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ORIGINAL RESEARCH

Antimicrobial Effectiveness of Three Herbal Mouthwashes: An *in vitro* Study

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ABSTRACT

Mouthwash is a chemotherapeutic agent used as an effective home care system by the patient to enhance oral hygiene. Mouthwashes are widely used as adjuncts to oral hygiene and in the delivery of active agents to the teeth and gums. Mouthwashes are used for various reasons: To freshen breath, to help prevent or control tooth decay, to reduce plaque, to prevent or reduce gingivitis, to reduce the speed that calculus forms on the teeth, or to produce a combination of these effects. The present study was conducted to comparatively evaluate the antimicrobial effect of three commercially available herbal mouthwashes on *Porphyromonas gingivalis, Streptococcus mutans*, and *Candida albicans* in *in vitro* conditions. Hi-Ora mouthwash manufactured by Himalaya, Colgate fresh tea extract mouthwash, Pepsodent herbal mouthwash, and 0.2% chlorhexidine (CHX) mouthwash were procured from medical store. Hi-Ora and CHX showed statistically significant difference in antimicrobial action against all the three microorganisms. Further *in vivo* studies should be conducted in the same direction for understanding better effect of these mouthwashes in oral conditions.

Keywords: Antimicrobial, Gingivitis, Herbal mouthwash, P. gingivais.

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INTRODUCTION

Mouthwash/mouth rinses are a helpful and effective addition for the maintenance of daily oral hygiene used at home by the patient to enhance oral hygiene. They are often used as an adjunct to toothbrushing for good breath, prevent or control tooth decay, reduce plaque, prevent or reduce gingivitis, reduce the speed that calculus forms on the teeth, or to produce a combination of these effects.[1]

The first known references to mouth rinsing are in Ayurveda and Chinese medicine, about 2700 BC, for the treatment of gingivitis. Later, in the Greek and Roman periods, mouth rinsing following mechanical cleansing became common among the upper classes, and Hippocrates recommended a mixture of salt, alum, and vinegar.[1]

The Jewish Talmud, dating back about 1800 years, suggests a cure for gum ailments containing "dough water" and olive oil. It was in the late 1960s when Harald Loe (at the time a professor at the Royal Dental College in Aarhus, Denmark) demonstrated that a CHX compound could prevent the buildup of dental plaque.[1]

Since then, commercial interest in mouthwashes has been intense and several newer products claim effectiveness in reducing the buildup in dental plaque and the associated severity of gingivitis, in addition to fighting bad breath. Many of these solutions aim to control the volatile sulfur compound-creating anaerobic bacteria that live in the mouth and excrete substances that lead to bad breath and unpleasant mouth taste.[2]

The daily removal of dental plaque is an important factor in the prevention of gingival inflammation and smooth surface enamel caries. However, since complete plaque removal is unrealistic, prevention may be achieved by either reducing the quantity of plaque below the individual's threshold for disease or by changing the quality of plaque to a more tissue-friendly composition (Kornman).[3]

To achieve sufficiently low levels of plaque, several mechanical oral hygiene aids like dental floss and interdental brushes are necessary in addition to toothbrush. Only few people are successful to achieve adequate and acceptable oral health according to the survey done by Lindhe and Koch. Many reviews have supported the feasibility of chemical approaches in the control of plaque formation.[3]

Mouthwashes without alcohol are the recent addition to a wide range of mouthwashes containing a numerous active ingredients available for case selection. While the manufacturers claim that their mouthwashes have

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antimicrobial properties, the aim of this study is to determine the antimicrobial properties of three commercially available herbal mouthwashes, i.e., Hi-Ora mouthwash, Colgate fresh tea extract mouthwash, and Pepsodent herbal mouthwash.

AIMS AND OBJECTIVES

The aim of the article is to assess the antimicrobial efficacy of commercially available alcohol-free mouthwashes (herbal) with CHX on *P. gingivalis*, *S. mutans*, and *C. albicans*.

MATERIALS AND METHODS

The present study was conducted to comparatively evaluate the antimicrobial effect of three commercially available herbal mouthwashes on *P. gingivalis*, *S. mutans*, and *C. albicans* in *in vitro* conditions. Hi-Ora mouthwash manufactured by Himalaya, Colgate fresh tea extract mouthwash, Pepsodent herbal mouthwash, and 0.2% CHX mouthwash were acquired from the retail medical store.

Microbial Type Culture Collection (MTCC) strain no. 890 (*S. mutans*) and no. 3017 (*C. albicans*) were procured from the MTCC, and ATCC strain no. 33277 (*P. gingivalis*) was obtained.

Malt yeast agar and brain heart infusion agar were obtained from HiMedia Laboratories Limited, for the culture of *C. albicans* and *S. mutans*. *Porphyromonas gingivalis* agar (AS-6422) is an enriched selective medium for the isolation and presumptive identification of *P. gingivalis*, which was obtained from Anaerobe Systems, USA.

Determination of microbic sensitivity can be done by two methods, i.e., dilution methods and diffusion methods. Ditch plate diffusion method was used in the present study as it has been proven to be more suitable for research purposes.

Totally, 16 Petri dishes (four for each of the three different media) were prepared and a narrow channel ditch of 6 mm diameter was made in each of them using a punch. These ditches were then filled with the 50 mL of full concentration of the four mouthwashes, which was coded from 1 to 4 so as to blind the microbiologist.

Petri dishes were incubated at 37°C for 24 hours and afterward examined for zone of inhibition. Plates with *P. gingivalis* agar were immediately placed in an anaerobic atmosphere and incubated at 37°C for 24 hours and then zone of inhibition was examined. The zone of inhibition was measured with the help of Hi Antibiotic Zone scale from HiMedia Laboratories Limited, Mumbai, which is certified to International Standards Organization and World Health Organization (WHO) Good Manufacturing Practice.

Table 1: Mean zone of inhibition

Mouthwashes	*S. mutans (mean ± SD)*	*C. albicans (mean ± SD)*	*P. gingivalis (mean ± SD)*
CHX	22.6 ± 0.57	20 ± 1.00	22 ± 1.0
Pepsodent	0 ± 0	0 ± 0	19 ± 1.0
Colgate	22 ± 1.0	13.33 ± 1.52	15 ± 1.0
Hi-Ora	19.6 ± 1.52	16 ± 1.00	30.33 ± 1.52

SD: Standard deviation

RESULTS

Table 1 represents the zones of microbial inhibition exhibited by the four different mouthwashes at full strength against the three microorganisms with mean diameter and standard deviation. Chlorhexidine and Colgate fresh tea extract mouthwash showed highest mean diameter of microbial inhibition against *S. mutans* (22.6 ± 0.57 and 22 ± 1.0 respectively) with no microbial inhibition found with Pepsodent herbal mouthwash.

Chlorhexidine reported the maximum mean inhibition against *C. albicans* (20 ± 1). Pepsodent mouthwash did not have any antimicrobial activity, whereas Colgate and Hi-Ora showed antimicrobial inhibition of 13.33 ± 1.52 and 16 ± 1.00 respectively. Highest mean zone of inhibition against *P. gingivalis* was seen with Hi-Ora mouthwash (30.33 ± 1.52). Pepsodent and Colgate showed mean inhibition of 19 ± 1.0 and 15 ± 1.0 respectively, which was found to be lesser than that with CHX (22 ± 1.0).

Comparison of different mouthwashes using one-way analysis of variance (ANOVA) showed that the difference in microbial inhibition was significantly different for all the four mouthwashes against the three microorganisms studied (Table 2).

Multiple comparisons using Bonferroni correction (Tables 3 to 5) showed that Pepsodent when compared with the other mouthwashes showed statistically significant difference in antimicrobial inhibition against all the three microorganisms except with CHX with which there was no statistically significant difference in action against *P. gingivalis*. Colgate when compared with Hi-Ora showed statistically significant difference in antimicrobial property only against *P. gingivalis*, whereas in comparison to CHX mouthwash, sufficiently great difference was observed against *C. albicans* and *P. gingivalis*. Statistically significant difference of microbial contamination was seen in CHX and Hi-Ora mouthwash.

Table 2: Mean comparison between the different mouthwashes using one-way ANOVA

Comparison	*p-value*	*Significance*
CHX–Pepsodent	0	S
CHX–Colgate	1.00	NS
CHX–Hi-Ora	0.03	S
Pepsodent–Colgate	0	S
Pepsodent–Hi-Ora	0	S
Colgate–Hi-Ora	0.105	NS

S: Significant; NS: Nonsignificant

Table 3: Multiple comparisons for *S. mutans* using Bonferroni

	Sum of squares	*df*	*Mean square*	*f-value*	*Significance*	*p-value*
S. mutans	Between different mouthwashes	1049.583	3	349.861	381.667	0
C. albicans	Between different mouthwashes	676.000	3	225.333	208.000	0
P. gingivalis	Between different mouthwashes	380.250	3	126.750	95.062	0

One-way ANOVA used for mean comparison between the different mouthwashes. The mean difference is significant at ≤0.05

Table 4: Multiple comparisons for *C. albicans* using Bonferroni

Comparison	*p-value*	*Significance*
CHX–Pepsodent	0.078	NS
CHX–Colgate	0.017	S
CHX–Hi-Ora	0	S
Pepsodent–Colgate	0.017	S
Pepsodent–Hi-Ora	0	S
Colgate– Hi-Ora	0	S

The mean difference is significant at ≤0.05; S: Significant; NS: Nonsignificant

Table 5: Multiple comparisons for *P. gingivalis* using Bonferroni

Comparison	*p-value*	*Significance*
CHX–Pepsodent	0	S
CHX–Colgate	0	S
CHX–Hi-Ora	0.009	S
Pepsodent–Colgate	0	S
Pepsodent–Hi-Ora	0	S
Colgate–Hi-Ora	0.083	NS

The mean difference is significant at ≤0.05; S: Significant; NS: Nonsignificant

DISCUSSION

In the current study, we compared the antimicrobial effectiveness of three herbal mouthwashes with CHX mouthwash against *S. mutans, C. albicans*, and *P. gingivalis*. According to our results, while CHX has shown substantive antimicrobial activity against all the three microorganisms, the three herbal mouthwashes has shown differing antimicrobial effectiveness against the microorganisms.

Pepsodent mouthwash did not reflect any signs of activity against *S. mutans and C. albicans*, but showed a zone of inhibition for *P. gingivalis*. Pepsodent herbal mouthwash contains hydrogenated castor oil, amino acetic acid, aloe vera powder, and betel leaf oil. Bhat et al[4] have shown that subgingival administration of aloe vera improves gingival condition.

Hi-Ora contains herbal extracts of *Salvadora persica* and *B myrobalan*,[5] which have been used for centuries as a natural toothbrush and its fibrous branches have been promoted by the WHO for oral hygiene use in its international consensus report on oral hygiene in 1986 and 2000.[6-11]

Salvadora persica contains numerous components like tri-methyamin, salvadrin, chloride, fluoride, silica, sulfur, mustard, vitamin C, and a small amount of saponine tanin. These components have antibacterial and antifever effects, in addition to acting against gingival irritation.[12]

Elvin-Lewis et al[13] and Almas[14] suggested that the antibiotic components detected in *S. persica* may have interaction with bacteria and prevent their attachment. *Salvadora persica* has strong activity against antibacterial compounds.[15]

Terminalia bellirica, known as "Bahera" or Beleric or bastard myrobalan in its fruit form, is used in the popular Indian herbal rasayana treatment Triphala. Susrutha utilized "Belric" as a catalyst in preparing for his surgeries, mainly due to its active antibacterial and antifungal effect.

Colgate fresh tea extract mouthwash contains green tea extracts and cetylpyridinium. Green tea extracts contain a wide range of anti-inflammatory characteristics, and therefore, it may be helpful in treating inflammatory conditions. Green tea has an inhibitory effect on *S. mutans*.[16] Subramaniam et al[17] in their literature reported aqueous extracts of green tea extracts reflected greater zone of inhibition in comparison with those of CHX.

CONCLUSION

Hi-Ora and CHX reported statistically significant difference in antimicrobial action against all the three microorganisms, and Pepsodent showed inhibition only against *P. gingivalis*. Further *in vitro/in vivo* research should be carried out to demonstrate the antimicrobial activity of these mouthwashes for better understanding and utilization.

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