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Comparative Study of the Antimicrobial Activity of Clove Oil and Clove Extract on Oral Pathogens

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ABSTRACT

Objective
The main objective of the present study was to do the comparative study of clove oil and clove extract on the oral micro-biota causing dental caries and also to assess the antifungal activity.

Materials and Methods
The antimicrobial activity of clove oil and clove extract was assessed against *Halobacterium sp.*, *Lactobacillus sp.*, *Pseudomonas sp.*, *Micrococcus sp.* and *Streptococcus mutans* (major causative bacteria of dental plaque) by the paper disc diffusion method. For each extract three replicate trials were conducted against each organism. The antifungal activity of clove oil and extract was also assessed against seven fungal species (*Aspergillus niger*, *A. fumigatus*, *Aspergillus sp.*, *Alternaria sp.*, *Rhizomucor sp.*, *Rhizopus sp.* and *Penicillium sp.*) by agar disc diffusion method.

Results
Both clove oil and clove extract was found to exhibit broad spectrum of antibacterial activity inhibiting all the ten test bacterial species involved in dental caries. Clove oil produced maximum inhibition zone of diameter (IZD) against themajor causative bacteria of dental plaque as compared to clove extract, thereby, showing that clove oil possesses strong bactericidal activity against oral pathogens. The highest inhibition zone of diameter was observed by clove oil as compared to clove extract against the test fungal species

Conclusion
The clove oil has the potential to be used as a natural antibacterial agent for oral pathogens.

Keywords
Syzygiumaromaticum; Clove extract; Clove oil; Antimicrobial; Oral pathogens.

INTRODUCTION

Dental caries, a chronic disease is unique among humans and is one of the most common important global oral health problems in the world today. Dental caries is caused due to the destruction of dental hard acellular tissue by acidic by-products from the bacterial fermentation of dietary carbohydrates and sugars. It progresses slowly in most of the people which results from an ecological imbalance in the equilibrium between tooth minerals and oral biofilms. This is characterized by microbial activity, resulting in fluctuations in plaque pH due to bacterial acid production, buffering action from saliva and the surrounding tooth structure. The microbial community of caries is diverse and contains many facultative and obligate-anaerobic bacteria. *Streptococcus mutans* isan important etiologic agent in dental caries. Dental caries can affect the humans in various ways such as presence of tooth pain, infection or dysfunction of the stomatognathic system which can limit the necessary ingestion of energetic foods, affecting the growth in children and adults including their learning, communication skills and recreational activities.[1]

Gum disease involves bacterial growth and production of metabolic substances that gradually destroy the tissue surrounding and supporting the teeth. Oral cavity pathogens include *Streptococcus mutans, Streptococcus salivarius, Halobacterium sp.*, *Veilonella sp.* etc. These bacteria grow and attack the tissues causing gingivitis, char-

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acterized by inflamed gums that bleed easily. The causative bacteria reside in plaque, the deposit that forms on the base of the teeth and hardens to form tartar. Poor oral hygiene is the major cause of gum disease.[2] Lifestyle, nutrition and ageing affect the immune response and increase the risk of gum disease.

In the present study, we have compared the antimicrobial activity of clove oil and clove extract on oral microbiota. The major difference between the two is mainly in the method of their extraction. Clove oil is extracted using steam distillation method (Clevenger Apparatus in lab) while clove extract is obtained through decoctionby immersing in a suitable organic solvent or *via* super critical fluid extraction.

MATERIALS AND METHODS

All chemicals used were of analytical-reagent grade and obtained from E. Merck (Mumbai, India). Readymade clove buds and clove oil was purchased from local market of Meerut (Uttar Pradesh, India).

Bacterial Strains

Ten bacterial strains 6 Gram positive (*Streptococcus mutans*, *Streptococcus salivarius*, *Lactobacillus spp*., *Bacillus spp*., *Micrococcus spp*., *Staphylococcus aureus*) and 4 Gram negative (*Halobacterium spp*., *Veilonella spp*., *Pseudomonas aeruginosa*, *Pseudomonas spp*.,) bacterial species that were commonly involved in dental caries were selected for the study. The bacterial stock cultures were obtained from the culture collection unit of Department of Microbiology, C.C.S University, Meerut, India. The stock on nutrient agar medium (Hi Media, Mumbai, India) was incubated for 24 h at 37 °C following refrigeration storage at 4°C until required for sensitivity testing.

Determination of Antibacterial Activity

Antimicrobial activity of the essential oil of clove and its extract was evaluated by the paper disc diffusion method.[3] Paper discs impregnated with 50 µL of a solution of 10mg/mL of chlorhexidine (positive control) as standard antimicrobials for dental caries were used for comparison. Sterile dimethyl sulfoxide (DMSO) served as negative control. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc. For each extract three replicate trials were conducted against each organism.

Determination of Antifungal Activity

The antifungal activity of clove oil and extract was determined by the method of Gupta et al[4] and Fiori et al[5] with minor modifications.

RESULTS

Table 1 shows the antimicrobial activity of clove oil and clove extract on the indigenous oral microbiota that cause dental caries. Both the clove oil and its extract were effective against both Gram positive and Gram negative bacteria.

However, the clove oil was more effective as compared to clove extract against all the test bacterial species. The highest inhibition zone was produced against *Halobacterium spp*. and *Lactobacillus spp*. with an IZD of 19.0 mm each. The second highest inhibition zone was produced against *Pseudomonas spp*. with an IZD of 18.0 followed by *Micrococcus spp*., *Streptococcus mutans*, *Staphylococcus aureus* and *Veilonella spp*. with an IZD of 17.0, 17.0, 16.0 and 15.0 mm respectively. The clove extract produced an IZD of 15.0 mm against *Streptococcus mutans*. Chlorhexidine, on the other hand was lesseffective producing an inhibition zone of diameter 13 mm. Amongst the Gram negative bacteria, the cloveoil showed highest activity against *Pseudomona sspp*. with an IZD of 18.0 mm while clove extract producedan IZD of 16.0 mm against *Pseudomonas spp*.

Table 2 shows the antifungal activity of clove oil and clove extract on the food spoilage fungi. Though both the clove oil and its extract inhibited all the tested fungi but clove oil again showed much better antagonistic activity than its extract counterpart. The oil produced the widest IZD against Aspergillus niger with an IZD of 42 mm followed by activity against Rhizopussp. and *Penicillium sp*. with an IZD of 40 mm each respectively. The

Table 1. *Comparison in Inhibition Zone Diameter (IZD) (mm) by Clove Oil and Clove Extract on Indigenous Oral Microbiota on Mueller-Hinton Agar Medium at 37 °C for 24 h;Volume of Oil in each Well=50 µL)*

S. No.	Bacteria	Clove Oil (mm)	Clove Extract (mm)	Chlorhexidine (+ve C) (mm)	DMSO
1.	*Bacillus sp.*	14.0	13.0	12.0	Nil
2.	*Halobacterium sp.*	19.0	15.0	14.0	Nil
3.	*Lactobacillus sp.*	19.0	14.0	15.0	Nil
4.	*Micrococcus sp.*	17.0	15.0	15.0	Nil
5.	*Pseudomonas aeruginosa*	15.0	14.0	13.0	Nil
6.	*Pseudomonas sp.*	18.0	16.0	15.0	Nil
7.	*Staphylococcus aureus*	16.0	13.0	14.0	Nil
8.	*Streptococcus mutans*	17.0	15.0	13.0	Nil
9.	*Streptococcus salivarius*	9.0	0.0	8.0	Nil
10.	*Veilonella sp.*	15.0	13.0	12.0	Nil

Table 2. *Comparison in Zones of Inhibition (mm) by Clove Ail and Clove Extract on Food Spoilage Fungi on Mueller-Hinton Agar Medium at 37 °C for 24 h;Volume of Oil in each Well=50 µL)*

S. No.	Fungus	Clove Oil (mm)	Clove Extract (mm)	Sodium Propionate (+ve C)	DMSO (-ve C)
1.	*Aspergillus niger*	42.0	27.0	25.0	Nil
2.	*Aspergillus fumigatus*	30.0	20.0	19.0	Nil
3.	*Aspergillus sp.*	36.0	21.0	20.0	Nil
4.	*Alternaria sp.*	28.0	30.0	22.0	Nil
5.	*Rhizomucor sp.*	35.0	26.0	21.0	Nil
6.	*Rhizopus sp.*	40.0	29.0	25.0	Nil
7.	*Penicillium sp.*	40.0	22.0	18.0	Nil

clove extract produced widest inhibition zone against *Alternaria sp*. followed by *Rhizopus sp*. The commonly used antibiotic chloramphenicol was used as positive control in the antifungal assay.

DISCUSSION

From this investigation, it was observed that both clove oil and its extractare active against both groups of bacteria (oral pathogens) and fungi though clove oilwas proved to be more effective than its extract counterpart.

Syzygiumaromaticum (Family-Myrtaceae) contains many compounds such as eugenol which is considered as one of essential component of clove oil and is known to possess antimicrobial activity against many pathogens. The other chemical constituents are eugenol acetate 4- allyl-2-methoxyphnol acetate β-caryophyllene; trans – (1R, 9S)-8-methylene-4, 11, 11-, bicycloundec-4-ene trimethylbicycloundec-4-ene, and 60-90% of another secondary compounds.[6]

Clove oil is one of the essential oil commonly used in seasoning of food. Antimicrobial activity is mainly attributable to eugenol, oleic acid and lipids present in it. In a similar study using oil extracts on dental caries causing microorganisms and found the zones of inhibition produced by these oil extracts ranged from of 14.7 mm to 33.7 mm which were comparable to the present study.[7]

The high-levels of eugenol contained in clove essential oil are responsible for its strong biological and antimicrobial activities. It is well known that both eugenol and clove essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast.[6,8]

The otherbioactive compounds present in clove are kaempferol and vanilic acid.[9] Chemically, the major bioactive constituents in clove belong to the group of secondary metabolites such as tannins, alkaloids and phenols, which are responsible for their antimicrobial[10] and antifungal activity.[9,11]

Due to medicinal properties of *S. aromaticum*, it is used in drugs for gums and toothache.[12] The oil of *S. aromaticum* has inhibitory activity against bacteria, fungi and insect repellent[13] and it is also used as an analgesic and a natural antiseptic in dentistry to reduce dental pain.[14]

CONCLUSION

In conclusion,clove oil was found to be a much better antagonistic agent, exhibiting broad range of antimicrobial activity against the microbes causing dental caries than clove extract and chlorhexidine. The antifungal effect of clove oil was also found to be more promising as compared to clove extract. Hence, it represents an alternative source of natural antimicrobial substances for use in chemotherapeutic agents.

Thus, clove oil has the potential to be used in oral dentifrices, mouthwashes, topical gels etc. However, future research efforts are needed for the evaluation of quality and efficacy of the plant products for their regular use in the oral hygiene products. It must be converted to viable form for daily use in toothpastes and mouth rinses. Further studies on the safety and efficacy of such products must be carried out to establish whether they offer therapeutic benefits, either alone or in combination with conventional therapies that can help to reduce the overall burden of oral diseases worldwide.

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None.

ETHICAL ISSUES

There is none to be declared.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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