

Comparative Evaluation of Antimicrobial efficacy of 0.2% Chlorhexidine, 2% Iodine and Homemade Mouthrinse as an Anti-Caries Agent- A Clinical Study

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Abstract

Aim

The aim of this study was to compare and evaluate the effectiveness of 0.2% Chlorhexidine, 2% iodine and homemade natural antiseptic mouth rinse solutions on patients who have risk of caries.

Materials and Method

The efficacy of three antiseptic mouth rinses was evaluated in this study on healthy volunteers. The subjects were stratified into three groups N=36. The antiseptic solutions used were Group I - 0.2% Chlorhexidine gluconate mouthwash, Group II - 2% iodine mouthwash and Group III - homemade essential oil mouthwash. The subjects were asked to rinse for 40 seconds respectively. The saliva samples were collected from the volunteers before and after 10 minutes after rinsing. Reduction of bacteria was then evaluated according to total number of colony forming unit (CFU) per ml.

Results

The results were tabulated and statistically analysed using one way ANOVA followed by Tukey HSD post hoc test using SPSS Software, version 22.0. The aqueous extract of essential oil mouthwash was as effective as Chlorhexidine and more effective than iodine mouthrinses.

Conclusion

The aqueous extract of essential oils was as effective anticaries mouthwash as chlorhexidine and iodine mouthrinses.

Keywords: Anticaries mouthwash, chlorhexidine, tea tree oil, lemon grass oil

INTRODUCTION

Management of dental caries focuses on limiting tooth demineralization by altering the dietary habits, preventing or modifying oral microbial growth, altering the salivary pH and buffering capacity¹. The primary etiological agents of dental caries are *Streptococcus mutans* and *Lactobacillus* species². They easily colonize the tooth and initiate acid production by producing extracellular polysaccharides from sucrose foods³. Hence, caries prevention program primarily should be aimed at reducing the cariogenic bacteria¹.

A mouth rinse is an agent used as an effective home care remedy to enhance oral hygiene and prevent dental caries by targeting the cariogenic bacteria. A variety of synthetic antimicrobial mouthwashes are available in the market to prevent dental caries. Studies have shown to inhibit plaque formation, also reduce gingival inflammation and prevent dental caries⁴. Chlorhexidine, being one of the most popular mouth rinse has shown to significantly reduce the level of oral *S. mutans*⁵. However, studies regarding the anticariogenic activity of chlorhexidine as a mouth rinse have reported large variations, inconsistencies and an inability to eradicate *S. mutans* in the oral cavity⁶.

The usage of antimicrobial herbal products in dentistry in prevention of dental caries has been reported⁷. In spite of

various commercially available anticaries agents, the search for an effective herbal antimicrobial mouth wash has continued. Hence in this study, we compared the aqueous extracts of oil of two medicinal plants tea tree and lemon grass as a mouthwash, on their efficacy against dental caries.

Hence, we aimed to evaluate and compare the effect of 2% aqueous extract of tea tree and lemon grass oils, 0.2% chlorhexidine and 2% iodine when used as an anticaries mouthwash. The objective of the study was to check the change in the salivary pH, buffering capacity and reduction in microbial count of the saliva before and after rinsing with the 2% oily aqueous extract of tea tree and lemon grass, 0.2% chlorhexidine and 2% iodine as a mouthwash in high caries risk individuals.

MATERIALS AND METHODS

In Vitro ANALYSIS

At first an in vitro study was done to check the antibacterial efficacy of the three mouthrinses. Commercially available 0.2% Chlorhexidine (Clohex, Chlorhexidine mouthwash), 2% Iodine (Betadine, Povidine-Iodine germicide gargle) and 2% wt. Natural essential oil mouthrinse.

Preparation of the sample

The 2% wt. homemade natural essential oil mouthrinse was prepared by:

- Distilled water – 1/2 cup
- Pure tea tree oil – 2 drops
- Pure lemon grass oil – 2 drops
- Glycerine - 6 drops (sweetening agent) (Fig. 1)



Fig. 1- Materials used in the study

Standard strains of *Streptococcus mutans* and *Candida albicans* were isolated for the study purpose and were compared with 0.5% McFarland standard. The study was performed in a microtitre plate. The solutions were then dispensed into wells present in the microtitre plate. 20uL containing bacterial suspensions is dispensed into 200uL solution of the test group (Fig. 2). They were then incubated for 4 hours at 37 degree Celsius.

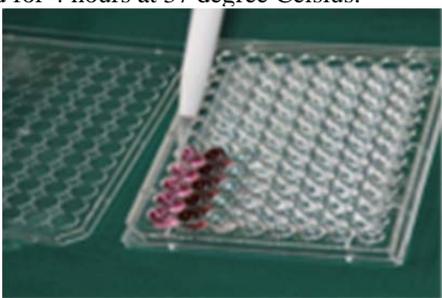


Fig. 2- 20uL of bacterial suspensions is dispensed into 200uL solution of test group

Culture medium

Brain heart infusion medium was prepared for subculture to check viability of bacteria.

10uL of each sample was then inoculated onto the medium with the help of sterile loops (Fig. 3). The culture plates were then incubated for 24 hours at 37 degree Celsius. The colony forming units (CFU) was then calculated and the antimicrobial efficacy was thereafter determined.



Fig. 3- 10uL of each sample is inoculated onto culture plate using sterile loops and incubated

The results of this in vitro study suggested that the natural essential oils mouthrinse was as effective as chlorhexidine and iodine mouthrinses. Therefore, an in vivo study was to be done as the next step to check their efficacy.

In Vivo ANALYSIS

Sample selection

A total of 36 patients who satisfied the selection criteria of high caries risk group were included in the study. Ethical Committee approval was obtained from the Institutional Review Board (Saveetha Dental College and Hospitals, Chennai). All patients subjected for the study were informed about study protocols and written consent was obtained. They were randomly divided into three groups using each containing 15 patients (n = 15); Group 1-2% aqueous extract of essential oil mouth wash; Group 2- 0.2% Chlorhexidine mouth wash; Group 3-2% iodine mouth wash.

Patients were asked to wash and retain the respective mouth rinse in the mouth for 40 seconds before expectorating it. Patients were not allowed to consume any diet or drink orally for following 10 minutes. A salivary sample from each patient was collected for before (pre-rinse) and after rinsing (post-rinse) after an interval of 10 minutes for microbial analysis. The mouth rinsing procedure and collection of salivary samples were handled by one investigator and analysis of the salivary parameters was carried out by different investigator who was not aware of the experimental groups to blind the study.

Microbial analysis

Pre-rinse and post-rinse salivary samples after 10 minutes were collected in the same manner as was carried out for the salivary buffering capacity analysis. The culture medium used for *S. mutans* was Mitis Salivarius agar. The technique used for assessing microbial analysis was the Dilution and Spread plate technique. The saliva samples were diluted and streaked on petri plates followed by incubation for 72 hours at 37°C. After incubation, the colony forming units were recorded and mean percentage of reduction (PR) was calculated.

Statistical analysis

The results were tabulated and statistically analyzed using one- way analysis of variance followed by Tukey HSD post-hoc test.

RESULTS

Table 1: Microbial analysis percentage of reduction

Groups	Streptococcus mutans	
	Percentage of reduction	SD
Group 1	71.6	5
Group 2	65	4
Group 3	75.6%	5.4

Table 1 shows the mean PR of *S. mutans*. There was a significant PR between the pre-rinse and post-rinse samples. Essential oils showed highly significant antibacterial activity compared with chlorhexidine and iodine against *S. mutans*. Essential oils (75.6%) and chlorhexidine (71.6%) showed antibacterial activity against *Streptococcus mutans* with no significant difference. Iodine showed least PR (65%) against the species when compared with the other two extracts.

DISCUSSION

The changes in the microbial activity before and after use of experimental mouthwashes were assessed because quantitative actions play a major role in the caries disease process. The time interval of 10 mins was selected for convenience and to evaluate the effect of mouthwash over a period of time.

Chlorhexidine gluconate is charged positively and shows high affinity for negative ions found in cell membranes of microorganisms. It indirectly affects the enzymatic function of dehydrogenase and adenosine triphosphatase present in the cell wall of bacteria thus causing disruption of cell membrane⁸. It is evident in this study that the chlorhexidine showed a definite reduction in the microbial activity resulting in effective anticariogenicity. However, chlorhexidine has reports of causing discoloration of teeth⁹ and an unpleasant taste¹⁰.

The evidence of efficacy of synthetic antimicrobial agents as mouthwashes remains uncertain for higher caries risk individuals. Hence, two medicinal plants of known and higher inhibitory action against common cariogenic pathogens were selected for this study to compare its efficacy with chlorhexidine. In this study, essential oils mouthrinse proved to be an effective anticaries mouthwash owing to its ability to inhibit *S. mutans*.

The antimicrobial efficiency was highest for essential oils followed by chlorhexidine and chlorhexidine. The aqueous extract of essential oils strongly inhibits the growth of *S. mutans*. The essential oil combination and 0.2% Chlorhexidine had been shown to have an inhibitory effect on plaque, gingivitis and growth of *S. mutans*. Thus, herbal extract can be suggested for management of dentinal caries as their action on *Streptococcus mutans* is more than chlorhexidine.

CONCLUSION

The aqueous extract of essential oils was as effective anticaries mouthwash as chlorhexidine and iodine mouthrinses. Future studies have to be aimed at increasing substantivity of herbal mouthrinses so that a potential alternative to 0.2% Chlorhexidine alone with intense antimicrobial activity and cost effective preventive strategies for caries can be used.

REFERENCES

- Hahn CL, Falkler WA Jr., Minah GE. Microbiological studies of carious dentine from human teeth with irreversible pulpitis. *Arch Oral Biol* 1991; 36:147-53.
- Pathak A, Aparna S, Kadam V, Rekadwad B, Karuppayil SM. Efficacy of some medicinal plants against human dental pathogens. *Indian J Nat Prod Resour* 2012; 31:123-7.
- Fardal O, Turnbull RS. A review of the literature on use of chlorhexidine in dentistry. *J Am Dent Assoc* 1986;112:863-9.
- Kocak MM, Ozcan S, Kocak S, Topuz O, Erten H. Comparison of the efficacy of three different mouthrinse solutions in decreasing the level of streptococcus mutans in saliva. *Eur J Dent* 2009; 3:57-61.
- Autio-Gold J. The role of chlorhexidine in caries prevention. *Oper Dent* 2008; 33:710-6.
- Twetman S. Antimicrobials in future caries control? A review with special reference to chlorhexidine treatment. *Caries Res* 2004; 38:223-9.
- Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices: Results of an in vitro diffusion method study. *J Am Dent Assoc* 2004; 135:1133-41.
- Badria FA, Zidan OA. Natural products for dental caries prevention. *J Med Food* 2004; 7:381-4.
- 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.
- Nair R, Chanda SV. Activity of some medicinal plants against certain bacterial strains. *Indian J Pharmacol* 2006;38:142-4.
- Hasan S, Danishuddin M, Adil M, Singh K, Verma PK, Khan AU. Efficacy of *E. officinalis* on the cariogenic properties of *Streptococcus mutans*: A novel and alternative approach to suppress quorum-sensing mechanism. *PLoS One* 2012; 7:e40319.
- Han Q, Song J, Qiao C, Wong L, Xu H. Preparative isolation of hydrolysable tannins chebulagic acid and chebulinic acid from *Terminalia chebula* by high-speed counter-current chromatography. *J Sep Sci* 2006; 29:1653-7.
- Aneja KR, Joshi R. Evaluation of antimicrobial properties of fruit extracts of *Terminalia chebula* against dental caries pathogens. *Jundishapur J Microbiol* 2009; 2:105-11.
- Srinagesh J, Pushpanjali K. Assessment of antibacterial efficacy of *Triphala* against *Mutans streptococci*: A randomised control trial. *Oral Health Prev Dent* 2011; 9:387-93.
- Jagtap AG, Karkera SG. Potential of the aqueous extract of *Terminalia chebula* as an anticaries agent. *J Ethnopharmacol* 1999; 68:299-306.
- McBain AJ, Bartolo RG, Catrenich CE, Charbonneau D, Ledder RG, Gilbert P. Effects of a chlorhexidine gluconate-containing mouthwash on the vitality and antimicrobial susceptibility of in vitro oral bacterial ecosystems. *Appl Environ Microbiol* 2003; 69:4770-6.
- Bagis B, Baltacioglu E, Özcan M, Ustaomer S. Evaluation of chlorhexidine gluconate mouthrinse-induced staining using a digital colorimeter: An in vivo study. *Quintessence Int* 2011; 42:213-23.