



EVALUATION OF EFFICACY OF CHLORHEXIDINE AND A HERBAL MOUTHWASH ON DENTAL PLAQUE: AN INVITRO COMPARATIVE STUDY

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ABSTRACT

An invitro study to compare the efficacy, safety and antimicrobial properties of a herbal mouthwash with chlorhexidine mouthwash invitro in patients with established plaque was conducted. A total of 20 plaque samples were collected from periodontitis patients and healthy patients and were streaked on blood agar plate. Well Diffusion method was used to compare chlorhexidine gluconate, herbal mouthwash and normal saline. The streaked blood agar plate was incubated at 37° for 24 h and examined for the zones of inhibition. There was no statistically significant difference $P=0.062(P>0.05)$ in the antimicrobial property between the two mouthwashes (Chlorhexidine and Herbal). It was concluded that the herbal and chlorhexidine mouthwash were equally effective invitro suggesting that the herbal mouthwash may be used therapeutically in the future to inhibit oral microbial growth.

KEYWORDS: antimicrobial, chlorhexidine gluconate, dental plaque, herbal, mouthwash, periodontitis



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INTRODUCTION

Dental plaque has been proved by extensive research to be paramount factor in initiation and progression of gingival and periodontal diseases. A direct relationship has been demonstrated between plaque levels and the severity of gingivitis. Since bacterial plaque is the principal causative factor in gingival and periodontal diseases, the most rational methodology towards the prevention of periodontal diseases would be regular effective removal of plaque by personal oral hygiene protocol.¹ Supra gingival plaque control is largely the responsibility of the individual, using tooth brushes and interdental cleaning devices. However, mechanical plaque eradication is considered for most as time consuming, requires motivation, skill and is more difficult for handicapped people.² Also, the prevalence of gingivitis from young age in all population, and the occurrence or recurrence of periodontal disease is high.³ These observations suggest that mechanical cleaning alone by a considerable proportion of individuals is insufficient to maintain gingival health and is difficult to prevent periodontal disease occurrence, progression or recurrence in susceptible individuals. A number of chemical agents which have antiseptic or antimicrobial action have been used, with variable success, to inhibit supragingival plaque formation and the development of gingivitis. Among these are; phenolic compounds, Bis-biguanides, pyrimidines, quaternary ammonium compounds, oxygenating agents, halogens, heavy metal salts.⁴ Among these agents, chlorhexidine (CHX) is the most effective antiseptic for plaque inhibition and prevention of gingivitis when used twice daily as mouth rinse.⁵ But in oral use as a mouth rinse chlorhexidine has been reported to have a number of side effects. Other chemical antiplaque agents have been tested but none has shown equal or better results than chlorhexidine without eliciting unfavorable side effects.^{6,7}

In order to overcome such side effects the World Health Organization (WHO) advice researchers to investigate the possible use of

natural products such as herb and plant extracts. Herbs and plant extract have been used in oral hygiene products for many years.⁴ A number of clinical studies have shown the effects of using mouth washes extracted from herbs such as *Sanguinarina*,⁸ *Myrtus communis*,⁹ *Qureucus infectoria*,¹⁰ *Capparis spinosa*¹¹ and *Cinnamon*¹² in the prevention of dental plaque accumulation and subsequent gingival inflammation. *Salvadora persica* (*S.persica*) is a medicinal plant whose roots have been used by many people in Africa, South America, Middle East and Asia. It has been demonstrated that extracts of *S. Persica* improved gingival health and inhibited growth of cariogenic bacteria.¹³ Another study showed that *Streptococcus mutans* was more susceptible to Miswaks antimicrobial activity than *Lactobacilli*.¹⁴ The widespread use of mouthwashes as an aid to oral hygiene is a relatively recent phenomenon in the developing countries of the world. Hence, herbal dental products are becoming popular amongst general public. A number of herbs have been recommended to help and maintain dental health.¹⁵ The purpose of the present study was to compare the efficacy, safety and antimicrobial properties of a herbal mouthwash with chlorhexidine mouthwash invitro in patients with established dental plaque in periodontitis` and healthy patients.

MATERIALS AND METHODS

Study design

An invitro study was conducted from the plaque samples of the patients attending Darshan Dental College and Hospital, Udaipur, Rajasthan, India. The ethical clearance was obtained from the ethical committee of Darshan Dental College and Hospital. The study participants were clearly informed about the study and a written informed consent was obtained.

Subject selection

A total of 20 adult patients between the age groups over 18 years of age were divided into two groups. The healthy group (group A) comprised of 10 adult patients with more than 3 teeth in each quadrant of the dentition, no periodontitis with no radiographic evidence of alveolar bone loss (as demonstrated by having fewer than 3 sites with probing pocket depth greater than 4 mm), and bleeding on probing in fewer than 10% of sites. The periodontitis group (group B) comprised adult patients with untreated periodontitis, radiographic evidence of alveolar bone loss in each quadrant of dentition, and more than 4 sites with probing pocket depth greater than 6 mm. Patients who received previous oral prophylaxis or any kind of periodontal treatment, patients with history of systemic diseases or antibiotic and oral drug therapy, chemical anti-plaque agents prior to six months of study initiation, physically and mentally handicapped patients, pregnant and lactating women were excluded from the study.

Plaque sampling

Supragingival plaque samples were collected in the morning between 9:00 am to 11:00 am from 20 adult patients. Participants were instructed to refrain from eating, drinking, and oral hygiene habits for 2 hours before samples were collected. Samples of supragingival plaque (approximately 1 mg) were collected with a sterile periodontal probe from the buccal and lingual lower molar surface of 16, 36 either the left or the right side of the mouth. It was then placed in a sterile container and chilled until carried to the laboratory for microbial investigation.

Antimicrobial Assay

A total of 20 blood agar plates were used. The plaque samples were pooled, streaked on blood agar plate, incubated at 37°C for 48 hrs. The cultured dental plaque was transferred into nutrient broth and incubated for 24 hrs at 37°C. Blood agar plate streaked by previously cultured broth with supragingival plaque using loop wire^{16,17}. The optical density was measured by spectrophotometer at a wavelength of 490nm

as a guide to microbial growth. Bacterial isolates were identified and characterized using conventional biochemical tests including Gram stain, catalase, coagulase, oxidase, indole and citrate utilization.

Antimicrobial evaluation of the mouthwashes

The modification of the disc diffusion method which is named as the "Well diffusion" method (WD) was used for antimicrobial susceptibility test in the present study. The streaked blood agar plate was incubated at 37° for 24 hrs. Three wells were bored equidistant into each agar plate using a sterile 8 mm diameter cork borer and 100% of the test mouthwashes (100µl volume) was placed into the wells at full strength. Two drops of each mouthwash were dropped into separate wells using a sterile dropper. The plates were left on the bench for 1 hr to allow for diffusion of the mouthwash samples into the blood agar medium. Orahex mouthwash (Abbott Healthcare Pvt. Ltd. Mumbai, India) containing Chlorhexidine (0.20 %), Hiora herbal mouthwash (The Himalaya Drug Company, Bangalore, India) which consists of the extracts of Pilu (*Salvadora persica*) 5mg, Bibhitaka (*Terminalia bellerica*) 10mg, Nagavalli (*Piper betle*) 10mg, essential oils namely Gandhapura taila (*Gaultheria fragrantissima*) 1.2mg, Ela (*Elettaria cardamomum*) 0.2mg, flavouring agents Peppermint satva (*Mentha*) 1.6mg and Yavani satva (*Trachyspermum ammi*) 0.4mg in 30ml weight by volume concentrations. Also, normal saline 0.9% as control were used. All the plates were then incubated at 37 °C for 24 h and the zones of inhibition measured using an accurately calibrated measuring scale. Results were recorded as the average diameter of inhibition zone surrounding the wells containing the test solution.

Statistical Analysis

Student's t-test was used to compare the zones of inhibition in Chlorhexidine and Herbal mouthwash in Group A and Group B. The test was considered statistically significant when the probability was less than 0.05 (P < 0.05).

Statistical Analysis was performed using a statistical package, SPSS windows version 16 by applying mean values.

RESULTS

In the present study, two mouthwashes Hiora herbal mouthwash and Orahex containing Chlorhexidine mouthwash and Normal saline as control were selected based upon their medicinal uses in the treatment of oral diseases

and their availability. The antibacterial activity and the effectivity of the mouthwashes against the dental plaque microflora was evaluated. The microorganisms detected in the plaque samples were namely *Staphylococcus aureus*, *Streptococcus salivarius*, *Candida albicans*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Figure 1 and Figure 2 shows the zones of inhibition of the CHX, herbal and Normal saline at 24 hrs in Group A(Figure 1) and Group B(Figure 2).



Figure 1

The zones of inhibition with herbal, chlorhexidine and normal saline in Group A



Figure 2

The zones of inhibition with herbal, chlorhexidine and normal saline in Group B

Table 1 shows the comparative effect of mouthwashes with the diameter of the zones of inhibition. Out of the total of 20 samples

analysed, the mean of CHX in Group A was 1.82 ± 0.489 cm as compared to the mean of herbal mouthwash as 1.33 ± 0.413 . The mean of

CHX in Group B was 1.48 ± 0.282 and mean of Herbal mouthwash was 1.49 ± 0.37 . The mean of normal saline was 0. The analysis using t-test indicated that there was no statistically significant difference $P=0.062$ ($P>0.05$) in the bacteriostatic property between the two

mouthwashes (Chlorhexidine 0.2% and Herbal). The total mean of CHX mouthwash was 1.65 ± 0.42 and the total mean of herbal mouthwash was 1.41 ± 0.39 . The efficacy of the CHX and Herbal mouthwash was found equally similar.

Table 1
Comparative effect of mouthwash with zones of inhibition

Group A- Healthy patient Group B- Periodontal patient		Zones of inhibition(in cm) Total N= 20			
Type	Mouthwash	Mean	SD	SEM	df
Group A	Chlorhexidine	1.82	0.489	0.089	18
	Herbal	1.33	0.413	0.130	18
	Normal Saline	0	0	0	0
Group B	Chlorhexidine	1.48	0.282	0.089	18
	Herbal	1.49	0.37	0.118	18
	Normal Saline	0	0	0	0

*P value= 0.062 (Chlorhexidine VS Herbal mouthwash, Paired T test)

** r =0.129(Chlorhexidine VS Herbal mouthwash)

Figure 3 shows the comparative evaluation of the zones of inhibition with Chlorhexidine and Herbal mouthwash in healthy patients. The results shows that the diameter of the zones of inhibition are maximum with chlorhexidine mouthwash of 2.6 cm and the mean of 1.82 cm (Range= 1.2-2.6) as compared to herbal with

the maximum diameter of zones of inhibition 1.9cm and mean 1.33 cm (range=0.8-1.9) There was no statistically significant difference $P=0.377$ between CHX and herbal mouthwash in healthy patients suggesting the efficacy of both the mouthwash were similar.

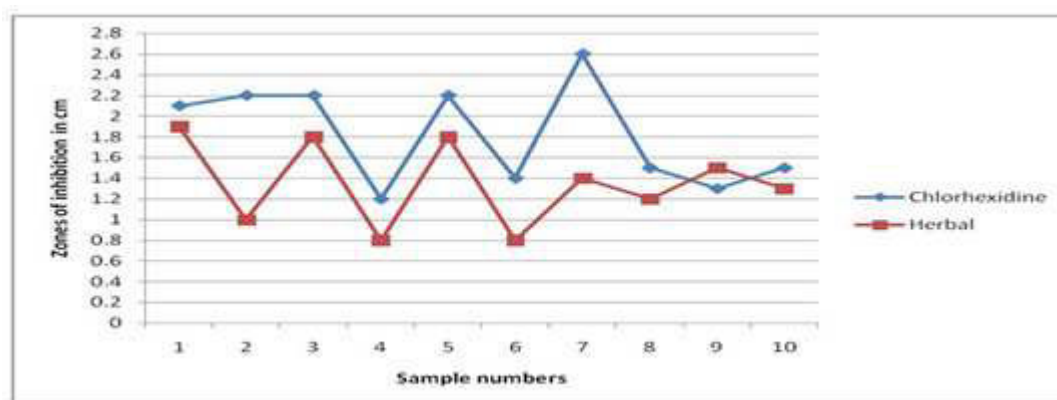


Figure 3
Comparative evaluation of the zones of inhibition with chlorhexidine and herbal mouthwash in healthy patients.

Herbal mouthwash in periodontal patients. The results shows that the diameter of the zones of inhibition are maximum with CHX mouthwash of 1.9 cm mean 1.48 (Range=1.2-1.9) as compared to herbal mouthwash (range=0.8-

1.8). There was no statistically significant difference $P=0.073$ between CHX and herbal in periodontal patients suggesting that both the mouthwashes are equally effective.

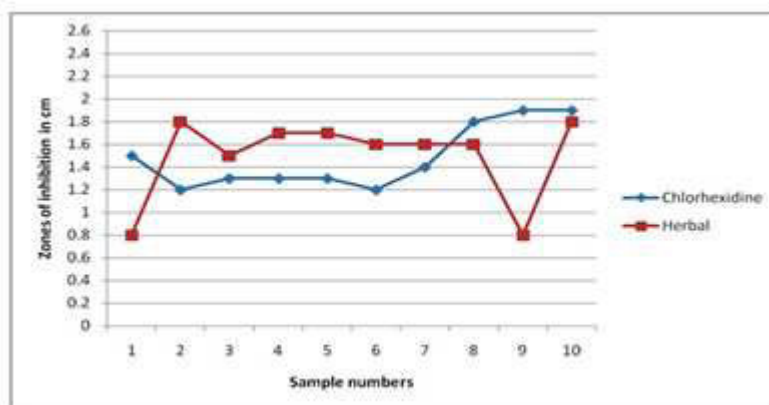


Figure 4
Comparative evaluation of the zones of inhibition with chlorhexidine and herbal mouthwash in Periodontal patients

DISCUSSION

There is a continued interest in identifying efficient antiplaque agents that could be used daily without side effects.¹⁸ In the present study, Hiora herbal mouthwash has shown a good potential as an anti-plaque agent in comparison to Chlorhexidine Gluconate mouthwash and has proven to be equally effective. Hiora mouthwash is a herbal preparation, made from natural herbs with their beneficial properties like anticariogenic and antiplaque (due to *S. persica* which contains trimethyl amine, salvadorine, chlorides, high amounts of fluoride and silica, sulphur, vitamin C, small amounts of tannins, saponins, flavonoids and sterols,¹⁹ antibiotic (due to the presence of *Piper betle* and *Elettaria cardamomum*) and anti-inflammatory and immunity booster (due to the presence of *Terminalia bellerica*). Also, *Mentha* and *Trachyspermum ammi* which are natural flavouring agents. A herbal mouthwash is a non-alcoholic preparation, with no added sugar, no artificial preservatives, no artificial flavors and colors and absolutely no side-effects. A study conducted reported that the mouth rinse with aqueous *Salvadora* twigs extract causes significant reduction (84%) in the adherence of bacterial cells (*Streptococcus mutans*) to buccal epithelial cells and it was also reported that *Piper betle*, *Elettaria cardamomum* also significantly inhibited the growth of oral

microflora and the reduction was up to 77%.²⁰ Similarly, a study conducted²¹ to determine the effect of *Triphala* extract against *Streptococcus mutans* and *Lactobacillus* by agar well diffusion method. There was no significant difference between the *Triphala* and the chlorhexidine mouthwashes with $P=0.145$ which was similar to present study in which the statistical difference between the herbal and CHX mouthwash $P=0.062$. Another study²² conducted which investigated the anti-microbial activity of herbal mouthrinse with Listerine and 0.12% Chlorhexidine Gluconate (Peridex) against *S. mutans*, *S. sanguis* and *A. viscosus*. It was found that herbal mouthrinse produced the largest zone of microbial inhibition when compared to Listerine against all the three bacteria tested. However, when compared to Peridex, herbal mouthrinse produced larger zones of microbial inhibition against two of three bacteria tested, and a similar zone of inhibition against the third bacteria tested. Similarly, a study²³ compared the effectiveness of herbal mouthrinse (The Natural Dentist, Medford, Mass) with 0.12% Chlorhexidine Gluconate and an essential oil mouth rinse. It was found that herbal mouthrinse (though less potent than CHX) was effective in inhibiting oral bacteria, predominantly *Actinomyces* sp., *E. nodatum*, *P. intermedia*, *P. melaninogenica*, *P. nigrescens*

and *T. forsythia*. Herbal mouthrinse was found to be a more effective antimicrobial agent than essential oil mouth rinse. As compared to the present study in which the herbal mouthwash was effective in inhibiting namely *S. aureus*, *S. salivarius*, *C. albicans*, *E. faecalis* and *P. aeruginosa*. However, other studies conducted^{22,24,25} reported that their mixed herbal extract mouthwash showed a weak antibacterial effect against oral bacteria on their experimental and dental plaque model study, is unlike our result and the different results is probably because of different conditions of the oral cavity and the invitro media. In vitro studies with methodology of culture in Petri dishes are still used to perform antibiograms and to discover new microorganisms.²⁶ These techniques, in many places, are not replaced by more advanced diagnostic in microbiology.²⁷ The results in this study should be carefully interpreted because it is difficult to deal with dental biofilm complexity, a limitation in any current technique, and why they do not deal any issue about the quality control of the products. However, it seems that there is the need for further clarification with other in vitro studies and also in health practice.

CONCLUSION

In the present study there was no significant difference in the efficacy of the Hiora and CHX

CONFLICT OF INTEREST

Conflict of interest declared none.

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mouthwash invitro in the plaque samples from healthy and periodontitis patients, suggesting that the herbal mouthwash may be used therapeutically in the future to inhibit oral microbial growth. As CHX rinsing can cause a number of local side-effects including extrinsic tooth and tongue brown staining, taste disturbance, enhanced supragingival calculus formation and, less commonly, desquamation of the oral mucosa. These side-effects limit its acceptability to users and the long-term use of CHX-containing mouth rinses. On the other hand, Hiora Herbal mouthwash due to its natural ingredients has no reported side-effects and can serve as a good alternative to patients who wish to avoid alcohol (e.g. Xerostomics), sugar (e.g. Diabetics), any artificial preservatives and artificial colors in their mouth rinses. Further research to establish the level of plaque inhibition,safety and microbial parameters is necessary before this product finds a place among the other agents for daily plaque removal. Even the results demonstrated relevant differences in the antimicrobial effect of the Herbal mouthwash there is need of other invitro and invivo investigations to confirm the results presented in this study. It should be pointed out that none of the studies have assayed the efficacy of Hiora herbal mouthwash invitro as compared to CHX on dental plaque leading to a very less reported data.

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