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Research Article

EVALUATION OF ANTIBACTERIAL PROPERTIES OF ESSENTIAL OILS FROM CLOVE AND EUCALYPTUS

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ABSTRACT

Objective: To substantiate the data for naturally available sources of antibacterial and bactericidal agents, this study was undertaken. The specific aim was to analyze antibacterial properties of two essential oils from clove (*Eugenia caryophyllata*) and Eucalyptus (*Eucalyptus globulus*) against *Sphingobium indicum*, *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria), which are clinically significant strains.

Methods: The analysis was performed by measuring the zone of inhibition or "halo" using disk diffusion method.

Results: Out of two oils used, clove oil was found to be more effective in inhibiting growth of all the four types of bacterial strains used in the study. *B. subtilis* was the most susceptible bacterial strain to clove oil showing maximum area of 7.54 cm^2 for the halo/zone of inhibition, whereas *E. coli* was the least susceptible with 5.14 cm^2 area of the halo. Eucalyptus oil inhibited growth in *S. indicum* and *E. coli* but the area of inhibition zone was much less in comparison to clove oil. *Staphylococcus* and *Bacillus* strains were found completely insensitive to eucalyptus oil.

Conclusion: New antibacterial agents are very valuable in multidrug-resistant bacteria and the present study provides additional support to the already available data to use essential oils against various strains of bacteria. The clove oil proved more suitable as antibacterial agent than eucalyptus oil.

Keywords: Essential oils, Antibacterial properties, Disk diffusion.

INTRODUCTION

Essential oils are chemically terpenes that are one of the largest group of plant secondary metabolites. They are volatile, limpid, colored and are soluble in lipids and organic solvents that have a lower density than water. They may be present in all plant organs of specific plant families, including buds, flowers, leaves, seeds, twigs, stems, flowers, fruits, roots, wood or bark and are generally stored by the plant in secretory cells, cavities, canals, glandular trichomes or epidermal cells. Essential oils of many medicinal plants have been used for evaluation of their antibacterial and antifungal activities [1,2]. Studies have shown that essential oils contain a wide series of compounds that can inhibit or slow the growth of bacteria, yeasts and molds inhibiting their transmission. Essential oils have been widely used in dentistry as an antiseptic and local anesthetic. Clove oil and eucalyptus oils are two very familiar names in this category of essential oils.

Clove oil is extracted from *Eugenia caryophyllata* of the Myrtaceae family. It has been used as a histological clearing agent. Medicinally, it is widely used for relieving toothache or cavity problems and in aromatherapy and as an antiseptic in oral infections [3,4]. The intake of the oil provides carminative and anti-plasmodic properties. In the stomach, the effect is carminative, relaxing the gastric sphincter, and it encourage eructation. Patients suffering from arthritis, rheumatism and leg ulcers are advised to take clove oil. Eucalyptus oil, on the other hand is extracted from *Eucalyptus globulus* of the Myrtaceae family. It showed antiseptic abilities and used in mosquito and vermin repellant products. Patient suffering from chronic bronchitis is advised to inhale the vapors of this renowned essential oil. It plays a crucial role in the preparation of thymol and menthol. Worldwide, it is used for the preparation of Aerosol that is used in the chemical and varnishing industry. The eucalyptus oil possesses a wide spectrum of biological activities such as antimicrobial and antifungal [5]. Antimicrobial potential of eucalyptus oil has been proved in some studies [6,7]. Mostly plant derived essential oils consist of chemical components such as terpenoids including monoterpenes, sesquiterpenes and their oxygenated derivatives. These compounds have the ability to easily diffuse across cell membrane to induce biological reactions [8]. The oil induces antimicrobial activity due to high level of eugenol and eucalyptol components [9].

The Gram-positive bacterium *Staphylococcus aureus* is mainly responsible for post-operative wound infection, toxic shock syndrome and food poisoning. *Escherichia coli*, a Gram-negative bacteria, is present in human intestines and causes urinary tract infection, coleocystitis or septicemia [10].

There is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [11] as bacteria over the years have developed increased antibiotic resistance [12]. It has also been seen that the synthetic antibiotics are costly, and cause some side-effects in the treatment of infectious diseases [13]. Therefore, the present study was undertaken to assess the antimicrobial properties of two essential oils (clove and eucalyptus oil) on four clinically significant bacterial strains.

METHODS

Plant material

Bacterial strains of *E. coli, Sphingobium indicum, Bacillus subtilis* and *S. aureus* were obtained from Laboratory of Microbiology, Department of Zoology, University of Delhi, Delhi. Commercial clove oil and eucalyptus oil were procured from Merck Scientific.

Method

Preparation of bacterial culture

To culture the bacterial strains, 5 ml of autoclaved liquid lysogeny broth (LB) medium was inoculated with four different bacterial cultures.

The inoculated tubes were incubated at 37°C at 120 rpm, overnight. Semisolid LB media plates were inoculated with 40 µl of overnight grown culture of each bacterial strain. Autoclaved Whatman paper disc saturated with the eucalyptus and clove oil were placed in plates freshly inoculated with bacterial culture. Discs saturated with ionized water were kept in the plates that served as the control. All experimental samples were cultured in triplicates. Petriplates were sealed with parafilm and incubated at 37°C for *B. subtilis* and at 28°C for *S. indicum, S. aureus* and *E. coli*.

Analysis

After 48 hrs of incubation, inhibition zone was measured around paper disc with the help of the scale for all plates. The readings were taken in triplicate.

RESULTS

Out of the two oils tested, clove oil was found to be most effective in inhibiting growth in all four strains of bacteria (Figs. 1 and 2). *B. subtilis* was the most susceptible bacterial strain showing largest area of 7.54 cm^2 for the inhibition zone followed by *S. aureus, S. indicum* and *E. coli* with 7.06, 6.42 and 5.7 cm^2 area each, respectively (Table 1). Eucalyptus oil failed to inhibit the growth in *S. aureus* and *B. subtilis,* but it showed moderate inhibition of growth in *S. indicum* and *E. coli*. *E. coli* appeared to be more sensitive to eucalyptus oil in comparison to *S. indicum* with an area of 4.37 cm^2 for the inhibition zone (Table 1 and Fig. 3).

DISCUSSION

The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak [14,15]. Vegetables, spices and fruits with a high level of Essential oils 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 plant is subjected to particular biotic or abiotic stress. Different amounts of specific compounds can affect the antimicrobial activity of essential oils. The presence of complex chemical structures constituted of several groups, such as terpenes and terpenoids, aromatic and aliphatic constituents, all characterized by low molecular weight, may explain their successful bacteriostatic and bactericidal action [16]. Phenolic acids such as thymol, eugenol and carvacrol are secondary plant metabolites that account for the antimicrobial activity of essential oils such as oregano, cinnamon, and clove [17].

The results have shown that the clove oil was more effective and created a halo or zone of inhibition in all the bacterial strains. In case of clove oil, the measurements for zone of inhibition (Halo) showed *Bacillus* (halo area 7.543 cm^2) to be most susceptible, whereas *E. coli* (halo area of 5.144 cm^2) was most resistant. With an area of 6.422 cm^2 *S. indicum,* proved its susceptibility to the clove oil. In the case of *Staphylococcus*, the measurement of zone of inhibition was 7.065 cm^2. Eucalyptus oil was found effective in inhibiting the growth only in the case of *E. coli* and *Sphingobium. Staphylococcus* and *B. subtilis* strains were found to be insensitive to eucalyptus oil. *E. coli* appeared to be more sensitive with a halo area of 4.372 cm^2, which was far more in comparison to that of

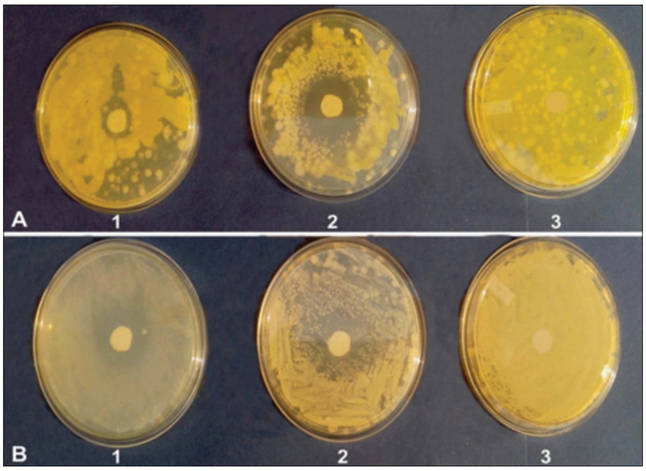

Fig.1: (a) Effect of eucalyptus oil (1), clove oil (2) on *Sphingobium indicum*, control (3), (b) Effect of eucalyptus oil (1), clove oil (2) on *Escherichia coli*, control (3)

Table 1: Zone of growth inhibition or "halo" for essential oils on different bacterial strains

Essential oil	Strain of the Bacteria	Bacteria	Diameter of the halo (cm)	Average diameter of the halo (cm)	Radius of the halo (cm)	Area of the halo (cm^2)
Eucalyptus oil	Gram-negative	*S. indicum*	1.9 2.0 2.1	2.0	1.0	3.14
	Gram-negative	*E. coli*	2.3 2.4 2.4	2.36	1.18	4.372
	Gram-positive	*S. aureus*	No halo No halo No halo No halo			
	Gram-positive	*B. subtilis*	No halo No halo No halo			
Clove oil	Gram-negative	*S. indicum*	2.7 2.7 2.7	2.7	1.43	6.422
	Gram-negative	*E. coli*	2.6 2.5 2.6	2.56	1.28	5.144
	Gram-positive	*S. aureus*	2.9 3.0 3.1	3	1.5	7.065
	Gram-positive	*B. subtilis*	3.1 3.2 3	3.1	1.55	7.543

S. indicum: Sphingobium indicum, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis

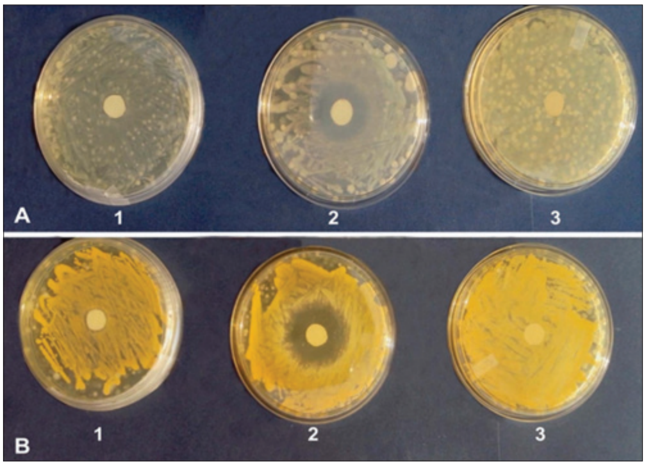

Fig. 2: (a) Effect of eucalyptus oil (1), clove oil (2) on *Staphylococcus aureus*, control (3), (b) Effect of eucalyptus oil (1), clove oil (2) on *Bacillus subtilis*, control (3)

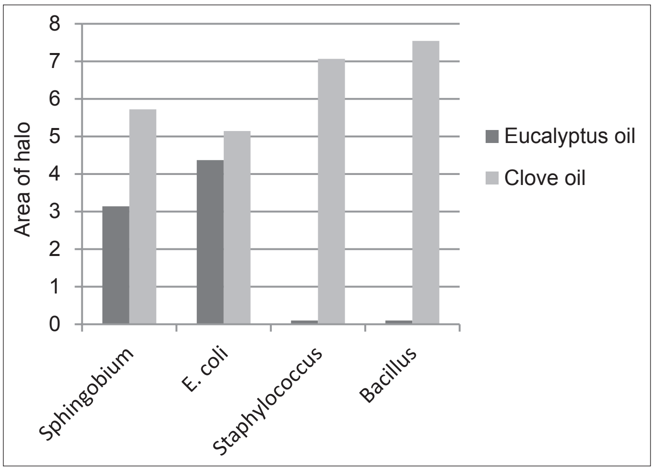

Fig. 3: Comparative antimicrobial activities of eucalyptus oil and clove oil

Sphingobium with an area of 2.268 cm^2. Eucalyptus oil has been found to have relatively strong antimicrobial properties against many important pathogens and spoilage organisms, including *S. aureus* and *E. coli* [18].

Results obtained with clove oil showed growth inhibition for all the four bacterial cultures, but the size of the halo was smaller for Gram-negative bacteria. This implies that Gram-negative are comparatively resistant to clove oil effect. Mostly in literature, Gram-negative bacteria were more resistant to essential oils than Gram-positive bacteria [19]. It is because the cell wall of Gram-negative bacteria is more complex. The presence of an outer membrane is one of the features that differentiate Gram-negative from Gram-positive bacteria. It is composed of a double layer of phospholipids that is linked to the inner membrane by lipopolysaccharides (LPS). The peptidoglycan layer is covered by an outer membrane that contains various proteins, as well as LPS. LPS consists of lipid A, the core polysaccharide, and the O-side chain, which provides the "quid" that allows Gram-negative bacteria to be more resistant to essential oils and other natural extracts with antimicrobial activity. Though small hydrophilic solutes are able to pass through the outer membrane via abundant porin proteins that serve as hydrophilic transmembrane channels, and this is one reason that Gram-negative bacteria are relatively resistant to hydrophobic antibiotics and toxic drugs.

The mechanisms of action of essential oils and/or their components are dependent on their chemical composition. The main constituents of clove essential oil are phenyl-propanoides such as carvacrol, thymol, eugenol and cinnamaldehyde [20]. The concentration of eugenol in clove oil is 79.2%. The inhibitory activity of clove is due to the presence of several constituents, mainly eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone [21]. The thymol and carvacrol have similar antimicrobial effects but have different mechanisms of action against Gram-positive and Gram-negative bacteria. Clove oil can inhibit the growth of *E. coli* and *Sphingobium* without disintegrating the outer membrane or depleting intracellular adenosine triphosphate. Similar to thymol and carvacrol, trans-cinnamaldehyde likely gains access to the periplasm and deeper portions of the cell. The location of one or more functional groups on these molecules can affect their antimicrobial activity. Essential oils and/or their constituents can have a single target or multiple targets of their activity. Several studies have demonstrated potent antibacterial effects of clove oil [22,23]. Essential oil of *E. globulus* constitutes oxygenated monoterpenes (46.5%) with terpinen-4-ol (23.46%) as the principal constituents. Other constituents of eucalyptus oil are α-pinene, β-pinene, α-phellandrene, 1, 8-cineole, limonene, terpine-4-ol, aromadendrene, epiglobulol, piperitone and globulol. Similarly, eucalyptus oil was used in various studies where its activity was found either moderate [24,25] or low [1].

CONCLUSIONS

The presence of 1,8-cineole and α-phellandrene coupled with low antioxidant activity and high cytotoxic effect makes eucalyptus oil less effective against the control of bacterial growth, which could be probably one of the reasons for complete resistance shown by *S. aureus* and *B. subtilis* to this oil. Thus, eucalyptus oil is not much suited for medicinal purposes, but can be used as repellant or anti-feedant in insecticidal formulation. New antibacterial agents are very valuable in multidrug resistant bacteria, and the present study provides additional support to the already available data to use essential oils against various strains of bacteria. However, it is very important to study the interaction of essential oils and their constituents *in vivo* to know their efficacy, as well as toxicity [26]. Therefore, further clinical trials are required to ascertain their use.

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REFERENCES

1. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. BMC Complement Altern Med 2006;6:39.
2. Aggarwal SG, Goyal S. Comparative analysis of antimicrobial activity of essential oil of *Ocimum kilimandscharium*. Asian J Pharm Clin Res 2012;5(1):53-5.
3. Meeker HG, Linke HA. The antibacterial action of eugenol, thyme oil, and related essential oils used in dentistry. Compendium 1988;9(1):32, 34-5.
4. Shapiro S, Meier A, Guggenheim B. The antimicrobial activity of essential oils and essential oil components towards oral bacteria. Oral Microbiol Immunol 1994;9(4):202-8.
5. Shahwar D, Raza MA, Bukhari S, Bukhari G. Ferric reducing antioxidant power of essential oils extracted from *Eucalyptusand curcuma* species. Asian Pac J Trop Biomed 2012;2:S1633-6.
6. Owlia P, Saderia H, Rasooli I, Sefidkon F. Antimicrobial characteristics of some herbal Oils on *Pseudomonas aeruginosa* with special reference to their chemical compositions. Iran J Pharm Res 2009;8(2):107-14.
7. Filoche SK, Soma K, Sissons CH. Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. Oral Microbiol Immunol 2005;20(4):221-5.
8. Lin KH, Lin MY, Shih MC, Yang K, Hwang SY. Major chemotypes and antioxidative activity of the leaf essential oils of *Cinnamomum osmophloeum* Kaneh from a clonal orchard. Food Chem 2007;105:133-9.
9. Kumar UK, Bhandari A, Kumar Y. Phytochemical investigation and comparison of antimicrobial screening of clove and cardamom. Int J Pharm Sci Res 2010;1:138-14.

10. Reddy LJ, Jose B, Jalli RD, Gopu S. Evaluation of antibacterial and DPPH radical scavenging activities of the leaf essential oil and leaf extracts of *Uvaria narum* (Dunal) Wall. Int J Res Pure Appl Microbiol 2012;2(2):13-9.
11. Agarwal G, Hore SK, Prakash O, Pant AK. Hypotensive activity of *T. connaroides* extracts in rats. J Vet Pharmcol Toxicol 2006;5:72-3.
12. De Esparza RR, Bye R, Meckes M, Torres Lopez J, Estrada J. Antibacterial activity of *Piqueria trinervia*, a Mexican medicinal plant used to treat diarrhea. Pharm Biol 2007;45:446-52.
13. Schinor EC, Salvador MJ, Ito IY, Dias DA. Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. Braz J Microbiol 2007;38:145-9.
14. Zaika LL. Spices and herbs: Their antibacterial activity and its determination. J Food Saf 1988;23:97-118.
15. Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M. Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. Int J Food Microbiol 2006;107(2):180-5.
16. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. Food Chem Toxicol 2008;46(2):446-75.
17. Barbosa LN, Rall VL, Fernandes AA, Ushimaru PI, da Silva Probst I, Fernandes A Jr. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathog Dis 2009;6(6):725-8.
18. Safaei-Ghomi J, Ahd AA. Antimicrobial and antifungal properties of the essential oil and methanol extracts of *Eucalyptus largiflorens* and *Eucalyptus intertexta*. Pharmacogn Mag 2010;6(23):172-5.
19. Jeyaseelan EC, Jashothan PT. *In vitro* control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L. Asian Pac J Trop Biomed 2012;2(9):717-21.
20. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, *et al.* The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): A short review. Phytother Res 2007;21(6):501-6.
21. Chaieb K, Zmantar T, Ksouri R, Hajlaoui H, Mahdouani K, Abdelly C, *et al.* Antioxidant properties of the essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. Mycoses 2007;50(5):403-6.
22. López P, Sánchez C, Batlle R, Nerín C. Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. J Agric Food Chem 2005;53:6939-46.
23. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, *et al.* Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res 2007;21(10):989-94.
24. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, *et al.* Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 2002;79(2):213-20.
25. Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol 2004;19(1):61-4.
26. Pednekar PP, Vakil BV, Sane RT, Datar AG. Development of analytical method for identification of essential oil from *Blumea eriantha* DC in plasma. Asian J Pharm Clin Res 2013;6(3):239-42.