Efficacy of Herbal Antimicrobial Mouthrinse in Comparison with Chlorhexidine and Essential Oil for Orthodontic Patients

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ABSTRACT

Introduction: The purpose of the study was to determine the efficacy of herbal antimicrobial mouth rinse compared to *chlorhexidine*, essential oil against *streptococci* genus in orthodontic patients.

Method: The samples were tested on 80 patients undergoing fixed orthodontic treatment with a mean age of 24.5 years. Patients were divided into 4 groups: Group A- placebo mouth rinse, Group B- Herbal mouth rinse, Group C-Essential oil and Group D-*Chlorhexidine*. Participants were asked to rinse twice a day in the morning and evening after brushing for 2 weeks. Swabs were collected on the first day, seventh day and fourteenth day from the labial surface of the maxillary lateral incisors. The collected samples were cultured through *Mitissalivarius* agar medium, the results were expressed in CFU and the collected data were subjected to statistical analysis.

Results: All three mouth rinses used in the study were effective against the *streptococci* genus in which Herbal mouth rinse showed the highest efficiency.

Conclusion: The efficacy of Herbal mouthwash was most consistent when compared with other groups.

Key Words: *Chlorhexidine* rinse, Colony-forming unit, Essential oil, Herbal mouth rinse, *Mitissalivarius* agar, *Streptococci* genus

INTRODUCTION

The biofilm that forms on the surface of teeth called dental plaque can induce some of the most common diseases which afflict the oral cavity including caries, gingivitis and periodontitis.¹

Streptococci are the primary inhabitants of the oral cavity which can be attained after birth and thus play a crucial role in the congregation of the oral microbiota. Also, they have a remarkable ability to metabolize carbohydrates via fermentation thereby generating acids as byproducts. This extreme acidification of the oral environment by aciduric species is associated with the progress of dental caries.^{2,3}

Streptococcus mutans, which has been implicated as a primary etiological agent of dental caries in humans, have a significant function in plaque formation and accumulation.⁴ Glucans synthesized from dietary sucrose by *glucosyltrans-*

ferases (GTFs) enhances the pathogenic potential of dental plaque. Furthermore commensal and pathogenic *streptococci* residing in the oral cavity can eventually gain access to the bloodstream and cause systemic infections such as infective endocarditis.⁵

Fixed orthodontic appliances lead to an increase in the volume and number of cariogenic *streptococci* in dental plaque,^{6,7} and the elevated levels of *streptococci* return to normal after removal of the appliance. This may be attributed to the difficulties in brushing due to malocclusion and fixed orthodontic appliance which can also increase the accumulation of microbial plaque and decrease salivary flow. This in addition facilitates the formation of dental caries and induces periodontal problems.⁸

The primary causative factor in the development of gingivitis during orthodontic treatment is the insufficient removal of supragingival plaque. Furthermore many orthodontic pa-

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tients neglect to floss since they find this practice tiresome in the presence of orthodontic archwires. The usage of a mouthwash is the easiest and convenient to perform, it could be a handy clinical adjunct for reducing the bacterial plaque accumulation.^{9,10}

Chlorhexidine gluconate is considered the most effective antiplaque mouth rinse and in preventing dental caries. Its mechanism of action is mainly based on the rupture of bacterial cell walls which subsequently causes precipitation of the cell cytoplasmic contents.^{11,4}

Essential oil mouthwashes have been used for years as an adjunct to brushing in addressing oral hygiene.^{12,13} They kill microorganisms by destroying their cell walls and inhibiting their enzymatic activity.^{14,15,16} Despite their advantage these mouthwashes have a complication such as a tooth staining, altered taste, dryness of mouth etc.¹⁷

Therefore search for other antimicrobial substances with fewer side effects continues.

Herbal mouthwashes are gaining popularity as they contain ingredients called *phytochemicals* that achieve the desired antimicrobial and anti-inflammatory effects. They are also capable of maintaining periodontal health, reducing dental plaque and decreasing bleeding upon brushing.^{18,19,20}

However, despite many studies on the efficiency of *chlorhexidine* and essential oil, there is scarce literature about the impact of herbal mouthwashes on the decrease in the colony-forming unit (CFU) counts of the *Streptococcus* genus. Therefore, the purpose of the study was to determine the antimicrobial efficacy of herbal mouthwash against *streptococci* genus in patients undergoing fixed orthodontic treatment.

MATERIALS AND METHODS

In this study, 80 patients (40 males and 40 females) who were under fixed orthodontic treatment in the Department of Orthodontics -Sathyabama Dental College and Hospital with a mean age: 24.5 years were selected. The informed signed consent was obtained from the patients after explaining to them the aim and process of the experiment. The study was presented before the institutional board and received approval (Ref No-Sathyabama University/IHEC/study no062) at Sathyabama Dental College & Hospital.

Inclusion/Exclusion criteria

Subjects willing to participate, who had started orthodontic treatment 6 months before, a lack of active dental caries and severe periodontal condition were included. Subjects taking any medication or mouth rinse, presence of any composite restorations near the gingival margin, and a history of previous orthodontic or periodontal treatments

and known hypersensitivity towards mouth rinse were excluded.

All subjects selected for this study were at the end of leveling and aligning. All the selected patients were bonded with 0.022 slots stainless steel MBT brackets (3 M Unitek, California, USA) on all teeth. Before the start of the protocol, a periodontal evaluation was done with the help of a single periodontist. After oral prophylaxis, the participants were randomly assigned to 4 groups of mouth rinses, with twenty subjects in each group (N= 20).

The groups and the assigned mouth rinses are as follows:

- A) Drinking water (Placebo)
- B) Herbal Mouthwash (Oro- fresh)
- C) Essential Oil (Listerine)
- D) 2% *Chlorohexidine Gluconate* (Hexidine).

The Orthodontist was the only person knowing the allocations, randomized the patients using a table of random numbers. The mouth rinses from their commercial/original containers were transferred to an identical glass bottle and given to the participants. All the participants and microbiologists were unaware of the randomization sequences.

Participants in all the groups were instructed to rinse twice (morning and evening) daily for about 1min after tooth brushing and the participants were asked to follow the same for 2 weeks. Participants were also instructed not to consume anything for 20 minutes after mouth rinse. Follow up was done every day through mobile communication twice a day morning and evening for the entire 14 days of mouthwash regimen.

Swabs were collected on the first day, seventh day and the fourteenth day from the labial gingival margin of the maxillary lateral incisors. The collected samples were cultured through *Mitisalivariusagar* medium (Figure1).

Streptococci genus sampling technique

The culture plates were incubated within 4 h at 37 °C for 48 h under 5–20% CO₂. *Streptococcus* genus colonies were identified as highly convex, spherical, raised, dark blue in color, ranging from a pinpoint to pinhead size with a rough surface using a digital colony counter. All plates were processed and examined by the same investigator. Confirmation of *streptococci* genus was performed under the light microscope. The results were expressed in colony-forming units (CFU), and the collected data were subjected to statistical analysis.

RESULTS

Out of 80 participants, 63 completed the 14-day study, 17 participants dropped out (Group A-4, Group B-5, Group C-3, Group D-5) since they did not show up for sample collection

on the 7th and 14th day. Therefore for statistical standardization, only 15 participants in a group were included.

Wilcoxon signed-rank test for Statistical analysis was performed using Statistical Package for Social Science (SPSS Inc., Chicago, Illinois, USA) version 21.0. The median and interquartile (IQR) range for each group was calculated.

When comparing the efficiency in reducing the Colony-forming unit among 4 groups between 1st and 7th day (Table 1), it was found that Group B exhibited consistent reduction ($P=0.0007$) for all the samples followed by Group C ($P=0.03$) and Group D ($P=0.27$).

When comparing the efficiency in reducing the Colony-forming unit among 4 groups between 1st and 14th day (Table 2) it was found that Group B ($P=0.0007$) exhibited consistent reduction for all the samples followed by Group C ($P=0.04$) and Group D ($P=0.24$).

Conversely in group A the CFU were increased on the 7th and 14th day when compared to 1st day which indicates that there is no effectiveness in reducing CFU.

Therefore it was evident that except Group A (Drinking water) Group B, C and D (Herbal mouthwash, Essential oil and *Chlorhexidine gluconate*) had an effective reduction in colony-forming count.

DISCUSSION

The occurrence of white spot lesions is unavoidable during orthodontic treatment when oral hygiene is deprived. This is due to the prolonged plaque retention around the brackets, which leads to decrease in pH. The presence of white spot lesions may lead to patient dissatisfaction at the end of orthodontic treatment and may necessitate cosmetic intervention by a dentist.²¹

The present study evaluated the antimicrobial efficiency against *streptococcus* among orthodontic patients. Results revealed that all the three mouth rinse except drinking water was effective in reducing CFU. Herbal mouth rinse was most consistent and efficiently followed by Essential oil and *Chlorhexidine*.

The ingredients of herbal mouth rinse used in the study are green tea, *Triphala*, *neem*, *pudhina*, *Nilgiri oil* and *alum* which have anti-inflammatory, anti-cariogenic, plaque reduction, anti-bacterial and anti-microbial properties respectively.^{22,23}

Whereas, ingredients of essential oil mouth rinse are *eucalyptol*, *menthol*, *methyl salicylate* and *thymol* which gives the effect of fresh mint smell, plaque control, analgesic and antiseptic agent respectively.²⁴ Likewise the Ingredients of *chlorhexidine* mouth rinse are *chlorhexidine gluconate*, *sodi-*

um fluoride and *zinc chloride* which has the properties such as anti-plaque, anti-cariogenic and odour-neutraliser.²⁵ However despite its advantage essential oil and *chlorhexidine* mouth rinse have a complication such as a tooth staining, altered taste, dryness of mouth etc.²⁶

Herbal mouthwashes are gaining popularity as they neither do have alcohol content nor colouring agents but contain naturally occurring ingredients called phytochemicals that achieve the desired antimicrobial and anti-inflammatory effects. Khan et al. reported that the effectiveness of herbal *Tachyspermum ammi* seeds against *Streptococcus mutans*, where they stated that these seeds serve as a great potential source to be used as a therapeutic agent against dental caries.²⁷ Additionally *Yadav S et al.* also suggested that the antibiofilm formation action of *Terminalia bellerica* plant extract against *Streptococcus mutans* and *streptococcus sobrinus* was found to be a strong inhibitor of *Streptococcus mutans*.

Moreover, our results were from the study done by *Jaidka S et al.* where he also compared the antimicrobial efficacy of *chlorhexidine*, *xylitol* and herbal (*Hiora*) mouth rinses on 7–14 years old children. He concluded that maximum reduction was seen in the herbal group (*Hiora*) followed by *xylitol* group and the least in *chlorhexidine* group.²⁷

On the contrary, *Sharma et al.* stated that the efficacy of *chlorhexidine*, was maximum followed by *Sodium fluoride* and then herbal mouth rinse against *Streptococcus mutans*.

Thus we recommend that more studies regarding the antimicrobial effects of these mouthwashes should be conducted with larger sample size and longer duration. Furthermore, adverse effects of these mouthwashes regarding taste and sensitivity should also be evaluated.

CONCLUSION

All three types of mouthwash used in the study were effective in reducing the colony-forming units of *streptococci* genus in patients undergoing fixed orthodontic treatment and the efficacy of Herbal mouthwash was most consistent when compared with other groups.

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Author Contribution

1. Dr. Faizee Shahul Hameed– Study Design, Write up, Discussion
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6. Dr. Ambika. S, CRRI-Discussion, Grammar check

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Figure 1: Mitis salivarius agar medium.

Table 1: Comparisons between 1st day and 7th day of all groups

Parameter	Day	N	Median	IQR	p-value
Group A (Distilled Water)					
	Day 1	15	176	(98,300)	0.4
	Day 7	15	300	(98,300)	
Group B(Herbal)					
	Day 1	15	112	(54,174)	0.0007
	Day 7	15	102	(24,130)	
Group C (Essential Oil)					
	Day 1	15	300	(40,300)	0.03
	Day 7	15	200	(80,280)	
Group D (Chlorohexidine)					
	Day 1	15	144	(26,270)	0.27
	Day 7	15	128	(80,250)	

Table 2: Comparisons between 1st day and 14th day of all groups

Parameter	Days	N	Median	IQR	p-value
Group A (Drinking Water)					
	Day 1	15	176	(98,300)	0.91
	Day 14	15	300	(90,300)	
Group B (Herbal)					
	Day 1	15	112	(54,174)	0.0007
	Day 14	15	80	(13,110)	
Group C (Essential Oil)					
	Day 1	15	300	(40,300)	0.04
	Day 14	15	150	(30,200)	
Group D (Chlorohexidine)					
	Day 0	15	144	(26,270)	0.24
	Day 14	15	100	(70,240)	