cardamom, dahurian angelica root and angelica) against four common meat spoilage and pathogenic bacteria (*Listeria monocytogenes, Escherichia coli, Pseudomonas fluorescens* & *Lactobacillus sake*) was studied and their results showed that individual extracts of clove, rosemary, cassia bark and liquorice contained strong antibacterial activity (Skrinjar and Nemet, 2009).

The essential oil from the rhizomes of turmeric (*Curcuma longa*) exhibited good to moderate activity against *Bacillus subtilis, Staphylococcus aureus* and *Corynebacterium diphtheria.* Oils of bay clove, cinnamon and thyme can act as potent inhibitors of *Listeria monocytogenes* and *Salmonella enteritidis.* Clove, cinnamon, bishop's weed, chili, horse raddish, cumin, tamarind, black cumin, pomegranate seeds, nutmeg, garlic, onion, tejpat, cellary, cmbodge have potent antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae* (Maurya *et al.,* 2013). The antimicrobial properties of fifty plant essential oils against 25 genera of bacteria were evaluated. Thyme, cinnamon, bay, clove, almond (bitter), lovage, pimento, marjoram, angelica and nutmeg essential oils displaced the greatest inhibitory properties. Clove extract showed remarkable antibacterial activity against all organisms tested and oregano and cinnamon exhibited wide inhibitory spectrum (Mohamed *et al.,* 2013).

7. Factors affecting antibacterial activity of Essential oil

In the antimicrobial action of essential oil components, the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of the main importance. The activity rank of essential oil components is as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons (Kalemba and Kunicka, 2003). In addition, both intrinsic and extrinsic conditions can be responsible to the susceptibility or resistant nature of the pathogen. Besides, fatty contents may also serve as barrier to the effectiveness of the pathogen while incorporating plant essential oils in foods due to having a higher population of pathogen in hydrophilic region. Hence, it was assumed that fatty composition may directly have an adversary effect on the efficacy of plant essential oils against the tested pathogens. In addition, interaction of terpenoid phenolics of plant essential oils with enzymatic substances might be a reason to limit the antimicrobial efficacy of the plant essential oils in food models (Bajpai *et al.,* 2012). There are a host of other factors, variability in composition or content of active agents that result from agronomic history, varietal differences, and maturity of the plant material studied; physical and chemical characteristics of the antimicrobial itself (hydrophobicity, volatility, compatibility in the test system); presence of protein, starch or lipid that may complex with and neutralize antimicrobial activity or partition the agent away from its target; and the inoculum size, genus of microorganism, species and even strain susceptibility as well as previous culture history (Holley and Patel, 2005).

The efficiency of an antimicrobial compound depends on the type, genus, species and strain of the target microorganism, besides the environmental factors such as pH, water activity, temperature, atmospheric composition and initial microbial load of the food substrate. The antimicrobial nature of phytochemical was determined by its chemical properties such as pKa value, hydrophobicity or lipophilicity ratios, solubility, and volatility. The pH and polarity are the most prominent factors influencing the effectiveness of a food antimicrobial (Negi, 2012). Hydrophobic properties of some antimicrobial substances can make their dissolution difficult in water limiting their use in foods. The concentration thresholds required for inhibition or inactivation of microorganisms will depend on the specific targets of the antimicrobial substance, including cell wall, cell membrane, metabolic enzymes, protein synthesis and genetic systems (Negi, 2012).

8. Synergic effect of Essential oil

Antimicrobial activity of essential oils was conditioned by the activity of their components (Kalemba and Kunicka, 2003). The interaction between plant essential oil compounds can produce four possible types of effects: indifferent, additive, antagonistic or synergistic effects. An additive effect was observed when the combined effect was equal to the sum of the individual effects. Antagonism was observed when the effect of one or both compounds was less when they are applied together than when individually applied. Synergism was observed when the effect of the combined substances was greater than the sum of the individual effects while the absence of interaction was defined as indifference.

Literature survey has demonstrated that an oil as a whole showed better antibacterial efficacy than only a combination of major volatiles of the oil. Hence, it might be said that the minor elements of the oil have crucial roles to increase the biological effectiveness of the oil, resulting in the synergism (Bajpai *et al.*, 2012). There are some generally accepted mechanisms of antimicrobial interaction that produce synergism: sequential inhibition of a common biochemical pathway, inhibition of protective enzymes, combinations of cell wall active agents, and use of cell wall active agents to enhance the uptake of other antimicrobials (Goni *et al.*, 2009).

Synergism between carvacrol and p-cymene, a very weak antimicrobial, might facilitate carvacrol's transportation into the cell by better swelling the *Bacillus cereus* cell wall. Thymol and carvacrol showed synergistic and antagonistic effects, in different combinations of cilantro, coriander, dill and eucalyptus Essential oils and mixtures of cinnamaldehyde and eugenol against *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp. and *Enterobacter* sp. Vacuum packing in combination with oregano Essential oils showed a synergistic effect against *Listeria monocytogenes* with 2 – 3 $\log_{10}$ reduction. Similar results have been recorded when clove and coriander Essential oils have been used against *Aeromonas hydrophila* on vacuum packed pork. Application of oregano Essential oils has a synergistic effect in modified atmosphere packaging including 40 % CO_2, 30 % N_2 and 30 % O_2 (Tajkarimi, *et al.*, 2010).

Application of nisin with carvacrol or thymol has been positively effective against *Bacillus cereus* with temperatures increasing from 8 to 30 °C. Application of nisin with rosemary extract enhanced the bacteriostatic and bactericidal activity of the nisin. Oregano Essential oils in combination with modified atmosphere packaging have effectively increased the shelf life of fresh chicken. Combinations of Essential oils of oregano and thyme, oregano with marjoram and thyme with sage had the most effects against *Bacillus cereus, Pseudomonas aeruginosa*, *Escherichia coli* and *Listeria monocytogenes* (Tajkarimi, *et al.*, 2010).

Synergistic effects of Eugenol or carvacrol and Eugenol or thymol might be due to the fact that carvacrol and thymol disintegrated the outer membrane of *Escherichia coli*, making it easier for eugenol to enter the cytoplasm and combine with proteins. It was also observed the synergistic effect of eugenol or cinnamaldehyde was probable due to the interaction of these components with different proteins or enzymes. Thymol or carvacrol could increase the permeability of the cytoplasmic membrane, and probably enable cinnamaldehyde to be more easily transported into the cell. Thymol or carvacrol could increase the number, size or duration of existence of the pores created by the binding of cinnamaldehyde to proteins in the cell membrane, so that a synergistic effect was achieved when these two components are used in combination. The synergistic combinations of Essential oils of oregano or basil against *Escherichia coli*, basil or bergamot against *Staphylococcus aureus*, oregano or bergamot against *Bacillus subtilis* and oregano or perilla against *Saccharomyces cerevisiae* significantly disrupted the integrity of cell membranes when compared with control untreated membranes (Bassole and Juliani, 2012).

The combination of clove and rosemary essential oils produced an additive effect against the Gram positive and Gram negative bacteria, namely *Staphylococcus aureus*, *Streptococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* (Faleiro, 2013). The exploitation of the synergetic action resulting from the combination of Essential oils with other biological antimicrobial substances such as bacteriocins has been investigated. The use of oregano or savory Essential oils showed a synergistic activity with a combination of cell - adsorbed bacteriocin of a *Lactobacillus curvatus* strain when applied to control *Listeria monocytogenes* in pork meat storage at 4 ºC. The association of the Essential oils components, thymol or cinnamaldehyde with low temperatures (≥8 ºC) also produces a synergistic effect on the control of the food borne bacteria *Bacillus cereus* (Faleiro, 2013).

Synergism between carvacrol and its biological precursor p-cymene has been noted when acting on *Bacillus cereus* vegetative cells. It appears that p-cymene, a very weak antibacterial, swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism p-cymene probably enables carvacrol to be more easily transported into the cell so that a synergistic effect is achieved when the two are used together. Synergism between NaCl and mint oil against *Streptococcus enteritidis* and *Listeria monocytogenes* has been recorded in taramosalata. The combined use of 2 – 3 % NaCl and 0.5 % clove powder (containing eugenol and eugenyl acetate) in mackerel muscle extract has been found to totally prevent growth and histamine production by *Enterobacter aerogenes* (Burt, 2004). Three plants extract of cornifructus, cinnamon and Chinese chive showed strong inhibitory effect on bacteria, on yeasts and on moulds, respectively. Hence, extracts of cornifructus, cinnamon and Chinese chive were mixed at the ratio of 1:1:1 (v/v/v), and proven to possess the distinctly antimicrobial effect on 15 test micro-organisms (Hsieh *et al.*, 2001).

9. Limitations of Essential oil

The limitation of the oil activity can be explained by the low water solubility of the oil and its components which limits their diffusion through the agar medium in the disc-diffusion method (Sokovic *et al.*, 2010). As has been shown by some other researchers, the use of antimicrobials can reduce or eliminate specific microorganisms but it may also produce favourable conditions for other microorganisms (Skrinjar and Nemet, 2009). It may be difficult to maintain quality consistency because the composition of an individual EO can vary due to several factors including the time of harvesting, variety, the part of the plant used, and method of extraction. In addition, the antimicrobial potency of EO constituents depends on pH, temperature, and level of microbial contamination (Jayasena and Jo, 2013).

In several reports, high concentrations of Essential oil components were needed to reduce growth of *Listeria monocytogenes* in cheese and minced meat, chicken, ham, and liver sausage. A similar need for higher concentrations of Essential oils has been reported for other food products such as fish, dairy products, and vegetables, although a few exceptions exist. The higher concentrations needed often cannot be used in practice because of the aroma connected to these Essential oils. It is generally believed that the higher fat and protein content of food compared with growth medium is the major cause of this higher microbial resistance to Essential oils.

One hypothesis is that the food product provides more nutrients to bacteria, thereby enabling them to repair damaged cells more quickly. Another suggestion was that Essential oils components can dissolve in the fat-lipid phase of the food, which would make these components less available to act on bacteria present in the water phase of these foods (Veldhuizen *et al.*, 2007). Although, essential oils possess antibacterial properties and may improve taste and some other characteristics of the meat, they should be used with care, because Essential oils may cause some side effects. Some essential oils, such as menthol, eugenol and thymol, depending on concentration, may cause irritation of mucous membranes, probably as a result of membrane lysis and surface activity, while cinnamaldehyde, carvacrol, carvone and thymol *in vitro* appear to have mild to moderate toxic effects at the cellular level (Marija *et al.*, 2013).

9. Conclusion

Many Essential oil possess antimicrobial activity and it has been proved by plenty of investigations in the recent years. The Essential oils are expected to be widely applied as an antibacterial and antifungal agent. The antimicrobial properties of the oil could be associated with the high percentage of phenolic components such as thymol and carvacrol which are known to possess strong antimicrobial activities. The essential oils of different herbal plants are composed of complex mixtures of various chemical compounds and many of them show a strong antimicrobial activity with different modes of action. This unique property of essential oils makes microorganism unable to show resistance. Further studies on essential oil are needed to identify promising compounds that could be used as lead to develop natural pharmacological agents.

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