A quantitative in vitro comparison of the effectiveness of essential oils on bacteria commonly found in the natural human flora of the skin and nasopharynx.

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ABSTRACT:

OBJECTIVE: To ascertain the effectiveness of various essential oils on common bacteria found on human skin and nasopharynx.

METHODS:
Six individual essential oils and one blend were selected from the Young Living Company due to their reputation for being a pure unadulterated oil. The six individual oils to be tested are: clove, cinnamon, eucalyptus radiata, oregano, tea tree and thyme. The blend of oils is called Thieves which contains cloves, lemon, cinnamon, eucalyptus radiata and rosemary. These oils will be tested at full strength, and blended with V6 oil to dilute the oils to 1/10 strength and 1/100 strength, on three different bacteria (*S. aureus, S. epidermidis and S. pneumoniae*). The diameter of growth inhibition after 48 hours of incubation was then measured and compared.

RESULTS:
All oils showed at least minimal inhibition of all three bacteria.

CONCLUSION:
These oils show antibacterial properties and further research should be considered to explore them as viable alternatives to topical antibiotics for common infections such as bacterial dermatitis, folliculitis, and impetigo.

KEY WORDS:
Essential Oils, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae,* bacterial inhibition

Introduction:
Essential oils (EOs), also called volatile, ethereal oils or aetherolea, are aromatic oily, hydrophobic liquids retrieved from various plant components: buds, flowers, fruits, twigs, bark, wood or roots. These oils are "essential" in that they carry the scent, or essence, of the plant they are obtained from.

Although essential oils have been used medicinally at different periods in history, their use has declined since the institutionalization of mainstream modern medicine. However; over the last few decades interest in essential oils has revived as alternative therapies and "green" consumerism have grown in popularity.[1] The antibacterial properties of these essential oils have been recognized for decades[2] and is currently an avid topic of research.[3,4,5]

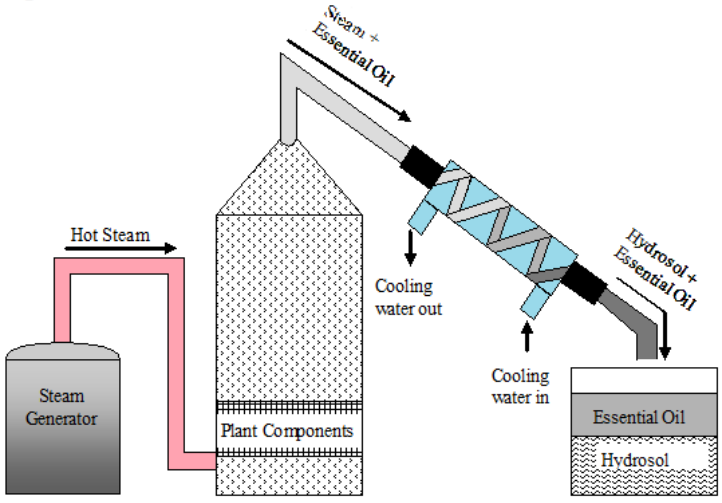
The objective of this study is to further that research; to increase the knowledge base for those looking for a natural alternative to drug based treatment of common infections. This paper should be an expansion and aggregate of the already wide collection of research on the anti-microbial effects of EOs, while focusing specifically on their effects on common bacteria of the human skin and nasopharynx.

Methods:

Essential Oils:
The following essential oils were used: clove, cinnamon, eucalyptus, oregano, tea tree, thyme and a blend of oils known as "Thieves" which contains cloves, lemon, cinnamon, eucalyptus radiata and rosemary. All oils were purchased from the Young Living Company which extracts these oils via steam distillation.

Commercially the vast majority of EOs are extracted by steam distillation; although some are obtained through fermentation, solvent extraction or enfleurage. Steam distillation involves boiling water and directing the steam through the plant components. The steam vaporizes the aromatic components as it passes through the plant components, carrying it to the top of the still. The mixture of aromatic compounds and vapor (now called hydrosol) then passes through a condenser where it is cooled and settles back into the liquid state. The liquid mixture flows down into a collection vessel where the hydrophobic essences separate from the hydrosol (figure 1)

Figure 1



The Apparatus:
The materials used include the following: essential oils, approximately 1300 (85 mm) petri dishes, nutrient agar, micropipettes (100-1000 µl), approximately 700 micropipette tips, 1300 blank discs, 1300 cotton swabs, Bunsen burner, forceps, incubator, micrometer, and three bacterial strains: *Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus pneumoniae*.

Procedure:
Seven essential oils were ordered from the Young Living Company: clove, cinnamon, eucalyptus, oregano, tea tree, thyme and a blend of oils known as "Thieves." The experiment was carried out over four separate three day sessions.

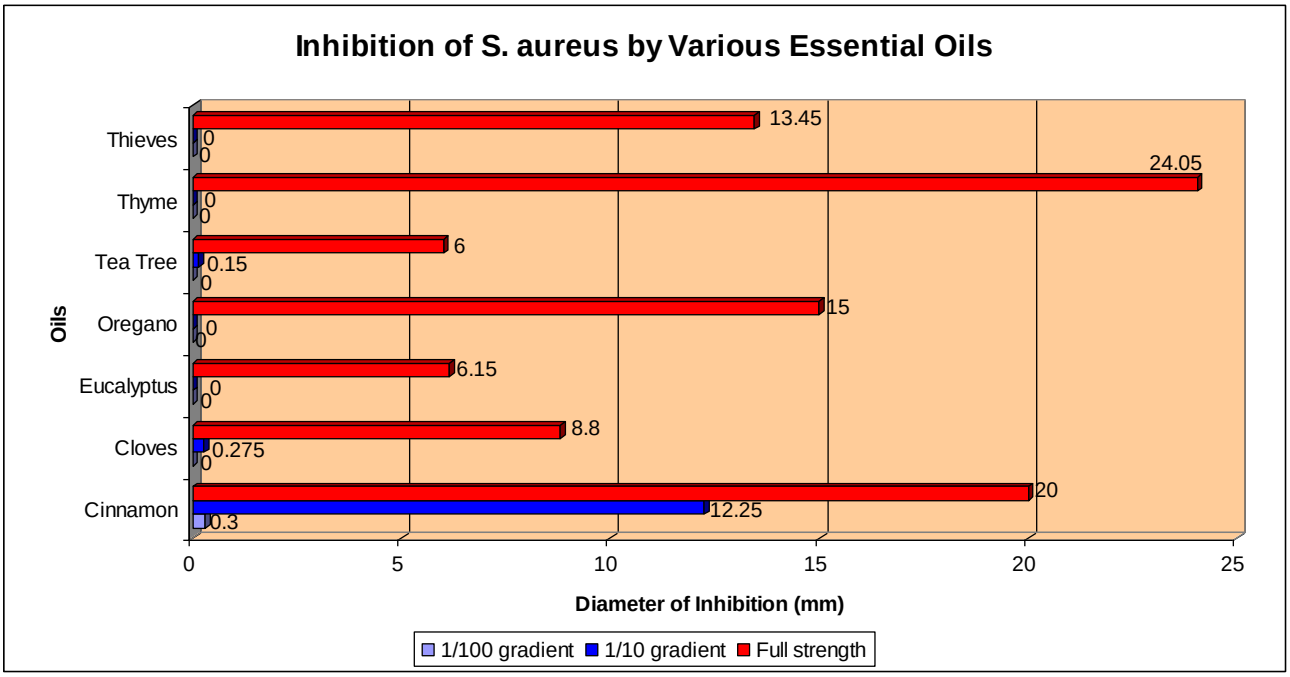
On the first day of each session, nutrient agar was prepared according to the manufacturer's directions, autoclaved at 120° C for 15 minutes and poured into the group of petri dishes that was to be made for that session. The agar set for at least 20 minutes to cool and solidify. The plates were labeled according to bacteria, oil, and oil concentration. The cultures of the bacteria were gram stained and examined under a microscope to confirm the presence of the expected bacteria and rule out contamination. *Staphylococcus aureus* were gram-positive and the bacteria were round and in the characteristic clusters. *Staphylococcus epidermidis* were gram-positive cocci, arranged in diplococci with two cells next to each other. *Streptococcus pneumoniae* were gram-positive, lancet formed diplococci, alpha-hemolytic and anaerobic. The oils were also blended into their respective dilutions with V6 oil.

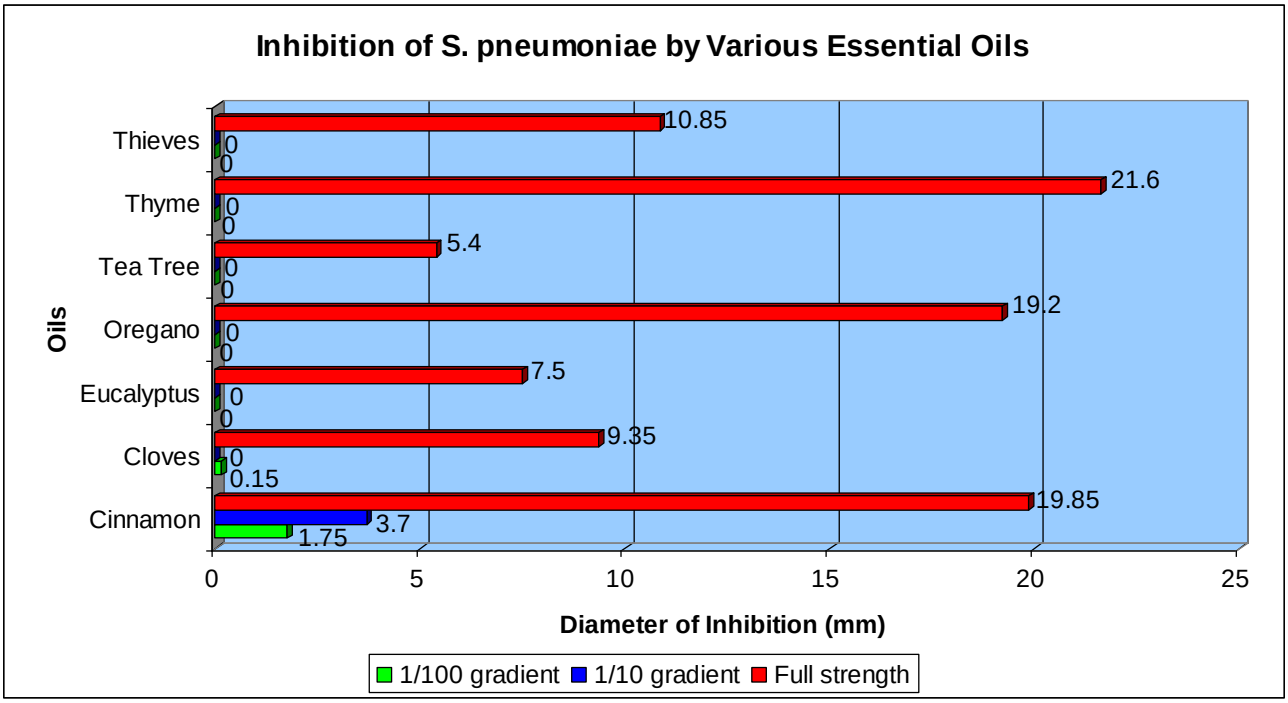
On the second day the bacteria were inoculated and swabbed onto the petri dishes using proper sterilization technique. One sterile cotton applicator was used with each inoculation and the plates were streaked for lawn growth. Eleven blank ¼" cotton discs were placed on an empty, sterile petri dish and 12 µl of essential oil was dropped on each blank disc. Then, using sterilized forceps, the disc was placed on the appropriate inoculated petri dish. The plates were then incubated at 37° C for at least 48 hours.

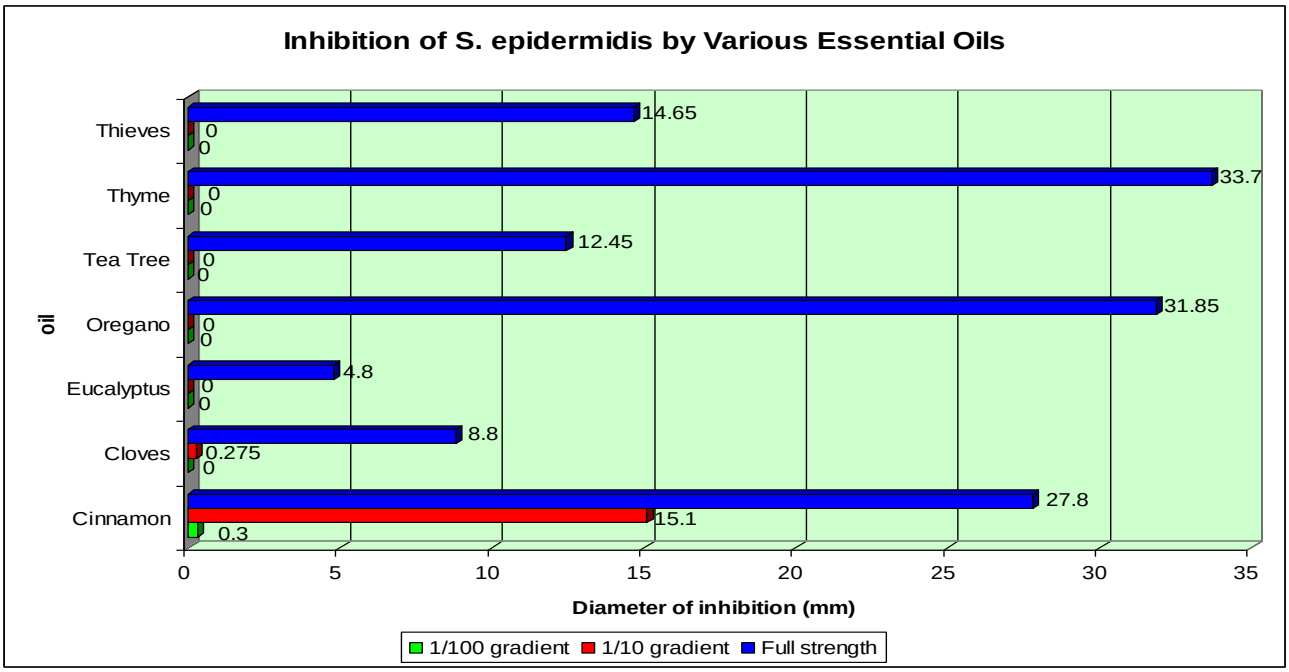
After the incubation period, the minimum diameter of inhibited growth was measured in millimeters using a micrometer. Each petri dish was measured independently by two examiners and correlated. In the case of a gross discrepancy a third examiner assessed the sample and an average was recorded.

Results:

Thyme achieved the overall broadest spectrum antibacterial action against the organisms tested. Cinnamon and oregano were the next most promising with only cinnamon showing a gradient effect as the concentration was reduced.







Discussion:

The oils:

Essential Oils have been used for medicinal purposes since ancient times. In the modern era, the vast majority of research into the aromatic substances is divided evenly between the two very different fields of food preservation and aromatherapy. There is a broad spectrum in both the quality and the pragmatic application of most of this research. There is hundreds of years[1] worth of high quality research that shows the efficacy of essential oils. This study aims to give the alternative health practitioner a scientifically astute, well-designed, pragmatic study of the effects of essential oils on common epidermal bacteria.

While the antimicrobial properties of essential oils have been adequately demonstrated, the mechanisms of action have only been explored in the last couple of decades[6]. Most research has focused on the disturbance of the lipid bilayer of the cytoplasmic membrane by hydrophobic hydrocarbons[7] causing leakage of ions and other cell contents[8,9].

Previous research suggests phenolic hydrocarbons possess the strongest antibacterial properties[3,6,10]. Examples of phenolic compounds include carvacrol, thymol and eugenol, the major constituents of oregano, thyme and clove oils respectively. This study supports that research with oregano and thyme consistently showing strong inhibition of all three bacteria. The mechanism of action of phenolics is generally agreed to be disturbance of the cytoplasmic membrane, disruption of the proton motive force, electron flow, active transportation and coagulation of cell constituents[11,12].

Eucalyptol (1,8-cineole) is a monoterpenoid known to have similar antibacterial mode of action to the phenolic hydrocarbons[13] and is found in concentrations between 34-92% in Eucalyptus essential oil[14,15]. This range exists because there are over 700 species of *Eucalyptus* plants[14] and some of these plants are known to have more than 20 active constituents which may vary from species to species and even within the same species depending on the age of the plant or even the season in which the oil was obtained[14,16,17,18]. Furthermore; while eucalyptol is the principle active compound, studies have shown other minor constituents have a sometimes additive, sometimes synergistic role to play as well[19,20,21].

Tea tree oil is a widely used antimicrobial in a wide variety of cosmetic and personal hygiene products with widely accepted antimicrobial properties[22,23]. The main active components of tea tree oil consist of oxygenated terpenoids[24,25]. The principle active terpinoid is considered to be terpinen-4-ol which makes up 40% of some tea tree oils[24,22] and seems to be more effective alone, than in concert with other tea tree components against at least a few species of bacteria[22]. Studies done with electron microscopy reveal that tea tree oils inhibit cell division and lead to apoptosis by coagulation of cytoplasmic constituents while leaving the cell wall and cytoskeleton relatively intact[26].

The active component of cinnamon oil; cinnamaldehyde, is known to be effective against *E. coli, Salmonella typhimurium* and *Photobacterium leiognathi*[27]. Unlike other lipophilic hydrocarbons, cinnamaldehyde seems to have no effect on the lipid bilayer but instead acts by competitively inhibiting the biotin binding site of carboxytransferase effectively blocking the cells ability to utilize ATP. This indirectly affects the cells ability to retain vital ion concentrations and maintain cell wall integrity[28,29].

This study can quantify and qualify the effectiveness of Thieves oil blend. It is a mixture of several different oils and because it is a brand blend there has been no research done on its mode of action. While one could describe the mode of action of each individual ingredient, analysis of possible synergistic or antagonistic effects of the combined oils is beyond the scope of this study.

While there is ample evidence in this study that these seven essential oils inhibit bacterial growth, there was not the kind of gradation one might hope for as the concentration was decreased. Cinnamon bark was the only oil that still showed effectiveness at 1/10 and 1/100 concentrations, and even then only against *S. aureus*. Because Minimum Inhibitory Concentrations (MIC) were not determined or investigated before the start of the experiment it is possible that the obtained oils were already at MIC.

Sources of potential error:

The largest source of potential error was made when the oregano and thyme were tested against the *S. epidermidis*. In these cases the cotton discs were placed on the agar before the oil was dropped on the disc. The discs may have had a harder time absorbing the oil

in these cases, possibly because it already soaked up some condensation from the agar. This may have caused some of the oil to seep off the disc and into the agar.

There was an incident on November 22, 2010 in which the researchers entered the lab and found the door to the incubator was not fully closed. No drop or rise in internal temperature of the incubator was noted and results for the affected samples were within expected values. Some, but not all, samples of *S. aurous* vs. tea tree, clove and eucalyptus oils may have been affected.

Potential future experiments

There are obviously many more bacteria and essential oils that can be tested. There is an indefinite number of combinations that can be explored with this type of research. However, while finding what oil works best against which bacteria would be interesting it would probably be more practical to find what oil inhibits the broadest spectrum of bacteria.

Testing the essential oils against *Streptococcus pyogenes,* a major cause of bacterial pharyngitis/tonsillitis may be used as a launch pad to a natural alternative to systemic antibacterial treatments for these conditions. The right blends of these essential oils may make an effective gargle for early upper respiratory tract infections as a prophylactic against acute otitis media.

Probably the most important avenue of future research would be to move from in-vitro to in-vivo trials. Some of this has already started with tea tree and eucalyptus oil in dental health[30]. Eucalyptus and tea tree oil already have uses in cosmetic and personal hygiene products like shampoo, could it be developed as a substitute for aluminum based deodorants, or as a non-alcohol based anti-bacterial hand sanitizer, or even surgical prep solution?

References:

1. Tuley De Silva K, A manual on the essential oil industry, presentations made by the resource persons at the Third Unido Workshop on Essential Oil and Aroma Chemical Industries held at the Anadolu University Medicinal and Aromatic Plant and Drug Research Centre in Eskisehir, Turkey, 1995; 1
2. Nychas GJE. New methods of food preservation: natural antimicrobials from plants. Gould GW, editor, London: Blackie Academic and Professional; 1995; 60-7
3. Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 2000; 88: 308-16.
4. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against *Eschericia coli* O157:H7. Lett Appl Microbiol, 2003; 36(3): 162-7
5. Lopez P, Sanchez C, Batlle R, Nerin C. Solid and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. J Agric Food Chem, 2005; 53(17): 6939-46
6. Lambert RJW, Skandamis PN, Coote PJ, Nychas JE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol, 2001; 91: 453-62.
7. Sikkeman J, De Bont JAM, Poolman B. Interaction of cyclic hydrocarbons with biological membranes. The Journal of Biological Chemistry, 1994 Mar 18; 269(11), pp 8022-28.
8. Skandamis P, Nychas GJE. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs, and oregano essential oil concentrations. Appl Environ Microbiol, 2000 Apr; 66(4): 1646-53.
9. Ultee A, Bennik MHJ, Moezelaar R, The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol, 2002 Apr; 1561-68.
10. Cosentino S. Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, et all. In-vitro antimicrobial activity and chemical composition of sardinian thymus essential oils. Lett Appl Microbiol 1999; 29: 130-5.
11. Sikkema J, De Bon JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews, 1995 Jun; 59(2): 201-22.
12. Heipieper H, Keweloh H, Rehm H. Influence of phenols on growth and membrane permeability of free and immobilized *Escherichia coli*. Appl Environ Microbiol, 1991 Apr; 1213-17.
13. Filoche SK, Soma K, Sissons CH. Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. Oral Microbiol Immunol. Aug 2005; 20(4): 221-5
14. Sefidkon F, Assareh M, Abravesh Z, Barazandeh M. Chemical composition of the essential oils of four cultivated eucalyptus in Iran as medicainal plants (*E. microtheca, E. spathulata, E. latgiflorens and E. torquata*). Iranian Journal of Pharmaceutical Research: IJRP. 2007; 6(2): 135-140.
15. Wildy TD, Pate JS, Bartle JR. Variations in composition and yield of leaf oils from alley-farmed oil mallees (eucalyptus spp.) at a range of contrasting sites in

the Western Australian Wheatbelt. Forest Ecology and Management. 2000 Sept; 134(1-3): 205-17
16. Silvestre AJD, Cavaleiro JA, Delmond B, Filliatre C, Bourgeois G. Analysis of the variation of the essential oil composition of *Eucalyptus globulus* lbaill. from Portugal using multivariate statistical analysis. Ind Crops Prod. 1997 Feb; 6(1): 27-33
17. Southwell IA. Variation in the leaf oil of *Eucalyptus punctata*. Phytochemistry. June 1973; 12(6) 1341-43
18. Bhatti HN, Iqbal Z, Chatha SAS, Bukhari IH. Variations in oil potential and chemical composition of *Eucalyptus crebra* among different districs of Punjab-Pakistan. International Journal of Agriculture and Biology. 2007; 09(1): 136-8
19. Hendry E, Worthington T, Conway B, Lambert P. Antimicrobial efficacy of eucalyptus oil and 1,8-Cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. J Antimicrob Chemother. 2009; 64: 1219-1225.
20. Takahashi T, Kokubo R, Sakaino M, Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*. Lett Appl Microbiol, 2004; 39: 60-4.
21. Nakayama R, Murata M, Homma S, Aida K. Antibacterial compounds from *Eucalyptus perriniana*. Agric Biol Chem. 1990; 54(1): 231-2
22. Cox SD, Mann CM, Markham JL. Interactions between components of the essential oil of *Melaleuca alternifolia*. Journal of Applied Microbiology. 2001; 91: 492-7.
23. Carson C, Mee B, Riley T. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrobial Agents and Chemotherapy. 2002 Jun; 46(6): 1914-20
24. Southwell IA, Hayes AJ, Markham J, Leach DN. The search for optimally bioactive austrailian tea tree oil. Acta Hort. (ISHS). 1993; 344: 256-65
25. Carson CF, Riley TV. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol. 1995 Mar; 78(3): 264-9
26. Reichling J, Schnitzler P, Suschke U, Saller R. Essentail oils of aromatic plants with antibacterial, antifungul, antiviral, and cytotoxic properties – an Overview. Forsch Komplementmed 2009; 16: 79-90
27. Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, et al. Characterization of the action of selected essential oil componenets on gram-negative bacteria. J. Agric. Food Chem. 1998; 46(9) 3590-5
28. Meades G jr, Henken RL, Waldrop GL, Rahman MM, Gilman SD, Kamatou GP, et al. Constituents of cinnamon inhibit bacterial acetyl CoA carboxylase. Planta Med. 2010 Oct; 76(14):1570-5
29. Wendakoon CN, Sakaguchi M. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active componenets in spices. J Food Prot. 1995; 58(3): 280-3
30. Rasooli I, Shayegh S, Astaneh S. The effect of *Mentha spicata* and *Eucalyptus camaldulensis* essential oils on dental biofilm. Int. J Dent Hyg. 2009; 7(3) 196-203.