



Antimicrobial Activity of Commercially Available Essential Oils Against *Streptococcus mutans*

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ABSTRACT

Introduction: Many essential oils have been advocated for use in complementary medicine for bacterial and fungal infections. However, few of the many claims of therapeutic efficacy have been validated adequately by either *in vitro* testing or *in vivo* clinical trials.

Objective: To study the antibacterial activity of nine commercially available essential oils against *Streptococcus mutans in vitro* and to compare the antibacterial activity between each material.

Methodology: Nine pure essential oils; wintergreen oil, lime oil, cinnamon oil, spearmint oil, peppermint oil, lemongrass oil, cedarwood oil, clove oil and eucalyptus oil were selected for the study. *Streptococcus mutans* was inoculated at 37°C and seeded on blood agar medium. Agar well diffusion assay was used to measure antibacterial activity. Zone of inhibition was measured around the filter paper in millimeters with vernier caliper.

Results: Cinnamon oil showed highest activity against *Streptococcus mutans* followed by lemongrass oil and cedarwood oil. Wintergreen oil, lime oil, peppermint oil and spearmint oil showed no antibacterial activity.

Conclusion: Cinnamon oil, lemongrass oil, cedarwood oil, clove oil and eucalyptus oil exhibit antibacterial property against *S. mutans*.

Clinical significance: The use of these essential oils against *S. mutans* can be a viable alternative to other antibacterial agents as these are an effective module used in the control of both bacteria and yeasts responsible for oral infections.

Keywords: *Streptococcus mutans*, Antibacterial property, Disk diffusion, Essential oils.

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INTRODUCTION

Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (buds, flowers, barks, seeds, leaves, twigs, wood, herbs, fruits and roots). An estimated 3000 essential oils are known to us, of which 300 are commercially important in fragrance market.¹ Essential oils are complex mixtures comprising of many compounds. Chemically, they are derived from terpenes and their oxygenated compounds. Each of them contributes to the beneficial effects of these oils.²

Essential oils have been shown to possess antibacterial, antiviral, insecticidal and antioxidant properties. Some oils have also been used in cancer treatment. They have been used as food preservatives, for aroma therapy and in the fragrance industry. They are a rich source of biologically active compounds.^{2,3}

The spread of drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential use as alternative remedies for the treatment of many infectious diseases.⁴ World Health Organisation (WHO) noted that a considerable part of the world's population depends on traditional medicine for primary care.²

Many essential oils have been advocated for use in complementary medicine for bacterial and fungal infections including boils, acne, gingivitis and vaginal candidiasis. However, few of the many claims of therapeutic efficacy have been validated adequately by either *in vitro* testing or *in vivo* clinical trials. Unless these claims have been substantiated scientifically, complementary medicines are unlikely to secure a place in conventional health care.⁵

OBJECTIVES

1. To evaluate the *in vitro* antibacterial efficacy of nine commercially available essential oils against *Streptococcus mutans*.
2. To compare the antibacterial activity between each material.

METHODOLOGY

Collection of Materials

The microbial strain (*Streptococcus mutans* ATCC 25175) selected for the present study was obtained from the American Type Culture Collection (ATCC). Nine pure essential oils commercially available were selected for the study:

1. Wintergreen oil (*Gaultheria fragrantissima*)
2. Lime oil (*Citrus aurantium*)
3. Cinnamon oil (*Cinnamomum camphora*)
4. Spearmint oil (*Mentha spicata*)
5. Peppermint oil (*Mentha piperita*)
6. Lemongrass oil (*Cymbopogon citratus*)
7. Cedarwood oil (*Cedrus atlantica*)
8. Clove oil (*Eugenia caryophyllus*)
9. Eucalyptus oil (*Eucalyptus globulus*).

All the oils were checked for authenticity by a pharmacognosist. The oils were not diluted and not altered chemically by any solvent or processing.

Agar Well Diffusion Assay

Agar well diffusion assay was the key process used to evaluate the antimicrobial potential of the oils. Petri dishes containing 18 ml of blood agar were inoculated with approximately 100 µl of *S. mutans* strain using swab technique. Wells of 8 mm diameter were cut into solidified agar media using a sterilized standard device. 100 µl of the oils were poured in the wells and the plates were incubated at 37°C for 48 hours. To ensure the consistency of all findings, the experiment was performed and repeated under strict aseptic conditions. The antibacterial activity of the solutions were expressed in terms of the mean diameters of

zone of inhibition (in mm) produced at the end of the incubation period.

Statistical Analysis

The mean and standard deviation of the diameter of inhibition zone were calculated. Statistical significance was measured by using one-way ANOVA followed by Tukey's post-hoc test. Analysis of data was done by SPSS (statistical package for social sciences) version 12.0.

RESULTS

Cinnamon oil showed highest activity against *Streptococcus mutans* followed by lemongrass oil and cedarwood oil. Eucalyptus oil showed the least antibacterial activity. Wintergreen oil, lime oil, peppermint oil and spearmint oil showed no antibacterial activity. The differences between each of the essential oils were significant at the end of 48 hours. Table 1 shows antibacterial activity (zone of inhibition in millimeters) of the essential oils against *S. mutans* at 48 hours respectively. Graph 1 shows the differences in antibacterial activity between the essential oils 48 hours.

DISCUSSION

Essential oils have been tested for *in vivo* and *in vitro* antimicrobial activity and some have demonstrated to be possessing potential antimicrobial potential. Their mechanism of action appears to be predominantly on the cell membrane by disrupting its structure thereby causing cell leakage and cell death, secondary actions maybe by blocking the membrane synthesis; and inhibition of cellular respiration.³ They readily penetrate into the cell membrane and exert their biological effect because of high volatility and lipophilicity of the essential oils.⁶

Usually, ethanol is added to the essential oils as solvent to enhance the volatility and aromatic properties.⁷ To avoid the possible effect of the solvent on the antimicrobial property, commercially available essential oils that were nondiluted and chemically not altered by any solvent or processing, were used in this study.

Table 1: Antibacterial activity (zone of inhibition in millimeters) of the essential oils against *S. mutans* at 48 hours

Sl No	Essential oil	Mean (mm)	Standard deviation	ANOVA	Tukey's post-hoc
1	Wintergreen oil	0.00	0.00	p-value = 0.001(HS)	3 > 6 > 7 > 8 > 9 = 1 = 2 = 4 = 5
2	Lime oil	0.00	0.00		
3	Cinnamon oil	12.51	0.66		
4	Spearmint oil	0.00	0.00		
5	Peppermint oil	0.00	0.00		
6	Lemongrass oil	10.07	0.71		
7	Cedarwood oil	7.42	0.53		
8	Clove oil	6.6	0.49		
9	Eucalyptus oil	3.44	0.37		

The essential oils that were used in this study were the ones commercially available in the local market. Four of the nine essential oils tested in this study demonstrated effective antibacterial activity against *S. mutans*, of which cinnamon oil was found to be the most effective. The antimicrobial activity of cinnamon oil may be explained by its active substances cinnamic aldehyde and eugenol.⁸ Antibacterial activity of cinnamon has been shown in studies conducted by Prabuseenivasan et al² in Chennai in 2006, Kalemba et al⁹ in 2006 in Poland and Kamal Rai Aneja et al¹⁰ in Haryana, India in 2009.

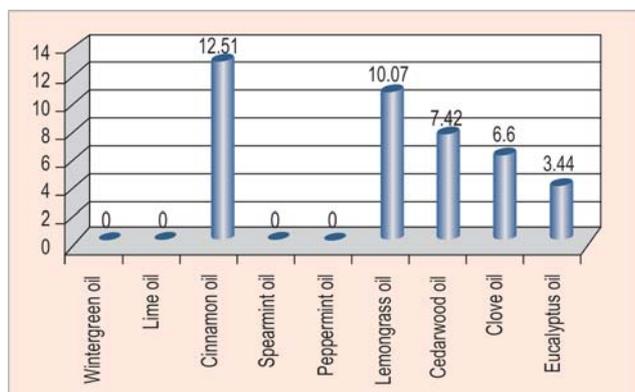
The main chemical components of lemongrass oil are citronellal, limonene and citral to which the antimicrobial activity might be attributed.⁶ A study conducted by Hammer et al¹¹ in 1999 explored the antibacterial activity of lemongrass oil and found it effective against a wide variety of bacteria.

The chemical components of cedarwood oil are a-cedrene, b-cedrene, sesquiterpenes and cedrol are thought to possess antimicrobial activity. Antimicrobial activity of cedarwood has been proved by a study conducted by Clark et al in 1990 in USA.¹²

Components of clove oil are eugenol, eugenol acetate, isoeugenol and caryophyllene. Clove oil is useful for its disinfecting properties, relieving of pain, especially toothache, arthritis and rheumatism. Studies conducted by Dorman et al¹³ in UK in 2000 and Betoni et al¹⁴ in Brazil in 2006 have proved the antimicrobial potential of clove oil.

Components of eucalyptus oil that are thought to be responsible for its antibacterial property are pinene, limonene, terpinenol, piperitone and globulol. Antimicrobial potential of eucalyptus oil has been proved in studies conducted by Sattari M et al¹⁵ in Iran in 2009 and Filoche SK et al¹⁶ in New Zealand in 2005.

In the agar well diffusion assay, the size of the effective inhibitory zone depends on the solubility and diffusion



Graph 1: Antibacterial activity (zone of inhibition in millimeters) of the essential oils against *S. mutans* at 48 hours

characteristics of the substances being tested. This makes the comparison of the different oils difficult. Therefore, the results of this study may not directly reflect the extent of the antibacterial potential of these oils. However, as these effective zones were clearly visible, this is a proof of their antibacterial efficacy.

Studies have also been conducted to assess the antifungal effectiveness of these essential oils against candida wherein all the oils used in the study have demonstrated varying amount of antifungal effectiveness.^{3,5,7,8,17,18}

Thus, as some of these essential oils have proved to have antimicrobial efficacy against oral bacteria and fungi *in vitro*, *in vivo* studies containing these oils are recommended so as to allow these essential oils to be incorporated within formulations marketed against oral infections.

CONCLUSION

Cinnamon oil, lemongrass oil, cedarwood oil, clove oil and eucalyptus oil exhibit antibacterial property against *S. mutans*. Cinnamon oil showed highest activity against *Streptococcus mutans* followed by lemongrass oil and cedarwood oil. Wintergreen oil, lime oil, peppermint oil and spearmint oil showed no antibacterial activity.

CLINICAL SIGNIFICANCE

The use of essential oils against oral pathogens can be a viable alternative to other antimicrobial agents as they offer a cheap and effective module used in the control of bacteria responsible for oral infections. There is a need to conduct *in vivo* studies to ascertain the safety and acceptability of these products.

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