Research Article

Comparative evaluation of antimicrobial efficiency of tea tree oil and chlorhexidine on *Streptococcus mutans*

S. Jayakeerthana, R. V. Geetha*

ABSTRACT

Introduction: *Streptococcus mutans* is the most common organism causing dental caries. Various chemotherapeutic agents are available that help in treating the bacteria, with each having their own merits and demerits. Tea tree oil is extracted from Mehknca leaves (Myrtaceae family). Cineole is a skin-irritating substance and terpinen-4-01 is presumed to be the most important antimicrobial agent of the tea tree oil. The aim of the study is to evaluate the antimicrobial efficiency of tea tree oil and chlorhexidine (CHX) on *S. mutans*. **Materials and Methods:** The antibacterial activity of tea tree oil was screened against *S. mutans* using agar well diffusion assay, and the zone of inhibition was recorded. **Result:** In this present study, the inhibition levels of tea tree oil and CHX were tested with *S. mutans*. The inhibition is different at different concentrations. However, it may also be noted that CHX is found to be more potent than tea tree oil in the majority of the concentrations. **Conclusion:** Tea tree oil has a significant antimicrobial effect compared to controlled group. The tea tree oil was as effective as an anticaries mouthwash as CHX.

KEY WORDS: Antimicrobial, *Streptococcus mutans,* Tree tea oil, Zone of inhibition

INTRODUCTION

Oral diseases such as dental caries and periodontal diseases are caused by microorganisms belonging to the resident microflora rather than by classic microbial pathogens.[1] Oral microbial flora is dominated by Gram-positive microorganisms, and hence dental plaque which is formed on the tooth surface contains Gram-positive cocci and bacilli.[2] With the exponential advancement in the field of dentistry, various preventive measures have emerged targeting the causative factors of the oral diseases.[3] Dental plaque which forms on the tooth surface exists in a state of biofilm which means the microorganisms present in the plaque live in communities and they are held together in a matrix. Plaque accumulation is one such factor which predisposes the individual to both dental caries and periodontal disease.[4-6] Salivary microfloras such as *Streptococcus mutans* and other predisposing factors lay an important role in the initiation and progression of dental diseases such as dental caries.[7,8] Chemotherapeutic and antimicrobial agents aiming at these predisposing factors, therefore, play a significant role in the prevention of these oral diseases and have a dramatic impact on improving the oral health of the individual.[9]

Among the plethora of oral hygiene products available, chlorhexidine (CHX) has been the mouthwash of choice due to its dramatic therapeutic effect, but its various side effects such as taste alteration, supragingival calculus formation, and desquamation of oral mucosa have restricted its usage in pediatric age group.[10] CHX, being one of the most popular mouth rinses, has shown to significantly reduce the level of oral *S. mutans*.[11] Moreover, it also causes extrinsic staining by attaching to the polyphenolic and tannin group of beverages such as tea and coffee. Alternative agents based on herbal extracts are, therefore, of particular interest. Tea tree oil is extracted from the tree *Melaleuca alternifolia* that grows in Australia and has been shown to have many beneficial medicinal uses as an antiseptic, antifungal, and antibacterial agent.[12] Studies indicate that *M. alternifolia* is extracted from the leaves and twigs by steam distillation, and the

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Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

***Corresponding author:** R. V. Geetha, Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India. Phone: +91-9710456203. E-mail: rvgeetha2015@gmail.com

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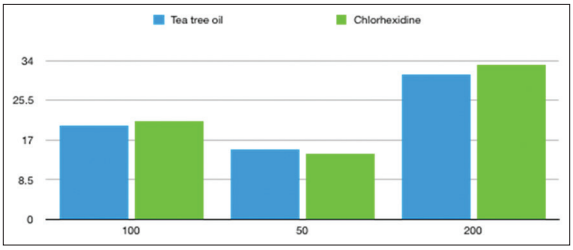

Figure 1: Antimicrobial activity of tree tea oil against *Streptococcus mutans* at different concentrations.

yield is about 1.8% and that the main chemical component to have antimicrobial activity in tea tree oil is attributed to terpinen-4-ol.[13] Hence, the aim of the present study is to evaluate and compare the effect of tea tree oil and CHX and when used as an anticaries mouthwash.

MATERIALS AND METHODS

Bacterial strain used was *S. mutans*. The organism was isolated using selective media Mutans-Sanguis agar and maintained in nutrient agar slope at 4°C in the Department of Microbiology, Saveetha Dental College.

Methodology

The tree tea oil was loaded onto sterile filter paper discs measuring 6 mm diameter in the following concentrations 50 µl, 100 µl, and 200 µl. The discs were dried and kept aseptically.

Screening of Antibacterial Activity (Disc Diffusion Technique)

Brain heart infusion medium was prepared for a subculture to check the viability of bacteria. 10 uL of each sample was then inoculated onto the medium with the help of sterile loops. The culture plates were then incubated for 24 h at 37°C. The colony forming units were then calculated, and the antimicrobial efficacy was thereafter determined. Disc diffusion assay was used to measure antibacterial activity. Various disk with different concentrations of 50, 100, and 200 was placed and control disk was placed and kept in an incubator after 24 h the zone of inhibition was calculated. Zone of inhibition was measured around the filter paper in millimeters with a Vernier caliper.

RESULTS

In this present study, the inhibition levels of tea tree oil and CHX were tested with *S. mutans* and the results were shown in Figure 1. When the concentration of tea tree oil and CHX was 50, the inhibition level of *S. mutans* was found to be 17 mm and 19 mm. When the concentration of tea tree oil and CHX was 100 µl, the inhibition level of *S. mutans* was found to be 15 mm and 13 mm. When the concentration of tea tree oil and CHX was 200, the inhibition level of *S. mutans* was found to be 29 mm and 33 mm.

DISCUSSION

In the present study, the inhibition levels of tea tree oil and CHX were tested with *S. mutans* species. From the above graph, it has been shown that the inhibition is different at different concentrations. However, it may also be noted that CHX is found to be more potent than tea tree oil in the majority of the concentrations. This factor has to be taken into consideration during preparation for oral care.

Groppo *et al*. conducted a study based on the comparision of the antimicrobial activity of garlic, tea tree oil, and CHX against oral microorganisms. Tea tree oil and garlic have been reported as an effective agent to be used as an alternative to CHX.[14] A bacterial lawn technique on agar plates for each tested bacterium has been carried out. In the results of the present study, it was reported that tea tree oil possess potent antibacterial action of the strains tested due to the presence of a significant zone of inhibition.[14] A study comparing agar and dilution techniques in broth presented statistical differences between these methods.[15,16] When evaluating the antimicrobial activity on planktonic cultures, the values for the minimum inhibitory concentration (MIC) of the tea tree oil, using the diffusion method in agar, *S. mutans* (ATCC 25175) and *S. mutans* (JC-2) were found to be 1000 µg/mL.[17] The previous study has identified MIC values lower than *Lactobacillus rhaminosus* (300 µg/mL) and higher for *S. mutans* (2500 µg/mL).[18] Differences in MIC values can be justified by the different strains tested, as well as by the method of obtaining the essential oil, its seasonality, or place of cultivation.

CHX is a potent chemotherapeutic agent considered to be the gold standard in the reduction of oral pathogens. CHX readily binds to the charged bacterial surfaces and acts against Gram-positive and Gram-negative bacteria. CHX gluconate is a cationic biguanide with broad-spectrum antimicrobial action, whose effectiveness in decreasing the formation of dental biofilm (plaque) and gingivitis have been demonstrated in several clinical studies. It is considered as the positive control (gold standard), to which all other anti-plaque agents should be compared. Its advantages are based on its substantivity property.[19] Its antibacterial action is due to an increase in cellular membrane permeability followed by coagulation of the cytoplasmic macromolecules.[20] The antibacterial action may also due to an increase in cellular membrane permeability followed by coagulation of the cytoplasmic macromolecules. It is representative of the cationic group that is highly effective against

S. mutans infection. Its superior effect is due to the fact that it retains its antimicrobial effect as its remains adsorbed to the tooth surface even after its clearance from saliva.[21,22]

CONCLUSION

Tea tree oil has a significant antimicrobial effect compared to controlled group. The tea tree oil was as effective as an anticaries mouthwash as CHX. Future studies have to be aimed at increasing substantivity of herbal mouthrinses so that a potential alternative to CHX alone with intense antimicrobial activity and cost-effective preventive strategies for caries can be used.

REFERENCES

1. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994;8:263-71.
2. Seymour RA, Heasman PA. Drugs and the periodontium. J Clin Periodontol 1988;15:1-6.
3. Carounanidy U, Satyanarayanan R, Velmurugan A. Use of an aqueous extract of *Terminalia chebula* as an anticaries agent: A clinical study. Indian J Dent Res 2007;18:152-6.
4. Rasooli I, Shayegh S, Astaneh S. The effect of *Mentha spicata* and eucalyptus camaldulensis essential oils on dental biofilm. Int J Dent Hyg 2009;7:196-203.
5. Mullally BH, James JA, Coulter WA, Linden GJ. The efficacy of a herbal-based toothpaste on the control of plaque and gingivitis. J Clin Periodontol 1995;22:686-9.
6. Pourabbas R, Delazar A, Chitsaz MT. The effect of German chamomile mouthwash on dental plaque and gingival inflammation. Int J Pharm Res 2005;2:105-9.
7. Agarwal P, Nagesh L, Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of tulsi (*Ocimum sanctum*) extract against *Streptococcus mutans*: An *in vitro* study. Indian J Dent Res 2010;21:357-9.
8. Vyas YK, Bhatnagar M, Sharma K. *In vitro* evaluation of antibacterial activity of an herbal dentifrice against *Streptococcus mutans* and lactobacillus acidophilus. Indian J Dent Res 2008;19:26-8.
9. Allaker RP, Douglas CW. Novel anti-microbial therapies for dental plaque-related diseases. Int J Antimicrob Agents 2009;33:8-13.
10. Eley BM. Antibacterial agents in the control of supragingival plaque-a review. Br Dent J 1999;186:286-96.
11. Khirtika SG, Ramesh S, Muralidharan NP/Comparative evaluation of antimicrobial efficacy of 0.2% chlorhexidine, 2% iodine and homemade mouthrinse as an anti-caries agent. J Pharm Sci Res 2017;9:2114-6.
12. Carson CF, Riley TV. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol 1995;78:264-9.
13. Brophy JJ, Davies NW, Southwell IA, Stiff IA, Williams LR. Gas chromatographic quality control for oil of *Melaleuca* terpinen-4-ol type (Australian tea tree). J Agr Food Chem 1989;37:1330-5.
14. Groppo FC, Ramacciato JC, Simões RP, Flório FM, Sartoratto A. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. Int Dent J 2002;52:433-7.
15. Alves EG, Vinholis AH, Casemiro LA, Furtado NA, Silva ML, Cunha WR, *et al*. Comparative study of screening techniques for antibacterial activity evaluation of plant crude extracts and pure compounds. Quim Nova 2008;31:1224-9.
16. Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T, *et al.* A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol 2004;19:61-4.
17. Filoche SK, Soma K, Sissons CH. Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. Oral Microbiol Immunol 2005;20:221-5.
18. Hammer KA, Dry L, Johnson M, Michalak EM, Carson CF, Riley TV, *et al.* Susceptibility of oral bacteria to *Melaleuca alternifolia* (tea tree) oil *in vitro*. Oral Microbiol Immunol 2003;18:389-92.
19. Herrera D, Roldán S, Santacruz I, Santos S, Masdevall M, Sanz M, *et al.* Differences in antimicrobial activity of four commercial 0.12% chlorhexidine mouthrinse formulations: An *in vitro* contact test and salivary bacterial counts study. J Clin Periodontol 2003;30:307-14.
20. Haraszthy VI, Zambon JJ, Sreenivasan PK. Evaluation of the antimicrobial activity of dentifrices on human oral bacteria. J Clin Dent 2010;21:96-100.
21. Brecx M, Brownstone E, MacDonald L, Gelskey S, Cheang M. Efficacy of listerine, meridol and chlorhexidine mouthrinses as supplements to regular tooth cleaning measures. J Clin Periodontol 1992;19:202-7.
22. Hennessy T. Some antibacterial properties of chlorhexidine. J Periodont Res 1973;8:61-7.

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