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INHIBITION OF ECOLI AND STAPH A OF ESSTENTIAL OILS, CINNAMON AND CLOVES

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ABSTRACT:

The selected essential oils were screened against four gram-negative bacteria and two gram-positive bacteria Staphylococcus aureus at four different concentrations using disc diffusion method. Essential oils are complex volatile compounds, synthesized naturally in different plant parts during the process of secondary metabolism. Essential oils have great potential in the field of biomedicine as they effectively destroy several bacterial, fungal, and viral pathogens. The presence of different types of aldehydes, phenolics, terpenes, and other antimicrobial compounds means that the essential oils are effective against a diverse range of pathogens. The reactivity of essential oil depends upon the nature, composition, and orientation of its functional groups. The aim of this article is to review the antimicrobial potential of essential oils secreted from MAPs and their possible mechanisms of action against human pathogens. This comprehensive review will benefit researchers who wish to explore the potential of essential oils in the development of novel broad-spectrum key molecules against a broad range of drug-resistant pathogenic microbes.

Keywords: Antimicrobial, Cinnamon, Cloves, Esstential Oils, Pathogenic Microbes, Staphylococcus aureus.

INTRODUCTION:

Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties. Some oils have been used in cancer treatment. Some other oils have been used in food preservation, aromatherapy and fragrance industries. Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential.

Essential oils (also called volatile oils) are aromatic oily liquids 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.

Essential oils such as aniseed, cinnamon, clove, eucalyptus, geranium, lime, mint, nutmeg, have been traditionally used by people for various purposes in different parts of the world. Cinnamon, clove and rosemary oils had shown antibacterial and antifungal activity; cinnamon oil also possesses

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antidiabetic property. Anti-inflammatory activity has been found in basil. Lemon and rosemary oils possess antioxidant property. Peppermint and orange oils have shown anticancer activity. Citronella oil has shown inhibitory effect on biodegrading and storage-contaminating fungi. Lime oil has shown immunomodulatory effect in humans. Lavender oil has shown antibacterial and antifungal activity; it was also found to be effective to treat burns and insect bites.

Table 1: List of selected essential oils and their properties.

Common name	**Botanical name (Family)**	**Properties**
Aniseed oil	*Pimpinella anisum* (Umbelliferae)	Carminative, stimulant, expectorant, condiment and flavouring agent.
Cinnamon oil	*Cinnamomum zeylanicum* (Lauraceae)	Carminative, stomachic, astringent, stimulant and antiseptic.
Clove oil	*Eugenia caryophyllus* (Myrtaceae)	Dental analgesic, carminative, stimulant and antiseptic.
Eucalyptus oil	*Eucalyptus globulus* (Myrtaceae)	Counter-irritant, antiseptic, expectorant, cough reliever.
Palmarosa oil	*Cymbopogon martini* (Graminae)	Cosmetic, anti rheumatism and insect repellent.

Chemical Composition of Essential Oils

Essential oils have the ability to hamper the growth of a diverse range of pathogens because of the presence of natural compounds produced by the organs of plants. Importantly, the unique aroma and other bioactive properties of an essential oil depend on its chemical constituents. In MAPs, essential oils generally accumulate in the secretary canals or cavities and glandular trichomes and sometimes in the epidermal cells. Essential oils and their chemical constituents exhibit more bioactivity when present in the oxygenated or active form. In general, the chemical composition of essential oils is relatively complex, and about 20 to 60 different bioactive components are observed in many of these essential oils. Many of these compounds are pharmaceutically appreciated for their numerous culinary properties. Usually, the chemical characterization of many essential oils reveals the presence of only 2-3 major components at a fairly high concentration (20–70%) compared to other components present in trace amounts.

Most essential oils are composed of terpenes, terpenoids, and other aromatic and aliphatic constituents with low molecular weights. Terpenes or terpenoids are synthesized within the cytoplasm of the cell through the mevalonic acid pathway. Terpenes are composed of isoprene units and are generally represented by the chemical formula $(C_5H_8)_n$. Terpenes can be acyclic, monocyclic, bicyclic, or tricyclic. Owing to the diversity in their chemical structures, terpenes are classified into several groups such as monoterpenes ($C_{10}H_{16}$), sesquiterpenes

($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), and triterpenes ($C_{30}H_{40}$).

The major component (~90%) of bioactive essential oils is constituted of monoterpenes. Some of the major compounds include monoterpene hydrocarbons (*p*-cymene, limonene, *α*-pinene, and *α*-terpinene), oxygenated monoterpenes (camphor, carvacrol, eugenol, and thymol), diterpenes (cembrene C, kaurene, and camphorene), sesquiterpene hydrocarbons (*β*-caryophyllene, germacrene D, and humulene), oxygenated sesquiterpenes (spathulenol, caryophyllene oxide), monoterpene alcohols (geraniol, linalool, and nerol), sesquiterpene alcohol (patchoulol), aldehydes (citral, cuminal), acids (geranic acid, benzoic acid), ketones (acetophenone, benzophenone), lactones (bergapten), phenols (eugenol, thymol, carvacrol, and catechol), esters (bornyl acetate, ethyl acetate), and coumarins (fumarin, benzofuran). The structures of some of these compounds are represented. The major and biologically important chemical constituents of MAPs.

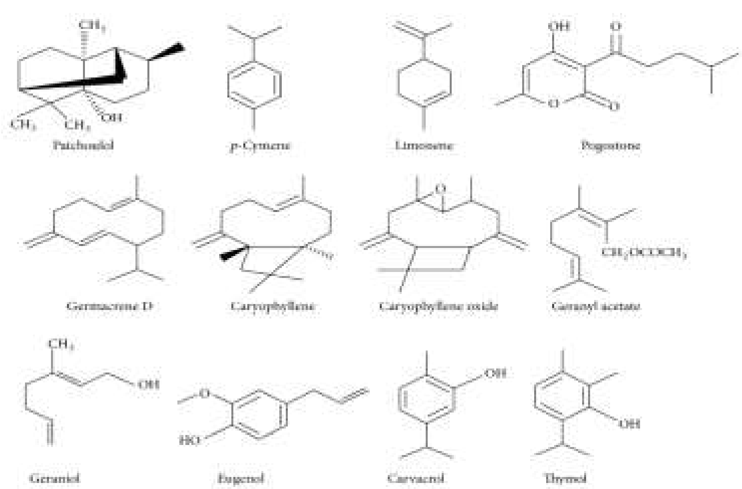

Figure 1: Structures of some important chemical compounds of essential oils.

The chemical constituents of plant essential oils differ between species. Some factors that can affect these constituents include the geographical location, environment, and stage of maturity. This chemical difference is directly related to differences in antimicrobial activities against various pathogenic microorganisms. For example, the major chemical constituents of origanum essential oil (carvacrol and thymol) were shown to differ in their origin as well as antimicrobial property.

Furthermore, the stereochemical properties of essential oils can vary and depend upon the method of extraction. However, extraction products may also vary qualitatively and quantitatively in their composition. Although essential oils can be recovered using fermentation, extraction, or effleurage processes, commercial production is preferably achieved by the steam distillation process. Likewise, the antimicrobial efficiency of essential oils depends on the type of microbes to be inhibited as well as the evaluation methods, including bioautography, diffusion, and dilution. Methods to evaluate the essential oil chemistry, their biological activities, and various factors that affect bioactivity are detailed in the literature.

Antibacterial Effects of Essential Oils

At present, many antibiotics are available for treating various bacterial pathogens. However, increased multidrug resistance has led to the increased severity of diseases caused by bacterial pathogens. In addition, low immunity in host cells and the ability of bacteria to develop biofilm-associated drug resistance have further increased the number of life-threatening bacterial infections in humans. Thus, bacterial infections remain a major causative agent of human death, even today. In addition, the use of several antibacterial agents at higher doses may cause toxicity in humans. This has prompted researchers to explore alternative new key molecules against bacterial strains. In this regard, plant essential oils and their major chemical constituents are potential candidates as antibacterial agents. Several types of essential oils and their major chemical constituents from various MAPs have been reported to possess a wide range of bacterial inhibitory potentials.

The effect of antibacterial activity of essential oils may inhibit the growth of bacteria (bacteriostatic) or destroy bacterial cells (bactericidal). Nevertheless, it is difficult to distinguish these actions. In relation to this, antibacterial activity is more frequently measured as the minimum bactericidal concentration (MBC) or the minimum inhibitory concentration (MIC). Rapid antibacterial screening of essential oils is usually conducted using the agar diffusion technique, where essential oils are added to filter paper discs or holes, which are put in agar that has been uniformly inoculated with a bacterial strain. After incubating, the inhibition zone represents the antimicrobial action. The effectiveness of essential oils differs from one type to another as well as against different target bacteria depending on their structure (Gram-positive and Gram-negative bacteria). For instance, sandalwood and vetiver oils exhibit higher inhibitory activity against Gram-positive bacteria; however, they fail to inhibit Gram-negative bacterial strains.

The essential oils of cinnamon, clove, pimento, thyme, oregano, and rosemary were shown to possess strong antibacterial activity against *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Clove oil was found to be the most effective among all the tested essential oils. The antimicrobial effect of these oils was correlated to the occurrence of the major compounds such as carvacrol, thymol, cinnamic aldehyde, eugenol, and *p*-cymene. Likewise, carvacrol, eugenol, and thymol obtained from MAPs have been shown to effectively inhibit food-borne pathogens such as *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Vibrio vulnificus*.

The compounds such as benzoic acids, benzaldehydes, and cinnamic acid have shown up to 50% inhibition of *Listeria monocytogenes* under anaerobic conditions. Ouattara et al. reported the antibacterial potential of clove, cinnamon, pimento, and rosemary essential oils against meat spoilage bacterial pathogens such as *Pseudomonas fluorescens*, *Serratia liquefaciens*, *Brochothrix thermosphacta*, *Carnobacterium piscicola*, *Lactobacillus curvatus*, and *Lactobacillus sake*. According to them, the 1/100 dilution of these essential oils was capable of inhibiting at least 5-6 of the tested microbes. The inhibitory effect of these oils was mainly correlated with the occurrence of eugenol and cinnamaldehyde in the essential oils. Other major compounds found were carvacrol, thymol, cinnamaldehyde, and camphor.

Arora and Kaur analyzed the antimicrobial activity of garlic, ginger, clove, black pepper, and green chilli on human pathogenic bacteria such as *Bacillus sphaericus*, *Enterobacter aerogenes*, *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus epidermidis*, *S. typhi*, and *Shigella flexneri*. They concluded that, among all these spices, the aqueous extract of garlic was sensitive against all the tested bacterial pathogens. The garlic extract inhibited 93% of *S. epidermidis* and *S. typhi* within 3 h of incubation time. Similarly, the effect of clove extracts on the production of verotoxin by *E. coli* was studied by Sakagami et al., who found that verotoxin production was inhibited by the clove extract (MIC value of >1.0% w/v).

The effectiveness of cardamom, anise, basil, coriander, rosemary, parsley, dill, and angelica essential oils against pathogenic and saprophytic microorganisms was examined. They concluded that essential oils extracted from oregano, basil, and coriander plants have an inhibitory effect against *P. aeruginosa*, *S. aureus*, and *Yersinia enterocolitica* in the range of 400 ppm concentration. Skandamis et al. observed the significance of oregano essential oils on the behavior of *S. typhimurium* in sterile and naturally contaminated beef fillets stored under aerobic and customized atmospheric conditions. The addition of oregano essential oils (0.8% v/w) reduced the majority of the tested bacterial pathogens. Hood et al. reported that the bacterial growth may be suppressed by the ample use of essential oils or their use at high concentrations and that their mode of action results in the decline of bacterial cells.

In study, *Achillea clavennae* essential oil exhibited maximum inhibitory activity against respiratory disease-causing microbes like *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *P. aeruginosa*. The oil contained eucalyptol (1,8-cineole) and camphor as major compounds. The major compounds with an antibacterial effect were found to be camphor, thymol, and carvacrol. The essential oil of *Salvia officinalis* contains *α*-thujone, camphor, and 1,8-cineole as the major chemical constituents and was shown to inhibit human bacterial pathogens such as *S. aureus* and *Providencia stuartii*. Some pathogenic bacteria (*Salmonella choleraesuis*, *Salmonella enteritidis*, *S. typhimurium*, and *E. coli*) were inhibited by the essential oils of thyme and oregano. The essential oils showed an MIC value of 0.25% to ≥2% v/v. In another study, *Salvia* spp. (*S. officinalis*, *S. sclarea*, and *S. lavandulifolia*) and *Thuja* spp. (*T. plicata* and *T. occidentalis*) essential oils exhibited potent antimicrobial properties against human pathogens. The major components (*α*-thujone and *β*-thujone) of these sage species demonstrated high inhibitory activity against *P. aeruginosa* and *K. pneumoniae*, whereas *S. aureus* and *E. coli* were moderately inhibited.

The antibacterial activity of oregano oil against *S. aureus*, *Bacillus subtilis*, *E. coli*, and *P. aeruginosa*. The MBC values ranged between 0.75 and 2.25 mg/mL. Carvacrol was the most effective compound with an MBC value of 0.75 to 1.53 mg/mL, followed by linalool with 1.04 to 1.75 mg/mL. Similarly, oregano essential oil was also shown to be effective against *Providencia stuartii* and *E. coli*. The essential oils of *Thuja* spp. (*T. plicata* and *T. occidentalis*) effectively inhibited *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *E. coli*. Moreover, Chaieb et al. revealed the antimicrobial potential of the essential oil of *Eugenia caryophyllata* against numerous multidrug-resistant *S. epidermidis* strains isolated from dialysis biomaterials. The presence of *n*-mentha-1,8-dien-10-al, limonene, geranial, and neral as the major constituents in *Dracocephalum foetidum* essential oil. The oil exhibited antibacterial activity against human pathogenic bacteria such as *S. aureus, B. subtilis, Enterococcus hirae, E. coli, Micrococcus luteus, Streptococcus mutans,* and *Saccharomyces cerevisiae*. The MIC value ranged from 26 to 2592 μg/mL. Likewise, Botelho et al. reported the antibacterial activity of *Lippia sidoides* oil against four strains of cariogenic bacteria, namely, *Streptococcus sanguis, S. mutans, Streptococcus salivarius,* and *Streptococcus mitis*.

The MIC value ranged from 0.625 to 10.0 mg/mL. Lopes-Lutz et al. reported that several species of *Artemisia* essential oil possessed strong activity against *E. coli*, *S. aureus*, and *S. epidermidis*. Likewise, *Momordica charantia* seed essential oil exhibited inhibitory action against *E. coli* and *S. aureus* with an MIC value of >500 and 125 μg/mL, respectively. The medicinal plant *Achillea ligustica* containing terpinen-4-ol, β-pinene, 1,8-cineole, and linalool showed effective inhibitory activity against *S. mutans* with an MIC ranging from 155 to 625 μg/mL. Many food-borne and spoilage bacterial pathogens were inhibited by *Satureja cuneifolia* essential oil and the MIC values were in the range of 600–1400 μg/mL.

The essential oil of *Coriandrum sativum* demonstrated an antimicrobial potential against a wide range of bacterial pathogens, but the highest inhibition was found against *Bacillus cereus* and *E. coli*. The MIC of oil for Gram-positive bacteria was observed to be 108 mg/mL and, for Gram-negative bacteria, it ranged from 130 to 217 mg/mL. Moreover, the essential oils extracted from thyme and mint leaves exhibited antibacterial activity against the *S. aureus, S. typhimurium, Vibrio parahaemolyticus, L. monocytogenes, E. coli, C. botulinum, C. perfringens, Shigella sonnei, Sarcina lutea,* and *Micrococcus flavus*. The Gram-negative bacterial strains showed more sensitivity towards the thyme oil. The MIC value ranged from 0.33 to 2.67 mg/mL. The essential oil of *Myrtus communis* was reported to inhibit various bacterial strains such as *S. aureus, L. monocytogenes, Enterococcus durans, S. typhi, Enterobacter cloacae, E. coli, B. subtilis, Mycobacterium tuberculosis, P. aeruginosa, K. pneumoniae,* and *Mycobacterium avium*. Similarly, Unlu et al. reported that diverse range of bacterial pathogens such as *S. aureus, Streptococcus pyogenes, S. pneumoniae, Enterococcus faecalis, Enterococcus faecium, B. cereus, Acinetobacter lwoffii, E. aerogenes, E. coli, K. pneumoniae, Proteus mirabilis, P. aeruginosa, S. typhimurium, C. perfringens,* and *Mycobacterium smegmatis* were inhibited by the essential oil of *Cinnamomum zeylancium*.

In a study by Shan et al., the essential oils of cinnamon, oregano, clove, pomegranate peels, and grape seeds were found to be effective against

S. enterica, but the clove extracts possessed the highest antibacterial activity. *Melaleuca alternifolia* (tea tree oil) and its major constituent, terpinen-4-ol, were shown to possess potential antibacterial properties against many pathogens including *E. coli, S. aureus, S. epidermidis, E. faecalis, P. aeruginosa, M. avium, H. influenzae, S. pyogenes,* and *S. pneumoniae*. Overall, it was shown that tea tree oil and terpinen-4-ol have limited influence on the development of antibacterial resistance and susceptibility. Ait-Ouazzou et al. studied the essential oil composition and antibacterial potential of *Mentha pulegium, Juniperus phoenicea*, and *Cyperus longus* and concluded that all these oils were effective against food-borne pathogens (*S. aureus, L. monocytogenes, E. faecium, S. Enteritidis, E. coli,* and *P. aeruginosa*).

According to them, *M. pulegium* exhibited the best antibacterial activity compared to *J. phoenicea* and *C. longus*. The MIC value of *M. pulegium* oil was <0.5 for *E. faecium* and 1 μL/mL for *S. aureus, L. monocytogenes, E. coli,* and *S. enteritidis*. Lawal et al. have reported the antibacterial activity of essential oil of *Ocimum gratissimum, O. kilimandscharicum, O. lamiifolium*, and *O. suave* against *S. aureus, Bacillus* sp., *E. coli, P. aeruginosa, S. typhi, K. pneumoniae,* and *P. mirabilis*. The MIC values varied between 1.25 and 10 mg/mL (flower oil) and between 0.16 and 10 mg/mL (leaf oil). The thyme oil obtained from leaves showed the presence of camphor, camphene, α-pinene, 1,8-cineole, borneol, and β-pinene, which exhibited effective antibacterial activity against *S. aureus, S. epidermidis, Streptococcus* sp., *Pantoa* sp., and *E. coli*. The thyme oil showed MIC and MBC values of 627.7 μg/mL and 990.2 μg/mL, respectively, against the *E. coli* strain.

The major compound thymol showed MIC and MBC values of 2786 μg/mL and 2540 μg/mL, respectively. Therefore, this study proposes the possible use of thyme oil as a potential antimicrobial agent for food preservation. The oil obtained from *Laurus nobilis* and *Lavandula intermedia* showed inhibitory potential against *Mycobacterium smegmatis* and *E. coli*. The bacterial strains (*Shigella sonnei*, *Sarcina lutea*, and *Micrococcus flavus*) were inhibited by the essential oil of *Origanum vulgare*. The zone of inhibition and MIC values of *O. vulgare* oil were in the range of 9–36 mm and 125–600 μg/mL, respectively. Several food-borne pathogens such as *Brochothrix thermosphacta*, *E. coli*, *Listeria innocua*, *L. monocytogenes*, *Pseudomonas putida*, *S. typhimurium*, and *Shewanella putrefaciens* were inhibited by some commercial essential oils including those of *Ocimum basilicum*, *Petroselinum sativum*, and *Rosmarinus officinalis*. The essential oil of *Syzygium cumini* was found to contain *α*-pinene, *β*-pinene, *trans*-caryophyllene, 1,3,6-octatriene, delta-3-carene, *α*-caryophyllene, and limonene as major chemical compounds and possessed effective antibacterial activity against pathogenic bacterial strains such as *E. coli*, *S. aureus*, *P. aeruginosa*, *Neisseria gonorrhoeae*, *B. subtilis, and S. aureus*.

The essential oil exhibited moderate inhibition zones (12–14 mm) against the tested microbes. Andrade et al. studied the antimicrobial activity of 27 different essential oils employed in aromatherapy procedures and found that *Cinnamomum cassia* essential oils were effective against *S. aureus* and *E. coli*, whereas *S. aromaticum* essential oil was efficient against *P. aeruginosa* strains. Khoury et al. have reported that *Juniperus excelsa* essential oil obtained from leaves and twigs was efficient at inhibiting *S. aureus* (MIC value of 64 mg/ml) and *Trichophyton rubrum* (MIC value of 128 mg/mL). Although the essential oil of *Mentha suaveolens* showed strong antibacterial activity against *S. xylosus* with an MIC value of 14.4 μL/mL, it showed no activity against lactic acid bacterial strains except *Lactococcus lactis*.

The essential oil of the herb *Struchium sparganophora* revealed the presence of β-caryophyllene, germacrene A, α-humulene, and germacrene D as major chemical constituents and it exhibited antibacterial activity against *S. typhi*, *B. cereus*, *B. subtilis*, *P. mirabilis*, and *P. aeruginosa*. The inhibitory zone for leaf oil ranged from 9.0 ± 1.0 to 14.3 ± 2.55 mm, whereas the essential oil from stem had inhibitory activity ranging from 18.5 ± 2.2 to 20.0 ± 0.0 mm. *Daucus littoralis* oil obtained from different parts of the plant has showed a strong antibacterial activity against *E. coli* and *S. aureus* with an MIC value ranging from 20 to 40 μL/mL. Likewise, Beatovic et al. have reported the antibacterial activity of *Ocimum basilicum* oil against *S. typhimurium* and *E. coli*. The essential oil of *Pogostemon cablin* was shown to have effective antibacterial activity against many pathogenic bacterial strains including *E. coli*, *S. aureus*, *K. pneumoniae*, and *H. pylori*. The GC-MS analysis of essential oils of *Foeniculum vulgare* (Fennel) showed the occurrence of *trans*-anethole, methylchavicol, limonene, and fenchone, whereas *Cuminum cyminum* L. had *γ*-terpin-7-al, *γ*-terpinene, *β*-pinene, and cuminaldehyde as the major constituents. Both essential oils were effective against *S. typhimurium* and *E. coli*. The *F. vulgare* oil exhibited the lowest MIC values of 0.062 and 0.031% (v/v) against *E. coli* and *S. typhimurium*, respectively, whereas *C. cyminum* oil showed MIC values of 0.250 and 0.125% (v/v) against *E. coli* and *S. typhimurium*, respectively.

The MICs ranged between 0.039 and 0.156 mg/mL for all tested bacterial strains. The essential oil extracted from seeds of *Trachyspermum ammi* showed activity against all 36 clinical isolates of *K. pneumoniae, E. coli,* and *S. aureus* isolated from patients suffering from urinary tract infections. An MIC value of 250 ppm was observed for *K. pneumoniae*, whereas it was observed to be 100 ppm for *E. coli* and *S. aureus*. The seed essential oils of *Nigella sativa* containing thymoquinone, *p*-cymene, *α*-thujene, thymohydroquinone, and longifolene as major phytocompounds were shown to exhibit strong antibacterial activity against *B. cereus, E. coli, P. aeruginosa,* and *S. aureus*. The oil was highly effective against *B. cereus, B. subtilis,* and *S. aureus* and showed a complete zone of inhibition at 3000 ppm concentration. Moreover, the zones of inhibition for *P. aeruginosa* and *E. coli* were 20 and 25 mm, respectively.

The essential oil displayed moderate antibacterial activity against *E. faecalis* (MIC = 250 μg/mL) and *S. salivarius* (MIC = 250 μg/mL). Meanwhile, *S. sobrinus* (MIC = 62.5 μg/mL), *S. sanguinis* (MIC = 62.5 μg/mL), *S. mitis* (MIC = 31.25 μg/mL), and *Lactobacillus casei* (MIC = 31.25 μg/mL) were significantly inhibited. Interestingly, the MIC value for *S. mutans* was found to be 3.9 μg/mL. In another study, the essential oil of *Fortunella margarita* was shown to inhibit *Streptococcus faecalis* and *P. aeruginosa* significantly with inhibitory zones of 30 mm and 28 mm, respectively. In addition, moderate activity was observed for *B. subtilis, S. aureus, Sarcina lutea,* and *E. coli* with inhibitory zones ranging from 20 to 25 mm. Similarly, *Achillea fragrantissima* essential

oil was effective against *S. aureus*, *S. epidermidis*, and *E. coli* with the highest inhibition zone of 26 mm, 16 mm, and 16 mm, respectively.

Table 2: Onion oil exhibited good antibacterial activity (MIC = 12 μg/mL) against *S. aureus*.

Plant extract	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Corynebacterium spp*	*Escherichia coli*	*Candida albicans*
Clove	1.56 (64)	0.78 (128)	0.39 (256)	0.78 (128)	0.20 (512)
Thyme	0.39 (256)	0.78 (128)	0.39 (256)	0.78 (128)	0.10 (1024)

Mechanisms of Action of the Essential Oils and/or Their Components

The antimicrobial activity of EOs, similar to all natural extracts, is dependent on their chemical composition and the amount of the single components. Many of the antimicrobial compounds are constitutively expressed by the plants, and others can be synthesised as mechanism of self-defence in response to pathogens. Vegetables, spices and fruits with high level of EOs are excellent sources of natural elements with activity against microorganisms of agricultural and health interest. 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. Different amounts of specific compounds can affect the antimicrobial activity of EOs. For example, high concentrations of cinnamic aldehyde, eugenol or citral confer antimicrobial properties to EOs. The monoterpenes and phenols present in thyme, sage and rosemary EOs possess noticeable antimicrobial, antifungal and antiviral activity. Some EOs, such as those found in basil, sage, hyssop, rosemary, oregano and marjoram, are active against *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* spp. but are less effective against *Pseudomonas* spp. due to the formation of exopolysaccharides that increase resistance to EOs. The mechanism of action of EOs depends on their chemical composition, and their antimicrobial activity is 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". In general, EOs act to inhibit the growth of bacterial cells and also inhibit the production of toxic bacterial metabolites. Most EOs have a more powerful effect on Gram-positive bacteria than Gram-negative species, and this effect is most likely due to differences in the cell membrane compositions.

RESULTS

The anti-bacterial activity of selected essential oils against six bacterial species is summarized. The results revealed that the selected essential oils showed antibacterial activity with varying magnitudes. The zone of inhibition above 7 mm in diameter was taken as positive result. Generally most of the tested organisms were sensitive to many of the essential oils. Cinnamon oil, lemon oil and clove oil showed maximum activity against all the bacterial species tested. On the other hand, aniseed oil, eucalyptus oil and camphor oil failed to inhibit any of the tested strains. Both gram-positive and gram-negative bacteria were sensitive to the potent essential oils. E *coli* in general cinnamon oil showed significant inhibitory effect against *P. aeruginosa* (33.3 mm), *B. subtilis* (29.9 mm), *P. vulgaris* (29.4 mm), *K. pneumoniae* (27.5 mm) and *S. aureus* (20.8 mm). Moderate effects were seen in lime oil, clove oil and lemon oil. No obvious difference in susceptibility was found between gram-negative and gram-positive bacteria. There was no inhibition of growth with the vehicle control (10% DMSO).

Table 3: Antimicrobial activity of 21 essential oils against *S. aureus, B. subtilis* and *K. pneumoniae* using disc diffusion method

Oil Name	*S. aureus*				*B. subtilis*				*K. pneumoniae*			
Aniseed oil	-	-	-	-	12.1 ± 1.0^{klm}	10.8 ± 0.2^{ij}	-	-	-	-	-	-
Cinnamon oil	20.8 ± 0.5^{a}	18.7 ± 0.2^{a}	14.8 ± 0.2^{b}	13.7 ± 0.28^{b}	29.9 ± 0.7^{a}	27.8 ± 1.1^{a}	24.1 ± 1.1^{a}	22.8 ± 0.2^{b}	27.5 ± 0.5^{a}	23.5 ± 0.5^{a}	20.9 ± 0.2^{a}	18.6 ± 0.5^{a}
Clove oil	16.3 ± 0.5^{b}	14.0 ± 0^{bc}	8.1 ± 1.1^{d}	9.8 ± 0.57^{c}	14.5 ± 0.2^{hij}	13.1 ± 0.2^{ef}	10.1 ± 1.0^{def}	8.9 ± 0.2^{f}	16.2 ± 0.5^{c}	14.4 ± 0.5^{b}	8.4 ± 0.5^{c}	-
Lime oil	14.2 ± 0.5^{cd}	$12.7 \pm .2^{bcd}$	$10.3 \pm 0.$	9 ± 0.00^{c}	23.9 ± 1.1^{c}	20.8 ± 0.7^{b}	15.8 ± 0.2^{b}	14.1 ± 1.1^{c}	16.1 ± 1.1^{c}	14.6 ± 0.5^{b}	12.9 ± 0.5^{b}	12.6 ± 0.5^{b}
Streptomycin **	20.9 ±				26.9 ±				20.9 ±			

	0.5				0.5				0.9				

Table 4: Minimum Inhibitory Concentration (MIC) of selected essential oils (mg/ml).

Oil name	*S. aureus*	*B. subtilis*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*E. coli*
Cinnamon oil	3.2	>1.6	3.2	>1.6	>0.8	>1.6
Clove oil	>6.4	>3.2	>6.4	>3.2	>1.6	>1.6
Geranium oil	>12.8	>6.4	12.8	>12.8	>12.8	>6.4
Lime oil	12.8	>6.4	>6.4	>3.2	>6.4	>6.4

Minimum inhibitory concentration (MIC) for selected seven oils ranged from 0.8 to 12.8 mg/ml. This study revealed that cinnamon oil showed maximum activity with MIC values ranging from 0.8 to 3.2 mg/ml followed by clove oil with MIC values ranging from 1.6 to 6.4 mg/ml against all the tested strains where as remaining oils showed moderate MIC values.

Discussion

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak.

In our study, cinnamon, clove, exhibited strong activity against the selected bacterial strains. Several studies have shown that cinnamon, clove and rosemary oils had strong and consistent inhibitory effects against various pathogens. Even though earlier studies have reported better antimicrobial activity for eucalyptus oil our study showed least inhibitory activity of eucalyptus in addition to aniseed and camphor oils. Among all oils analyzed in this work, the essential oil of cinnamon was the most effective as an antibacterial agent. The antibacterial activity has been attributed to the presence of some active constituents in the oils. Our GC-MS study revealed cinnamaldehyde to be the major constituent of cinnamon oil. Cinnamon extract had a regulatory role in blood glucose level and lipids and it may also exert a blood glucose-suppressing effect. Cinnamon oil is locally applied with much benefit in neuralgia and headache. As an antiseptic it is used as an injection in gonorrhea; as germicide it is used internally in typhoid fever. This oil is also used in the treatment of cancer and other microbial diseases. It can be incorporated into creams, lotions, drops, etc. which are applied externally on the body to treat diseases caused by *Aspergillus niger*.

An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death. Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria. In the present study, cinnamon, lime, geranium, rosemary, orange, lemon and clove oils were found to be equally effective against both gram-positive and gram-negative organisms.

Conclusion

From this study it can be concluded that many essential oils possess antibacterial activity. Cinnamon oil has the most potential bactericidal properties. We believe that the present investigation together with previous studies provide support to the antibacterial properties of cinnamon oil. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agents. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral applications.

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