



Comparison of Efficacy of 0.2% Chlorhexidine Gluconate and Herbal Mouthrinses on Dental Plaque: An *in vitro* Comparative Study

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the efficacy and antimicrobial properties of a five herbal mouth rinses with chlorhexidine gluconate mouthrinse *in vitro* in healthy and periodontitis patients with established dental plaque.

Materials and Methods: A total of 20 dental plaque samples were collected from periodontitis patients and healthy subjects and were streaked on blood agar plate. Well Diffusion method was used to compare 0.2% chlorhexidine gluconate, herbal mouthrinses [hiora, *Punica granatum*

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(Pomegranate), *Azadirachta indica* (Neem), *Caryophyllus aromaticus* (Cloves) and *Ocimum sanctum* (Tulsi)] and distilled water. The streaked blood agar plate was incubated at 37° for 24 h and examined for the zones of inhibition.

Results: The present study resulted out statistically non significant differences between chlorhexidine, hiora and pomegranate ($p>0.005$). Statistically significant differences were observed between chlorhexidine, tulsi, clove and neem ($p<0.005$).

Conclusion: Herbal mouthrinses (Hiora and Pomegranate) and chlorhexidine mouthrinse were equally effective *in vitro* suggesting that the herbal mouthwash may be used therapeutically in the future to inhibit oral microbial growth.

Keywords: Chlorhexidine gluconate; hiora; *Punica granatum*; *Azadirachta indica*; *Caryophyllus aromaticus*.

1. INTRODUCTION

It is well known that microbial plaque is a paramount factor in initiation and progression of periodontal diseases [1]. The results of the clinical trials and analysis of literature indicates a strong correlation between microbial plaque levels and severity of gingivitis [2-4]. Plaque control has long been considered as the cornerstone of its management [5,6]. Regular effective removal of microbial plaque by the personal oral hygiene protocol is the most rational methodology towards the prevention of periodontal diseases [7,8].

Supra gingival plaque control is largely the responsibility of the individual. However, mechanical plaque eradication is considered for most as time consuming, requires motivation, skill and there are large group of individuals, such as the handicapped and elderly, for whom maintaining adequate oral hygiene can be an insurmountable problem [9,10]. These observations suggest that mechanical cleaning alone is insufficient to maintain gingival health. Chemical plaque control approach is desirable to overcome the deficiency of mechanical plaque control. A number of chemical agents which have 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. Chlorhexidine (CHX), a bis-biguanide is the most effective antimicrobial for plaque inhibition when used twice daily as mouth rinse, [11] but it is not a 'Magic Bullet' and it also comes with certain side-effects [12]. Other chemical antiplaque agents have been tested but none has shown equal or better results than chlorhexidine without eliciting unfavorable side effects [13,14].

In order to overcome such side effects the World Health Organization advice researchers to investigate the possible use of natural products such as herb and plant extract [15]. In the midst of growing evidence of the connection between oral health and whole body health, herbal medicines with their 'naturally occurring' active ingredients offers a gentle and enduring way for restoration of health [16]. Natural herbs have been used alone or in combination and have been scientifically proven to be safe against various oral health problems like bleeding gums, halitosis, mouth ulcers and decay [17]. Also Pomegranate (*Punica granatum*) has shown antibacterial properties [18].

Thus in view of this, the present study was carried out to compare the efficacy and antimicrobial properties of a five herbal mouthwash with chlorhexidine mouthwash *in vitro* in healthy and periodontitis patients with established dental plaque.

2. MATERIALS AND METHODS

The study was designed and conducted in the Department of Periodontics, Genesis Institute of Dental sciences and Research, Ferozepur, Punjab, India from November 2014 to March 2015. Approval from the Institutional Ethics Committee was obtained before initiating the study.

2.1 Subject Selection

A total of 20 adult patients between the age groups over 18 years of age were selected. All volunteers' subjects were informed about the study protocol and informed consent was obtained. Participants were divided into two groups:

Group A: The healthy group comprised of 10 adult subjects 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 lesser than 4 mm), and bleeding on probing in fewer than 10% of sites.

Group B: The periodontitis group 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.

2.2 Exclusion Criteria

Patients who had received previous oral prophylaxis or any kind of periodontal treatment, patients with any history of systemic diseases or condition, or antibiotic and oral drug therapy, or had used chemical anti-plaque agents prior to six months of study initiation were excluded from the study.

2.3 Study Design

2.3.1 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 abstain from eating, drinking, and oral hygiene habits two hours before samples were collected. Samples of supragingival plaque were collected with a sterile scaler or curette from the buccal aspect of upper molar and lingual aspect of 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 kept in freezer until carried to the laboratory for microbial investigation.

2.3.2 Antimicrobial assay

A total of 20 blood agar plates were used. Plaque samples were pooled, streaked on blood agar plate, incubated at 37°C for 48 hrs. Microorganisms were stained with Gram staining and were detected under high power microscope. Conventional test such as catalase, coagulase, oxidase, indole were used to identify specific microorganisms.

2.3.3 Preparation of test solution

Five herbal (Himalaya HiOra, clove, neem, tulsi, pomegranate) and 0.2% chlorhexidine mouthrinse (Hexidine) were the test solution used in the present study. Out of which 0.2% chlorhexidine (Hexidine) and Hiora mouthrinse (Himalaya Drug Company, Bangalore, India) are

commercially available. Aqueous preparations of the other mouthrinses was prepared in the laboratory. Distilled water (D.W.) was used as control in the study.

2.3.4 Preparation of Herbal extracts

Fresh leaves were thoroughly cleaned twice using distilled water. They were cut into pieces with the help of scissors/knife and were dried at room temperature and thereafter powdered. Aqueous plant extracts were prepared by dissolving the powdered form of plant materials in sterile distilled water using magnetic stirrer, respectively, in the ratio of 1:5 i.e., 20 gm of plant material in 100 ml of water in a sterile 250 ml glass flask [19]. Flasks were then plugged with cotton and kept in refrigerator at 4°C for 24 h. These were then filtered and kept in a hot air oven for 5-7 days at 30±2°C to completely evaporate the solvent. The various preparation used in the study is shown in Fig. 1.

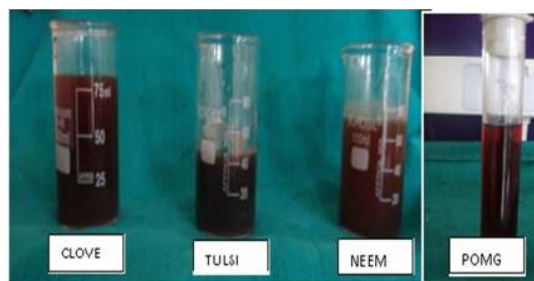


Fig. 1. Laboratory preparation of test solution

2.3.5 Antimicrobial evaluation of the mouthwashes

The modification of the disc diffusion method, 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. 7 wells were made equidistant to each other. 2 ml of test solution was poured in each well. Thereafter, the zones of inhibition were measured using an accurately calibrated measuring transparent scale. Results were recorded as the average diameter of inhibition zone surrounding the wells containing the test solution. The present study is an *in vitro* double blind study where an experienced investigator selected the patients in group A and group B and also poured drops of mouthrinse in all the wells, thereafter a single trained investigator who was masked about the type of mouthrinse in each well, measured zone of inhibition in both the groups.

2.4 Statistical Analysis

Statistical Analysis was performed using a statistical package for Social Sciences software (SPSS inc, Chicago, IL, windows version 16) by applying mean values. The test was considered statistically significant when the probability was less than 0.05 ($P < 0.005$). Student's t-test was used to compare the zones of inhibition in Chlorhexidine and Herbal mouthrinses in Group A and Group B. Significance was reported at 95% confidence interval.

3. RESULTS

In the present study, five herbal mouthrinses and Hexidine containing 0.2% Chlorhexidine mouthrinse were selected based upon their medicinal uses in the treatment of oral diseases and their availability and distilled water as control as all herbal preparations are aqueous preparation. The antibacterial activity and the effectiveness of the mouth rinses were compared on dental plaque micro flora.

The microorganisms detected in the plaque samples were mainly *Staphylococcus aureus*, *Streptococcus salivarius*, *Candida albicans* and *Enterococcus faecalis*. Microorganisms which were detected under high power light microscope after Gram's staining and conventional tests in subjects from both the groups are shown in Table 1. Results of the present study showed that microorganisms detected under light microscope were aerobes and were similar in both the groups. However, Group A showed more Gram positive microorganism whereas Gram negative microorganisms were more in Group B subjects.

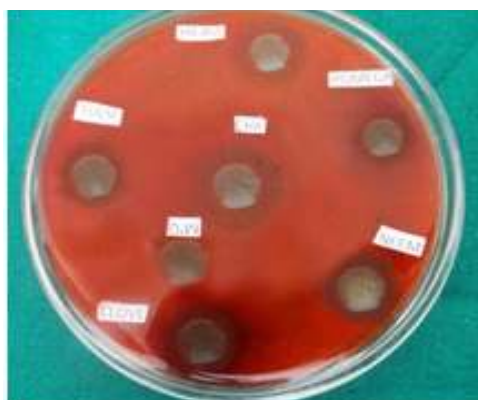


Fig. 2. Zone of inhibition of mouthrinses in group A

3.1 Comparison of Mouthrinses in Group A

Comparative evaluation of the zones of inhibition with Chlorhexidine and 5 herbal mouthrinses in healthy subject is depicted in Table 2, Figs. 2 and 4. Results showed that the mean diameter of the zones of inhibition are maximum with chlorhexidine mouthrinse (2.9 cm) followed by hiora (1.99 cm), Pomegranate (1.95 cm), Tulsi (1.62 cm), Clove (1.52 cm), Neem (1.28). Non-significant difference in zone of inhibition was observed between chlorhexidine and hiora, and chlorhexidine and pomegranate. Chlorhexidine when compared to tulsi, clove and neem showed statistically significant differences as shown in Table 3, suggesting overall the efficacy of hiora and pomegranate similar to Chlorhexidine gluconate.

3.2 Comparison of Mouthrinses in Group B

Comparative evaluation of zone of inhibition with chlorhexidine and 5 herbal mouthrinses in periodontitis group is shown in Table 2, Figs. 3, 5. Result of the present study showed mean diameter of zone of inhibition is maximum with chlorhexidine mouthrinse (2.09 cm), followed by hiora (1.94 cm), pomegranate (1.94 cm), tulsi (1.63 cm), clove (1.5 cm), neem (1.31 cm). Non-significant difference of the zone of inhibition between chlorhexidine and hiora, and chlorhexidine and pomegranate were observed. Compared of tulsi, clove and neem with chlorhexidine showed statistically significant differences (Table 4), suggesting hiora and pomegranate efficacy similar to chlorhexidine.

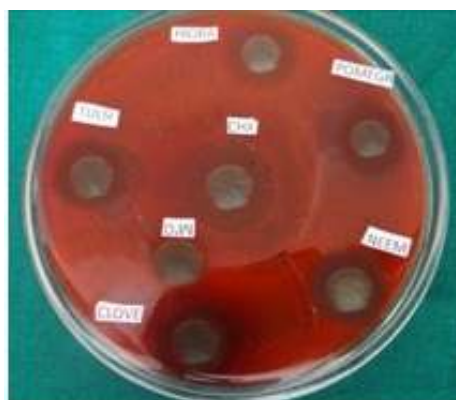


Fig. 3. Zone of inhibition of mouthrinses in group B

Table 1. Showing microorganisms detected in group A and group B

Patients in group A	Microorganisms on blood agar	Patients in group B	Microorganisms on blood agar
1	Gram positive cocci ++ Gram negative cocci + <i>Streptococcus salivarius</i> , <i>Staphylococcus aureus</i> ,	1	Gram positive cocci and bacilli ++ Gram negative cocci ad bacilli++ <i>Streptococcus salivarius</i> , <i>Staphylococcus aureus</i> , Candida albican
2	Gram positive cocci and bacilli ++ <i>Streptococcus salivarius</i> ,	2	Gram positive cocci and bacilli ++ Gram negative cocci and bacilli ++ <i>Streptococcus salivarius</i> , <i>Staphylococcus aureus</i> , Candida albican
3	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Staphylococcus aureus</i> , Candida albican <i>Enterococcus faecalis</i>	3	Gram positive bacilli + Gram negative cocci ++ <i>Streptococcus salivarius</i> , <i>Staphylococcus aureus</i> ,
4	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Staphylococcus aureus</i> , Candida albican	4	Gram positive cocci and bacilli ++ Gram negative cocci ++ <i>Staphylococcus aureus</i> , Candida albican <i>Enterococcus faecalis</i>
5	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Staphylococcus aureus</i> , Candida albican	5	Gram positive cocci and bacilli + Gram negative cocci ++ <i>Staphylococcus aureus</i> , Candida albican <i>Enterococcus faecalis</i>
6	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Staphylococcus aureus</i> , <i>Streptococcus salivarius</i> Candida albican	6	Gram positive cocci and bacilli + Gram negative cocci ++ <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i>
7	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i>	7	Gram positive cocci and bacilli + Gram negative cocci ++ <i>Staphylococcus aureus</i> , Candida albican <i>Enterococcus faecalis</i>
8	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Staphylococcus aureus</i> , Candida albican	8	Gram positive cocci and bacilli + Gram negative cocci ++ <i>Staphylococcus aureus</i> , Candida albican <i>Enterococcus faecalis</i>
9	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Streptococcus salivarius</i> Candida albican	9	Gram positive cocci and bacilli ++ Gram negative cocci ++ <i>Enterococcus faecalis</i>
10	Gram positive cocci and bacilli ++ Gram negative cocci + <i>Staphylococcus aureus</i> , <i>Staphylococcus salivarius</i>	10	Gram positive cocci and bacilli + Gram negative cocci ++ <i>Staphylococcus aureus</i> , Candida albican <i>Enterococcus faecalis</i>

Table 2. Showing mean values of zone of inhibition in group A and group B

Mouthrinses	Group A Mean±S.D. (cm)	Group B Mean±S.D. (cm)	'p' value	Significance
CHX	2.09±0.29	2.09±0.33	0.52 (p>0.05)	NS
Hiora	1.99±0.34	1.94±0.38	0.23 (p>0.05)	NS
Pomegranate	1.95±0.41	1.94±0.49	0.36 (p>0.05)	NS
Tulsi	1.62±0.38	1.42±0.42	0.37 (p>0.05)	NS
Clove	1.52±0.47	1.50±0.36	0.29 (p>0.5)	NS
Neem	1.28±0.52	1.31±0.42	0.27 (p>0.05)	NS

S.D.: Standard deviation; NS: Non-significant

Table 3. Comparison of CHX with other other mouthrinses in group A

Comparison of CHX with other mouthrinses in group A	'p' value	Significance
CHX & Hiora	0.259 (p>0.05)	NS
CHX & pomegranate	0.210 (p>0.05)	NS
CHX & Tulsi	0.004 (p<0.05)	S
CHX & Clove	0.002 (p<0.05)	S
CHX & Neem	0.0002 (p<0.05)	S

p: Probability; NS: Non-Significant; S: Significant

Comparison of group A and group B showed non-significant differences in the zone of inhibition as shown in Table 2 (p>0.005), suggesting that antimicrobial efficacy of mouthrinses in both the group were similar.

Table 4. Comparison of CHX with other other mouthrinses in Group B

Comparison of CHX with other mouthrinses in group B	'p' value	Significance
CHX & Hiora	0.06450 (>0.05)	NS
CHX & pomegranate	0.08445 (>0.05)	NS
CHX & Tulsi	0.00012 (<0.05)	S
CHX & Clove	0.00004 (<0.05)	S
CHX & Neem	0.00003 (<0.05)	S

4. DISCUSSION

Chlorhexidine (CHX) is the most common and extensively studied chemical agent for plaque control to date. Its efficacy as a mouth rinse and as a local drug delivery agent to inhibit dental plaque and gingivitis has been well documented [12]. In spite of potent antimicrobial and anti-plaque properties of chlorhexidine, its widespread and prolonged use is limited by its

local side effects. Thus, there is a continued interest in identifying efficient antiplaque agents that could be used daily without side effects. In view of this, herbal products are steadily gaining interest in the present era as they are naturally occurring, hence economical. They also claim to have little or no side effects. Herbal mouthrinse has shown antibacterial and anti-inflammatory effect in few studies [16]. Thus the present study was carried out to compare the efficacy of chlorhexidine gluconate and five herbal mouthrinses on dental plaque. Earlier studies has compared chlorhexidine and hiora mouthrinses; [20,21] chlorhexidine and clove; [22] chlorhexidine and tulsi; [23] chlorhexidine and neem; [24] chlorhexidine and pomegranate [25]. To the best of our knowledge the present study is the first to compare the efficacy of chlorhexidine with all earlier mentioned herbal mouthrinses together. The active ingredients and mechanism of actions of various mouthrinses in present study is addressed in Table 5.

The study resulted out that chlorhexidine, hiora and pomegranate are equally efficacious and has shown nostatistically significant differences (p<0.005). The results of the study are in agreement with the study conducted by Nagesh Bhat et al. [21] who compared the efficacy, safety and antimicrobial properties of a hiora mouthwash with chlorhexidine mouthwash *in vitro* in patients with established plaque and concluded that there was no statistically significant difference in the antimicrobial property between the two mouthwashes. Another study investigated the anti-microbial activity of herbal mouthrinse with Listerine and 0.12% Chlorhexidine gluconate (Peridex) against *S. mutans*, *S. sanguis* and *A. viscosus* [26]. Author reported that herbal mouthrinse produced the largest zone of microbial inhibition when compared to Listerine against all the three bacteria tested. Results of the present study are in contrast to a study which revealed weak antibacterial effect of hiora mouthrinse against

oral bacteria [27]. In view of pomegranate, the finding of the present study are in agreement to Ahuja S, et al. [25] who reported *Punica granatum* is a better antingivitis agent than chlorhexidine.

In the present study tulsi, clove and neem mouthrinse had also shown antimicrobial activity, but their antimicrobial property was less and had shown statistically significant values when compared to chlorhexidine ($p < 0.005$). Similar results were observed in parallel study by Mistry Sk, et al. [26] who compared antimicrobial

activity of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Mimusops elelengi* (Babul), *Tinospora cardifolia* (Giloy) and Chlorhexidine gluconate (CHX) and concluded CHX was the most consistent of all the medicaments testes. In a study Balappanavar AY compared the effectiveness of 0.5% tea, 2% neem, and 0.2% chlorhexidine mouthwashes on oral health and concluded effectiveness of 0.5% tea was more compared to 2% neem followed by 0.2% chlorhexidine mouth rinse [24]. Results of clove and chlorhexidine are in contrast to the study by Dalirsani Z [22].

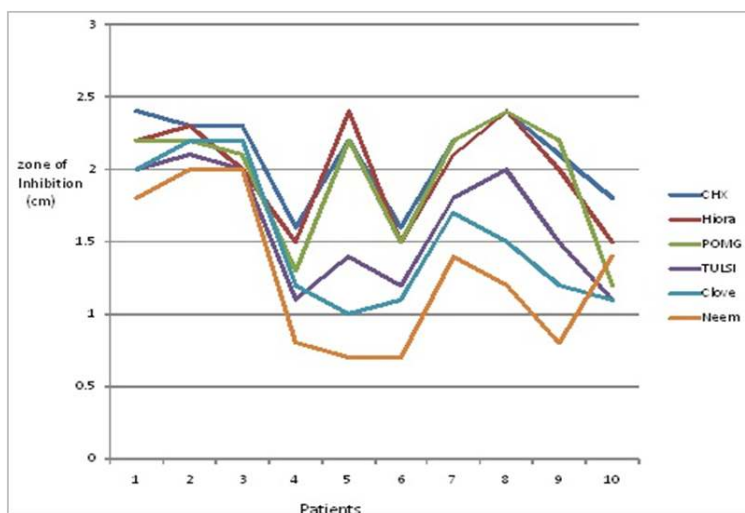


Fig. 4. Comparative evaluation of zone of inhibition (cm) of different mouthrinse in group A

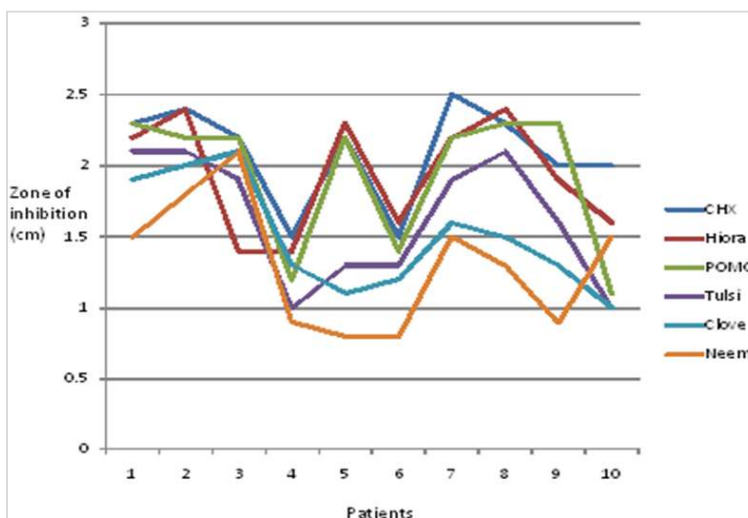


Fig. 5. Comparative evaluation of zone of inhibition (cm) of different mouthrinse in group B

Table 5. Different mouthrinses, their active ingredients and mechanism of action

Mouthrinses	Active ingredient	Mechanism of action
Hexidine mouthwash	0.2% Chlorhexidine gluconate	Chlorhexidine has an affinity for bacteria probably because of an interaction between the positively charged chlorhexidine molecule and negatively charged groups on the bacterial cell wall (e.g. phosphate groups). This interaction increases the permeability of the bacterial cell wall and thus permits the agent to penetrate into the cytoplasm and cause the death of the microorganism.
HiOra mouthwash	Miswak (<i>Salvadora persica</i>), bibhitaka (<i>Terminalia bellerica</i>), gandhapura taila, nagavalli (<i>Piper betle</i>), ela.	Hiora mouthwash has antiplaque, analgesic, antimicrobial, antiseptic, and refreshing properties. It has active herbal ingredient that act against common strains of oral bacteria and fungi and prevent gum and tooth disease. It helps in the prevention and treatment of gum disease. Silica in Miswak acts as an abrasive material to remove stains giving the teeth whiteness. Tannins also inhibit the action of glucosyl transferase thus reducing plaque and gingivitis. The alkaloid present in <i>Salvadora persica</i> is Salvadorine, which yields trimethylamine on hydrolytical cleavage. It exerts a bacteriocidal effect and stimulatory action on the gingiva. The mild bitter taste stimulates the flow of saliva, which is antiseptic. The sulfur compounds present in Miswak as shown by their pungent taste and smell have a bactericidal effect.
Pomegranate (<i>Punica granatum</i>)	Flavonoids (flavonols, flavanols and anthocyanins), condensed tannins (proanthocyanidins) and hydrolysable tannins (ellagitannins and gallotannins).	Hydrolysable tannins account for 92% of its antioxidant properties. Also has broad spectrum activity against both bacteria and fungus. According to Ross et al. the anti-inflammatory effect of pomegranate may be attributed to its considerable immune-regulatory activity over macrophages and T- and B-lymphocyte subsets.
<i>Ocimum sanctum</i> (tulsi) - It is the Queen of Herbs"	Alkaloids, glycosides, tannins and volatile oil (eugenol, thymol, Urosolic acid).	Tannins act as an antioxidant and scavenger against reactive oxygen species and free radicals. Urosolic acid helps in increasing leukocyte count and significantly protects mast cell membrane; thus, preventing cell degradation and histamine release. Linolenic acid present in <i>O. sanctum</i> has the capacity to block both the cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism and hence responsible for its anti inflammatory activity.
<i>Caryophyllus aromaticus</i> (clove)	Eugenol beta-caryophyllene, flavonoids	Eugenol, the primary component of clove's volatile oils, functions as an anti-inflammatory substance. It has beta-caryophyllene, which is a mild anaesthetic as well as an anti-bacterial agent. Clove also contains a variety of flavonoids, including kaempferol and rhamnetin, which also contribute to clove's anti-inflammatory and antioxidant properties. It also has antiseptic property.
<i>Azadirachta indica</i> (Neem)	Phenolic group	Active component phenolic group phenolic group can destroy bacterial cell membrane by disrupting osmotic balance.

Though chlorhexidine has shown maximum efficacy in this study, it has certain side effects on long term use. The reported side effects of CXH are alteration in taste, increase of calculus formation, staining of teeth and mucous membranes and, more rarely, oral mucosa desquamation and parotid swelling. However, the most obvious and important local side effects are the brown staining of the teeth, restorative materials and dorsum of the tongue as well as supragingival calculus formation [28]. Use of chlorhexidine mouthwash with tea, coffee and red wine must be avoided. Also its usage is restricted in cases of anterior composite restorations and glass ionomer restorations [29]. Studies has proved that there should be a 30 minute lapse between the usage of a dentifrice and chlorhexidine mouth wash [30]. It is so advised because the toothpastes contain detergents which are predominantly anionic agents. Chlorhexidine molecule being dicationic in nature tends to bind with the anionic agents leading to a reduction in the substantivity of chlorhexidine mouth rinse. An *In vitro* study revealed that chlorhexidine is toxic to the cultured fibroblast [31].

On the other hand, herbal mouthrinses 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. Hiora and pomegranate which are proved to be equally efficacious to that of chlorhexidine can be substituted with chlorhexidine for such patients.

Chlorhexidine is most active against Gram-positive bacteria, but also has activity against Gram-negative bacteria, anaerobes, fungi and some enveloped viruses. Evidence of chlorhexidine activity against mycobacteria is inconclusive and it has limited activity against non-enveloped viruses. The agent is not active against bacterial spores. Chlorhexidine is known to be less effective in the presence of organic material, such as serum concluding that it a strongest antimicrobial agent we have. It is prescribed to every patient by dentist. Reduced susceptibility to chlorhexidine in Staphylococci species has been documented [32]. We anticipate that clinical use of chlorhexidine will continue to increase, and it will be important to be alert to the possibility that this may lead to the emergence of new clones with reduced susceptibility. Indiscriminate chlorhexidine use should be discouraged. Patients who have gingivitis, mild periodontitis can be prescribed

hiora and pomegranate mouth rinses which have proved equal efficacy.

Chlorhexidine is a second generation mouthwash acting on nonspecific mouthwash killing even the healthy microflora of the oral cavity thus increasing the chances of opportunistic infections like *Candida albicans*. Numerous studies have proved tulsi mouthrinse active against *Candida albicans* infection [33]. The various indications where chlorhexidine a potent antimicrobial can be substituted for herbal mouthrinses with equal efficacy are suggested as:

1. Patient's less compliance to chlorhexidine
2. Healthy, gingivitis and mild periodontitis patient
3. After periodontal surgery as chlorhexidine interfere with fibroblast activity.
4. Patient who regularly use mouthwashes
5. Tulsi has shown antimicrobial activity against *Candida albicans*
6. Patient who have got anterior composite restoration

Taking only periodontal diseases into consideration in this study, use of the chlorhexidine in moderate to severe periodontitis or in conditions where substantivity is utmost importance, is suggested to prevent the resistance of microorganism against this strong antimicrobial agent.

Strength of the present study is that, this study has compared chlorhexidine mouthwash with five different mouth rinses unlike other studies where chlorhexidine was compared with one or two herbal mouthrinses in *in vitro* studies. Results of this study provides a standard, the results of which can be compared with similar other studies.

In interpreting the findings of the present study, it is important to acknowledge possible limitations. The present study has assessed only aerobic microorganism. The results may not correspond to the actual behavior of mouth rinses in *in vivo* because they are not exposed to the same conditions found in the oral cavity. Substantivity exists or not could not be ascertained in this study. Further researches are needed which focus mainly on these areas of herbal mouthrinses.

5. CONCLUSION

In the present study, chlorhexidine gluconate, hiora and pomegranate has shown equal efficacy

to that of chlorhexidine and followed by tulsi, clove and neem has got least among all mouthrinses. Since chlorhexidine has side effects on long term use, use of mouthrinses with equal efficacy (Hiora and pomegranate) has been recommended. Futhermore laboratorial studies are needed to support the performance of further clinical investigations with much larger sample size.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Loe H. Experimental gingivitis in man. J. Periodontol. 1965;36:177.
2. Breuer MM, Cosgrove RS. The relationship between gingivitis and plaque levels. J Periodontol. 1989;60:172-1755.
3. Theilade E, Wright WH, Jensen SB, Løe H. Experimental gingivitis in man: A longitudinal clinical and bacteriological investigation. J Periodontal Res. 1966;1:1-13.
4. Ramires-Romito ACD, Romito GA, Mayer MPA, Rodrigues RMD. Correlation study of plaque and gingival indexes of mothers and their children. J Appl Oral Sci. 2005;13:227-231.
5. Darby I. Non-surgical management of periodontal disease. Aust Dent J. 2009;54:86–95.
6. Blinkhorn A, Bartold PM, Cullinan MP, Madden, Marshall RI, Raphael SL. Is there a role for triclosan/ copolymer toothpaste in the management of periodontal disease? Br Dent J. 2009;3:117-125
7. Bassiouny G, Barrak HA. The anti-plaque effect of miswak and myrrh mouthwashes versus chlorhexidine in the treatment of chronic gingivitis; a comparative clinical trial. Medical Science. 2014;9:28-31.
8. Mali AM, Behal R, Gilda SS. Comparative evaluation of 0.1% turmeric mouthwash with 0.2% chlorhexidine gluconate in prevention of plaque and gingivitis: A clinical and microbiological study. J Indian Soc Periodontol. 2012;16:386-391.
9. Bayalty FH, Kaubaisi AH, Wahid NA, Abdulla MA. Effect of mouthwash extracted from *Salvadora persika* on dental plaque formation: A clinical trial. J. Med. Plant. Res. 2010;4:1446-1454.
10. Malhotra R, Grover V, Kapoor A, Saxena D. Comparison of the effectiveness of a commercially available herbal mouthrinse with chlorhexidine gluconate at the clinical and patient level. J Indian Soc Periodontol. 2011;15:349-352.
11. Goutham BS, Manchanda K, Sarkar AD, Prakash R, Kand J, Mohammed S. Efficacy of two commercially available Oral Rinses - chlorohexidine and listrine on plaque and gingivitis - a comparative study. J Int Oral Health. 2013;5:56-61.
12. Van Strydonck DAC, Slot DE, Van der Velden U, Van der Weijden F. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: A systematic review. J Clin Periodontol. 2012;39:1042–1055.
13. Hellden L, Comosci D, Hoch J, Tinanoff N. Clinical study to compare the effect of stannous fluoride and chlorhexidine mouth rinses on plaque formation. J Clin Periodontol. 1981;8:12-26.
14. Addy M, Moran M, Newcomb R, Warren P. The comparative tea staining potential of phenolic, chlorhexidine and antiadhesive mouth-rinses. J Clin Periodontol. 1995;22:929-934.
15. Gupta D, Bhaskar DJ, Gupta RK, Karim B, Jain A, Singh R, Karim W. A randomized controlled clinical trial of *Ocimum sanctum* and chlorhexidine mouthwash on dental plaque and gingival inflammation. J Ayurveda Integr Med. 2014;5:109-116.
16. Nagappan N, John J. Antimicrobial efficacy of herbal and chlorhexidine mouth rinse -A systematic review. JDMS. 2012;2:5-10.
17. Shetty S, Pillai S, Sridharan S, Satyanarayana A, Rahul A. Comparative efficacy of chlorhexidine and a herbal mouth rinse in patients with gingival inflammation - a clinical & microbiologic study. Asian Journal of Pharmaceutical Technology & Innovation. 2013;1:1-8.
18. Dahham SS, Ali MN, Tabassum H, Khan M. Studies on antibacterial and antifungal activity of pomegranate. American-Eurasian J. Agric. & Environ. Sci. 2010;9: 273-281.
19. Doddanna SJ, Patel S, Sundarrao MA, Veerabhadrapa RS. Antimicrobial activity of plant extracts on *Candida albicans*: An *in vitro* study. Indian J Dent Res. 2013;24:401-415.
20. Bhat N, Mitra R, Reddy JJ, Oza S, Vinayak KM. Evaluation of efficacy of chlorhexidine and a herbal Mouthwash on dental plaque:

- An *in vitro* comparative study. Int J Pharm Bio Sci. 2013;4:625-632.
21. Vahabi S, Najafi E, Alizadeh S. *In vitro* antimicrobial effects of some herbal essences against oral pathogens. J. Med. Plant. Res. 2011;5:4870-4878.
 22. Dalirsani Z, Aghazadeh M, Amirchaghmaghi M, Pakfetrat A. *In vitro* comparison of the antimicrobial activity of ten herbal extracts against *Streptococcus mutans* with chlorhexidine. Journal of Applied Sciences. 2011;11:878-882.
 23. 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;21:3357-3359.
 24. Balappanavar AY, Sardana V, Singh M. Comparison of the effectiveness of 0.5% tea, 2% neem and 0.2% chlorhexidine mouthwashes on oral health: A randomized control trial. Indian J Dent Res. 2013;24:26-34.
 25. Ahuja S, Dodwad V, Kukreja BJ, Mehra P, Kukreja P. A comparative evaluation of efficacy of *Punica granatum* and chlorhexidine on plaque and gingivitis. J Int Clin Dent Res Organ. 2011;3:29-32.
 26. Kaim JM, Gultz J, Do L, Scherer W. An *in vitro* investigation of the antimicrobial activity of an herbal mouthrinse. J Clin Dent. 1998;9:46-48.
 27. Van der Weijden GA, Timmer CJ. The effect of herbal extracts in an experimental mouth rinse on established plaque and gingivitis. J Clin Periodontol. 1998;25:399-403.
 28. Zanatta FB, Antoniazzi RP, Rösing CK. Staining and calculus formation after 0.12% chlorhexidine rinses in plaque-free and plaque covered surfaces: A randomized trial. J Appl Oral Sci. 2010;18:515-521.
 29. Balagopal S, Arjunker R. Chlorhexidine: The gold standard antiplaque agent. J. Pharm. Sci. & Res. 2013 ;5:270-274.
 30. Kolahi J, Soolari A. Rinsing with chlorhexidine gluconate solution after brushing and flossing teeth: A systematic review of effectiveness. Quintessence Int. 2006;37:605-612.
 31. Ghabanchi J, Moattari A, Darafshi R, Andisheh Tadbir A, Khorshidi H, Shakib M. Effects of three commercial mouth rinses on the cultured fibroblasts: An *in vitro* study. Dent Shiraz Univ Med Scien. 2013;14:64-67.
 32. Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: Is it increasing and does it matter? J Antimicrob Chemother. 2012;1-13.
 33. Subramanian G, Tewari BB, Gomathinayagam R. Studies of antimicrobial properties of different leaf extract of tulsi (*Ocimum tenuiflorum*) against human pathogens. AIJCR. 2014;4:149-57.

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