ANTIBACTERIAL EFFECT OF LEMONGRASS OIL ON ORAL MICROORGANISMS: AN IN VITRO STUDY
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ABSTRACT
Aim of the study was to know antibacterial effect of lemongrass oil on oral microorganisms: *Streptococcus mutans*, *Prevotella intermedia* and *Porphyromonas gingivalis*. The disc diffusion test for the *Streptococcus mutans*, was carried out at various concentrations of lemongrass oil as neat, 1:2, 1:5, 1:10, 1:20 and 1:25. The punch hole diffusion technique was used for *Prevotella intermedia* and *Porphyromonas gingivalis* at various concentrations of lemongrass oil as neat, 1:2, 1:5, 1:10, 1:20 and 1:25. The disc diffusion test for *Streptococcus mutans* showed the zone of inhibition of >40mm, >40mm, >20mm, 14mm, 14mm at the concentration of neat, 1:2, 1:5, 1:10, 1:20 respectively. The punch hole diffusion technique used for *Prevotella intermedia* and *Porphyromonas gingivalis* showed Zone of inhibition of >30mm, >20mm, >10mm at the concentrations of neat, 1:2, 1:5, and 1:10 respectively. The study has demonstrated that essential oil of lemongrass has significant antimicrobial potential against oral microorganisms *S. mutans*, *P. intermedia* and *P. gingivalis*.

Keywords: Essential oil, oral microorganisms, antimicrobial effect.

INTRODUCTION
The antibacterial activity of various essential oil has formed the basis for their widespread application in dentistry. The use of Listerine mouthwash, containing thymol, menthol and eucalyptol, as antiseptic agent is well documented. Lemongrass oil is important essential oil, extracted from Lemon grass which belongs to the section of *Andropogon* called *Cymbopogon* of the family Germinae. The two of the major species are *Cymbopogon citrates* and *C. flexuosus*. It has plethora of medicinal uses. It is said have antibacterial, antifungal, antioxidant, anti-septic, astringent, anti-inflammatory, analgesic, antipyretic and carminative property. Because the herb has not been studied extensively, its effectiveness is based mainly on its centuries-old reputation as a folk remedy.

Exploring its antibacterial property specifically for oral microorganism can help us incorporate this essential oil in dentistry in particular as an adjunctive chemical agent against oral microorganisms. *Streptococcus mutans* is considered to be one of the most important the causative agent for dental caries. *Porphyromonas intermedia* and *Prevotella intermedia* are established periopathogens involved in periodontal disease. Thus in this study the invito antimicrobial effect of lemongrass essential oil against the three microorganism was evaluated.

MATERIAL AND METHODS
The pure lemongrass oil (*Cymbopogon Citratus*) was obtained from Falcon company, Bangalore, India which is a member of Basic chemicals, pharmaceuticals and cosmetics export promotion council, Government of India and registered with the United States Food And Drug Administration, USA. The test microorganisms: *Streptococcus aureus*, *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* were obtained from the clinical isolates.

Disc diffusion test
Nutrient agar was inoculated with a microbial cell suspension (200 µl in 20ml of medium) and poured into sterile petri dishes. Sterile filter paper discs 6 mm in diameter were impregnated with 20 µl of each oil concentration (neat, 1:2, 1:5, 1:10, 1:20, 1:25), which were prepared using the same solvents, then sterilized via pasteurization and membrane filtration and placed on the inoculated agar surface. After pre-incubation for 2 h in a refrigerator the plates were incubated overnight at 37 °C for 18-24 h. At the end of the incubation period antimicrobial activity was evaluated by measuring the zones of inhibition.

Punch hole diffusion test
Modified nutrient agar was used as a medium. The plates were inoculated with the test organisms. The holes were punched out in the plates. Three drops of the various concentrations of essential oil (neat, 1:2, 1:5, 1:10, 1:20, 1:25) are dropped into the punched holes using pipettes. The plates were incubated for 18-24 h in anaerobic hood. At the end of the incubation period antimicrobial activity was evaluated by measuring the zones of inhibition.

RESULTS
The zones of inhibition representing the antimicrobial activity of lemongrass essential oil on *Streptococcus mutans*, *Prevotella intermedia* and *Porphyromonas gingivalis* are presented in the table 1-3 and fig 1-3. The zones of inhibition were measured in millimetres. The zone of inhibitions for *S. mutans* were >40mm, >40mm, >20mm, 14mm, 14mm at the respective concentrations of neat, 1:2, 1:5, 1:10 and 1:20. The zones of inhibition for *P. intermedia* were >40mm, >30mm, >10mm and >5mm at the respective concentrations of neat, 1:2, 1:5 and 1:10.

The zones of inhibition for *P. gingivalis* were >40mm, >30mm, >10mm and >5mm at the respective concentrations of neat, 1:2, 1:5 and 1:10.
DISCUSSION

Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. 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.

The vitro study was done to find out the effectiveness of the lemongrass oil against oral microorganisms: S. mutans, P. intermedia, P. gingivalis. The study showed that the organisms were sensitive to lemongrass oil at various concentrations of neat, 1:2, 1:5, 1:10, 1:20 and 1:25. The S. mutans was inhibited at all the concentration of except at 1:25. The P. intermedia and P. gingivalis were inhibited at the concentrations of neat, 1:2, 1:15, 1:10. No zones of inhibitions were found at concentrations of 1:20 and 1:25. These results indicate that lemongrass oil can be tried as an alternative adjunctive chemical agent against oral microorganisms.

This is one of the first invitro studies where the antimicrobial effect of lemongrass oil was tested against the oral microorganisms in particular the two important periopathogens P. intermedia and P. gingivalis. Various other invitro studies have been done earlier for testing the antimicrobial effect of lemongrass oil on many organisms involved in systemic diseases.

Akin-Osanaiya BC, Agbaji AS, and Dakare MA studied the antimicrobial potentials of essential oils and extracts from the leaves of Cymbopogon citrates (lemon grass) and Eucalyptus against Salmonella typhi, Staphylococcus aureous and E. coli. Zone of inhibition measurement showed that eucalyptus oil was effective against Staphylococcus aureous and E. coli. Lemongrass oil was observed to possess highest antimicrobial activity against all three microorganisms. The punch hole diffusion technique was used. Without dilution lemongrass oil almost showed no growth. The aqueous extracts were not found to be effective.

Prabuseenivasan S et al. studied the in vitro antibacterial activity of few plant essential oils by the disk diffusion method. The result obtained for lemongrass oil for P. vulgaris was 14.6 ± 0.7, 12.1 ± 0.2 and 9.5 ± 0.5 at 1:1, 1:5 and 1:10 dilution and for P. aureus was 23.4 ± 1, 19.6 ± 0.5, 9.1 ± 0.5 at 1:1, 1:5 and 1:10 dilution.

K.A. Hamme et al. studied the antimicrobial activity of essential oils and plant extracts. They found that Lemongrass, oregano and bay inhibited all organisms at ≤2-0% (v/v). The organisms tested in the study were Staphylococcus aureus, Escherichia coli, Candida albicans organisms.

Antifungal and antiviral activities of lemongrass oil against candida species and HSV type 1 respectively have also shown positive results. Lemongrass oil completely inhibited the HSV 1 replication at concentration of 0.1%. These studies are noteworthy as candida species and HSV 1 are involved in oral diseases.

CONCLUSION

The present study has demonstrated that essential oil of lemongrass has significant antimicrobial potential against oral microorganisms S. mutans, P. intermedia and P. gingivalis.

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