

ORIGINAL RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF COMMERCIALY AVAILABLE ESSENTIAL OILS AGAINST CANDIDA ALBICANS - AN IN VITRO STUDY

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ABSTRACT

Background: 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. The objective of the study was to study the antifungal activity of nine commercially available essential oils against *Candida albicans* in vitro and to compare the antifungal 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. *Candida albicans* was inoculated at 37°C and seeded on Sabourauds dextrose agar medium. Sterilized filter paper saturated with the oils were placed on the seeded agar plates and inoculated for 48 hours. Zone of inhibition were measured around the filter paper in millimeters with vernier caliper.

Results: Cinnamon oil showed highest activity against *Candida albicans* followed by Lemongrass oil and cedarwood oil. Wintergreen oil, lime oil, peppermint oil and spearmint oil showed no antifungal activity.

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

Key words: *Candida albicans*, anti fungal property, disc diffusion, essential oils.

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INTRODUCTION:

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.^[1]

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. World Health Organisation (WHO) noted that majority of the world's population depends on traditional medicine for primary care.

Medicinal and aromatic plants which are widely used as medicines, constitute a major source of natural organic compounds.

Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticides and antioxidant properties. Some oils have been used in cancer treatment. Some other oils have been used as food preservative, aroma therapy and fragrance industries. Essential oils are a rich source of biological active compounds.^[2,3]

There has been an increased interest in looking at antimicrobial properties from extracts of aromatic plants particularly essential oils. Therefore it is reasonable to expect a variety of plant compounds, in their 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, barks, herbs, wood, fruits and roots). An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market. Essential oils are complex mixtures comprising of many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of them constitutes and contributes to the beneficial or adverse effects.^[2]

Hence this study was conducted with the objective of assessing the antifungal activity of nine commercially available essential oils against *Candida albicans* in vitro and to compare the antifungal activity of these materials.

MATERIALS AND METHODS:

Collection of materials: The following pure essential oils commercially available in Davangere city were selected for the study: wintergreen oil, lime oil, cinnamon oil, spearmint oil, peppermint oil, lemongrass oil, cedarwood oil, clove oil and eucalyptus oil. All oils were non diluted and chemically not altered by any solvent or processing. Pure cultures of *Candida albicans* were obtained from Department of Microbiology, Jagadguru Jayadeva Murugarajendra Medical College, Davangere.

Antimicrobial tests: The Disc diffusion method was used to determine the antimicrobial activity. A volume of tested microorganisms grown in Sabourauds dextrose agar (at 37°C for 24 hours), were inoculated on Sabourauds agar growth media, and then spread on the entire surface of the dish using a sterile cotton swab. Sterile paper disc, 6 mm. diameter with absorbed oil (30 µl /disc) was placed onto the agar at certain intervals by pressing gently. After the plates were incubated at 37±0.1°C for 48 hours, the inhibition zones around the discs where no growth occurred were measured on all four sides of the filter paper in millimeters using a vernier caliper. The experiments were repeated and ten readings were taken per essential oil used.

Statistical analysis:

The mean and standard deviation of the zones of inhibition around the discs were tabulated. Statistical significance was measured by using one way ANOVA followed by Tukey's post hoc test. p value <0.05 was considered statistically significant. The analysis of data was done by SPSS Version 16.0 (Statistical Package Software).

RESULTS:

Cinnamon oil showed highest activity against *Candida albicans* followed by Lemongrass oil and cedarwood oil. Eucalyptus oil showed the least antifungal activity. Wintergreen oil, lime oil, peppermint oil and spearmint oil showed no antifungal

activity. The differences between each of the essential oils were significant at the end of 48 hours.

Table I; shows antifungal activity (zone of inhibition in millimeters) of the essential oils against *C. albicans* at 48 hours respectively.

Graph I; shows the differences in antifungal activity between the essential oils 48 hours.

DISCUSSION:

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 antifungal activity against *C. albicans*, of which cinnamon oil was found to be the most effective.

Numerous essential oils have been tested for in vivo and in vitro antifungal activity and some demonstrated to be potential antifungal agents. Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing cell leakage and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration. Because of high volatility and lipophilicity of the essential oils, they are readily attached to penetrate into the cell membrane and exert their biological effect.^[3]

Usually ethanol is added to the essential oils as solvent to enhance the volatility and aromatic properties.^[4] Thus to avoid the possible effect of the solvent on the antimicrobial property, commercially available essential oils that were non diluted and chemically not altered by any solvent or processing were used in this study.

According to this study, cinnamon oil was found to be the most effective against *C. albicans*. Numerous studies have shown similar results.^[1,4,5,6,7] The antimicrobial activity of cinnamon may be explained by its volatile oil components. The most important active substance found in cinnamon oil are cinnamic aldehyde and eugenol.^[6]

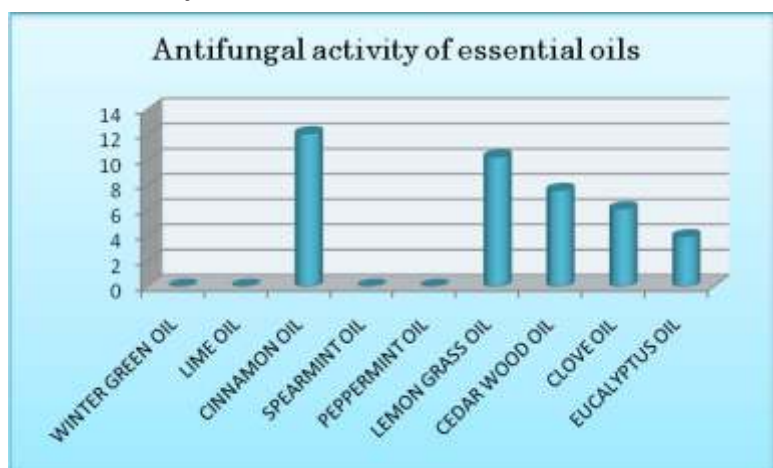
This study also demonstrated the anti fungal properties of lemongrass oil, cedarwood oil, clove oil and eucalyptus oil. The results obtained are comparable to various similar studies conducted.^[3,4,5,6]

In the agar disc diffusion tests, the size of the effective inhibitory zone depends on the solubility and diffusion 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 antifungal potential of these oils. However, as these effective zones were clearly visible, this is a proof of their antifungal efficacy.

Table I shows antifungal activity (zone of inhibition in millimeters) of the essential oils against *C. albicans* at 48 hours respectively.

Sl. No.	ESSENTIAL OIL	MEAN	STANDARD DEVIATION	ANOVA	TUKEY'S POST HOC
1	WINTER GREEN OIL	.00	.00	F value = 1294.07 p value = 0.001 (HS)	3>6>7>8>9 =1=2=4=5
2	LIME OIL	.00	.00		
3	CINNAMON OIL	12.11	.66		
4	SPEARMINT OIL	.00	.00		
5	PEPPERMINT OIL	.00	.00		
6	LEMON GRASS OIL	10.27	.71		
7	CEDAR WOOD OIL	7.62	.53		
8	CLOVE OIL	6.16	.49		
9	EUCALYPTUS OIL	3.94	.37		

Graph I: shows the differences in antifungal activity between the essential oils at 48 hours.



Studies have also been conducted to assess the antibacterial effectiveness of these essential oils against oral bacteria wherein all the oils used in the study have demonstrated varying amount of antibacterial effectiveness.^[5,6,7,8]

Thus as some 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 antifungal property against *C. albicans*. Thus the use of these essential oils against *Candida* can be a viable alternative to other antifungal agents as these offers an effective module used in the control of both bacteria and yeasts responsible for oral infections such as caries, periodontal disease and stomatitis.

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