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Original Article

Comparative Evaluation of the Antimicrobial Efficacy of a Herbal Mouthwash and Chlorhexidine Mouthwash on Oral Pathogens: An *in vitro* Study

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Abstract

Introduction: Tea tree oil (TTO) is an ethereal oil widely used due to its curative nature. The topical use of TTO has shown great results due to its antibacterial as well as antiviral properties.

Purpose: This study was carried out to see the effect of TTO on oral pathogens.

Materials and Methods: Blood and MacConkey agar plates were prepared to inoculated the test organism, that is, *Streptococcus mutans*. Streak plate method is used in this study for the antimicrobial assessment. The plates were incubated at 37°C for 24–48 h. The readings were subjected to statistical analysis using the Wilcoxon signed-rank test and Chi-square test. *P*-value was considered significant at $P < 0.05$.

Results: Maximum antibacterial efficacy was exhibited by 2% chlorhexidine (CHX), followed by herbal mouthwash and least was shown by placebo.

Conclusion: According to this study, herbal mouthwash can be used as an alternative to CHX mouthwash although long-term *in vivo* studies are needed.

Key words: Antibacterial efficacy, *Melaleuca alternifolia*, *Streptococcus mutans*

INTRODUCTION

Tea tree (*Melaleuca alternifolia*) oil is well known and used as it has numerous therapeutic properties.[1] Tea tree oil (TTO) also known as melaleuca oil, is an essential oil obtained by the process of steam distillation of leaves and terminal branches of tea tree plant. TTO is volatile in nature and is made up of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. Terpenes are aromatic hydrocarbons and are contemplated as polymers of isoprene, with chemical formula C_5H_8. The antimicrobial activity of TTO is attributed mainly to terpinen-4-ol. The minimum inhibitory concentration of TTO against *Streptococcus mutans* and *Streptococcus salivarius* is equivalent to 1940.16 μg/ml and 3977.34 μg/ml, respectively.[2] Various studies conducted in the past have shown antimicrobial properties of *M. alternifolia*.[3-6] During the initial stage of development, biofilm comprises mainly of streptococci and actinomyces which acts as commensals. However, further colonization leads to rapid increase in lactobacillus species, or Gram-negative anaerobes resulting in the beginning and development of dental caries and periodontal diseases.[7] Ethereal oils have abundant biologically active compounds.[8] Clinically, TTO has shown the capability to put a stop on the hospital acquired infections caused by Gram-positive as well as Gram-negative microorganisms.[9] Due to the presence of antimicrobial terpenes, *M. alternifolia* is well known to fight against acne. The essential oil is popularly used for the treatment of dermatophyte infection or tinea, tinea pedius, and fungal nail infections.

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Increasing evidence of microbicidal, fungicidal, antiviral, and anti-inflammatory effects of *M. alternifolial* in various *in vitro* studies presents a need to determine its efficacy as a mouthwash on oral pathogens. Hence, this study was conducted aimed to discover and compare the result of an herbal mouth wash preparation containing TTO in controlling microbial growth with chlorhexidine (CHX) mouthwash which is considered as the gold standard.

MATERIALS AND METHODS

An *in vitro* study was executed in the department of public health dentistry. The Institutional Ethical Committee Clearance (Ref. No: TMDCRC/IEC/SS/19-20/PHD01) and informed consent were obtained from all the participants before commencement of the study. Forty samples of saliva were taken to determine the antimicrobial effect of herbal (tea tree) mouthwash on bacterial strains.

Herbal mouthwash was prepared using tea tree essential oil (pure TTO) purchased from Moradabad (India), baking soda, 80% alcohol, methylparabene, sodium chloride, propylparabene, peppermint flavor, and distilled water to study the antibacterial activity. Various aerobic and anaerobic strains of bacteria that are associated with distinguishable phases of periodontal disease were selected to study the antimicrobial effect of the herbal preparation. To compare the antimicrobial efficacy, three groups were formed Group 1, Group 2, and Group 3 representing CHX mouthwash, tea tree mouthwash, and placebo, respectively. Each saliva sample collected was mixed with three mouthwashes placed in different test tubes. To determine the antimicrobial activity against aerobic and anaerobic bacteria, the samples from test tubes were inoculated on blood and MacConkey agar plates. Then, the samples were incubated at a temperature of 37°C for 24-48 hours.

Inclusion Criteria

The following criteria were included in the study:

- Subjects aged between 20 and 30 years
- Subjects having at least 22 teeth were selected.

Exclusion Criteria

The following criteria were excluded from the study:

- Subjects who showed allergic reactions to test products were excluded
- Any systemic illness
- Antibiotic and periodontal therapy in the past month
- Subjects with destructive periodontal disease.

Intervention

Preparation of tea tree/herbal mouth rinse: Herbal mouth wash was prepared by dissolving 1 ml of TTO in 5 ml of 80% alcohol. In second beaker, 20 ml of distilled water was taken and 0.5 ml of methylparaben and 1 ml of propylparaben were added with constant stir. After that, 1 g of baking soda and 1.5 g of sodium chloride were added. In second beaker, peppermint flavoring agent was dissolved in 10 ml of water. Alcoholic solution of TTO was added in the second beaker and stirred for 10 min. Finally, the preparation was filtered with muslin cloth.

Preparation of placebo mouth rinse: Placebo mouth rinse was prepared by dissolving peppermint flavoring agent in distilled water.

Preparation of CHX: Commercially available 0.2% CHX mouth rinse was used.

Statistical Analysis

The results were tabulated and statistically analyzed using Wilcoxon signed-rank test and Chi-square test. $P < 0.05$ was considered significant.

RESULTS

Table 1 shows that the qualitative count of bacteria detected by both the mouthwashes was significantly different. The qualitative count of bacteria with CHX mouthwash was none in 36 samples. However, Herbal Mouthwash was able to detect moderate and heavy bacterial growth in two samples, respectively. The qualitative count of bacteria was mild in 32 out of 40 samples with herbal mouthwash. The Chi-square test of association shows that there is an association between the qualitative bacterial count and the kind of mouthwash used as the $P < 0.05$.

Table 2 shows the difference between the two mouthwashes to detect the qualitative count of bacterial

Table 1: Qualitative bacterial count by different mouthwashes

Crosstab						
Groups	Nil	Qualitative count				Total
		No Growth	Mild	Moderate	Heavy	
Group						
Chlorhexidine						
Count	4	36	0	0	0	40
% of Total	5.0	45.0	0.0	0.0	0.0	33.3
Herbal mouthwash						
Count	4	0	32	2	2	40
% of Total	5.0	0.0	40.0	2.5	2.5	33.3
Placebo						
Count	4	0	0	0	36	40
% of Total	5.0	0.0	0.0	0.0	0.0	33.3
Total						
Count	12	36	32	2	38	120
Chi-square (*P* value)			4.235 (0.037)			

growth. There were 4 cases where the qualitative count with Herbal Mouthwash is more than the qualitative count with CHX, while 36 samples where the qualitative count with Herbal Mouthwash is equal to the qualitative count with CHX. The Wilcoxon signed-rank test shows that there is a difference in the two mouthwashes in the detection of the qualitative count of bacterial growth as $P < 0.05$, herbal mouthwash was more efficient in the detection of the more moderate and heavy count of bacterial growth.

DISCUSSION

S. mutans is the most commonly isolated microorganism from the mouth. It possesses various virulence factors which enable it to survive and persist as a pathogen in the oral cavity. Hence, *S. mutans* was chosen as the test organism for this study and was obtained using the subculture method. Streak plate method was used in this study for antimicrobial assessment. The streak plate method is a rapid qualitative isolation method. This method is used for segregation into pure culture from a mixed population of microorganisms. In this study, the experiment was repeated 40 times making the results more reliable, and there were four dropouts due to contamination of the samples. In our study, we found that the qualitative growth of bacteria with CHX mouthwash was none in 36 samples. However, herbal mouthwash was able to detect moderate and heavy bacterial growth in two samples, respectively. The qualitative count of bacteria was mild in 32 out of 40 samples with herbal mouthwash. The qualitative count of bacteria was heavy in 36 out of 40 samples with a placebo as shown in [Figure 1]. CHX exhibited the best antibacterial efficacy followed by herbal mouthwash and least by placebo in our study as shown in [Figures 2-4] respectively, which is in support of previously published studies.

M. alternifolia is responsible for the disintegration and deprivation of membrane integrity and function by outflow of ions and the obstruction of respiration in bacterium. *M. alternifolia* shows broad-spectrum antimicrobic activity which can be chiefly ascribed to terpinen-4-ol.[10] In 2006, Carson *et al.*, Carson and Riley, and Cox *et al.* found that the *M. alternifolia* has shown obstructive results on bacteria with *Escherichia coli*.[1,11,12] In 2010, Fitzpatrick conducted a study on the effectiveness of TTO against five bacteria: *Bacillus subtilis*, *E. coli*, *Micrococcus roseus*, *Suillus luteus*, and *Serratia marcescens*. In his

Table 2: Difference between the two mouthwashes to detect qualitative bacterial growth

Ranks			
Qualitative count with herbal Mouthwash – qualitative count with Chlorhexidine	***n***	**Mean rank**	**Sum of ranks**
Negative ranks	0[a]	0.00	0.00
Positive ranks	4[b]	2.50	10.00
Ties	36[c]		
Total	40		

[a]Qualitative count with herbal mouthwash <qualitative count with chlorhexidine, [b]Qualitative count with herbal mouthwash >qualitative count with chlorhexidine, [c]Qualitative count with herbal mouthwash = qualitative count with chlorhexidinem, Z (*P* value)= −1.857 (0.034)

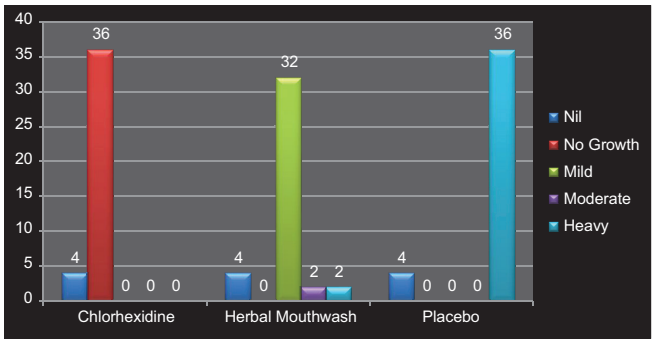

Figure 1: Bacterial growth in three different mouthwashes

Figure 2: Antimicrobial activity by chlorhexidine

Figure 3: Antimicrobial activity by herbal/tea tree mouthwash

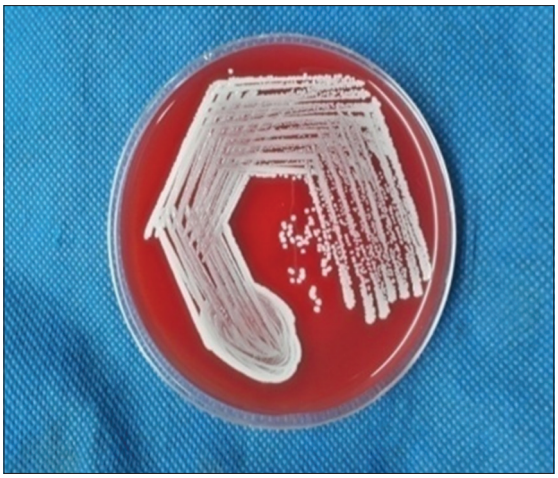

Figure 4: Antimicrobial activity by placebo

study, he found that TTO showed persistent preventive action in all bacteria, excluding *S. luteus*.[13] Results of our study show that TTO exhibits good antibacterial efficacy, which is in support of the study by Kamath *et al*., in 2013.[14] Studies like those conducted by Leite *et al*., in 2017, found that TTO presented antibacterial activity on oral microflora like *S. mutans*, *S. salivarius,* and Lactobacillus rhaminosus.

CONCLUSION

Based on the results of our study, it can be concluded that 2% CHX showed the maximum antibacterial activity. Herbal mouthwash demonstrated significant antibacterial activity in opposition to *S. mutans*. Thus, *M. alternifolia* can be employed as a substitute for CHX. Since herbal products are easily extracted and are cost-effective, this study opens new path for the use of herbal products. Further, preclinical and clinical trials are needed to be aware of the antimicrobial activity of numerous herbal products accessible and to encourage the unearthing of new natural resources.

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