

Evaluation of the Antibacterial and Antifungal Properties of Herbal Mouthwash - A Prospective Study

Running Title- Antibacterial activity of Herbal Mouthwashes

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ABSTRACT

Aim: To Compare and evaluate the antibacterial and antifungal properties of herbal mouthwashes.

Methodology: The mouthwashes tested were Persica and Triphala 6% with reference bacterial strains of *Enterococcus faecalis* (ATCC: 29212) *Staphylococcus aureus* (ATCC: 25923) and *Candida Albicans*. Chlorhexidine gluconate (CHX) 0.2% was used as control. 1 ml of organism suspension made equivalent to Mc Farland 0.5 was contacted with 1 ml of each mouthwash was removed in 5, 8 & 10 min. After 72 hrs of incubation colony counts were measured using a microscope. Kruskal Wallis test and Post hoc tests i.e. Mann Whitney U test and Duncan's-test of multiple comparisons were done.

Results: The number of CFUs dropped to zero after 5 minutes contact time with persica mouthwash with high significance (P = 0.000). CFUs dropped to zero after 10 minutes and remained zero with Triphala mouthwash. Control i.e. Chlorhexidine (CHX) showed significant growth at all time intervals.

Conclusion: Persica mouthwash as a an effective herbal mouthwash.

Keywords: Persica, Herbal, Antibacterial, Mouthwash.

INTRODUCTION

Oral infectious diseases, such as periodontal inflammation, caries, and gingivitis, can be developed by dental biofilm formation.^[1] These kinds of dental problems can cause other serious health diseases.^[2] The recent research suggests that periodontal diseases may contribute to the development of heart diseases^[3] and stroke^[4] and they can compromise the health of patients with diabetes, respiratory diseases, or osteoporosis.^[5] Biofilm bacteria have increased antimicrobial resistance^[6] and, for this reason, the increase in demand for new antimicrobials has led to several investigations toward the search for antimicrobial effects of phytochemicals extracted from a range of species of botanical origin.^[7,8]

Periodontitis is a more severe and destructive gum disease which may progress irreversibly in breaking down supporting periodontal structures.^[9] As it is impossible to eliminate oral bacteria causing dental plaque, it is important to achieve plaque control by limiting growth of harmful bacteria. Complex oral flora makes it difficult for the antimicrobial agent to be targeted at a particular organism in the plaque.^[10]

More than 500 species of bacteria are found in the oral cavity, among them approximately 22 dominant species are recognized. In healthy adults, *Streptococcus* is the dominant aerobic microorganism in oral flora.

Enterococci are regarded as a part of oral microbial flora and form most of the primary dental root infections. They have been isolated from root canals of treated teeth, which had failure after treatment. *Enterococcus faecalis* is a gram positive voluntary anaerobe observed in most human infections with enterococcus.^[11,12]

On the other hand, candidiasis is the most common oral fungal infection which is caused by *Candida*; *Candida albicans*, found in the normal mouth flora, is the most important in this regard.^[13,14] Using mouthwashes is one of the effective methods for reducing oral microbes. In addition to mechanical methods, such as brushing and dental floss, mouthwashes play an important role in reducing dental caries and infectious diseases.^[15] Thus a non specific plaque removal or inhibition has been accepted as the practical approach to control dental plaque.

In the recent years, use of mouthwash has been on the increase as it is relatively easy to use for maintaining oral hygiene. Mouthwashes decrease the risk via reducing the number of microorganisms, their transmission and colonization. Among the mouthwashes, chlorhexidine is considered as the gold standard. The major advantage of chlorhexidine over most other compounds lies in its substantivity. It binds to soft and hard tissues in the mouth, enabling it to act over a long period after application of a formulation.^[16]

Nevertheless, it has several adverse effects such as undesirable tooth discoloration, unpleasant taste, dryness and burning sensation in the mouth, mucosal irritation, dryness and injuries, allergies etc. Therefore, there has always been a tendency to use mouthwashes that have antibacterial effects like chlorhexidine and at the same time have less unwanted effects. To overcome the above disadvantages naturally occurring antimicrobial herbs can be used individually or in combination.^[17]

Thus, in the midst of growing evidence of the connection between oral health and whole body health, herbal medicines with their 'naturally occurring' active ingredients offer a gentle and enduring way for restoration of health by the most trustworthy and least harmful way. Herbal medicine is both promotive and preventive in its approach. It is a comprehensive system, which uses various remedies derived from plants and their extracts to treat disorders and to maintain good health. Natural herbs like triphala, tulsi patra, jyestiamadh, neem, clove oil, pudina, ajwain and many more used either as whole single herb or in combination have been scientifically proven to be safe and effective medicine against various oral health problems like bleeding gums, halitosis, mouth ulcers and preventing tooth decay. The major strength of these natural herbs is that their use has not been reported with any side-effects till date and by use of a herbal mouth rinse, ingredients which causes halitosis are avoided which itself is one step forward towards better oral hygiene and better health.^[18]

Persica® mouthwash contains three medicinal plants, *Salvadora persica*®, Yarrow and Mint. The antibacterial effects of persica® are *In vitro* and on the pathogens that are important in dentistry. It has been shown that using this herbal medicine or its extract would support periodontal health, and reduces the accumulation of microbial plaques^[19], and bleeding during brushing^[20,21] and controls gingivitis and periodontal diseases.^[20-22]

Triphala is a key ingredient used in Ayurveda since time immemorial. It is a botanical preparation consisting of equal parts of three herbal fruits: Harada (*Terminalia chebula*), amla (*Emblica officinalis*), and bihara (*Terminalia bellerica*). Much revered in Ayurveda, triphala has been proven to have antibacterial, antiviral, and antifungal actions. It is also said to possess antihistamine, anti-inflammatory, antioxidant, antitumor, blood pressure lowering, cholesterol lowering, digestive, diuretic, and laxative properties.^[23,24]

The aim of this study is to evaluate the antimicrobial effects of herbal mouthwashes i.e. persica and triphala mouthwash.

MATERIALS AND METHODS

MOUTHWASH

The mouthwash tested in the study were persica and triphala mouthwash. Triphala mouthwash 6% was prepared by dissolving sixty grams of pure triphala churna in 1 liter of distilled water to obtain 6% of the extract. Chlorhexidine 0.2% is served as control.

BACTERIAL INOCULATION OF SPECIMENS

Reference bacterial strains of *Enterococcus faecalis* (ATCC: 29212), *Staphylococcus aureus* (ATCC: 25923) and *Candida Albicans* was obtained from IMTECH, Chandigarh. All the bacterial strains were plated on BHI (Brain Heart Infusion) broth supplemented with 1.5% (wt/vol) agar (Himedia laboratories, Mumbai, India) and incubated anaerobically at 37°C for 24 hours. A single colony of bacteria from a BHI agar plate was collected and suspended in

sterile BHI broth at 37°C. Microbial cells were diluted with distilled water to reach the concentration of 1.6×10^8 CFU/ml (adjusted to Mc Farland 0.5).

1 ml of each organism suspension was contacted with 1 ml of mouthwash and subsequently, one hundred microliters of each mixture was removed in 5 min(t5), 8 min(t8) & 10 min(t10) time interval. Each contact period sample is taken and plated on Brain Heart Infusion agar to determine the number of colony forming unit (CFU) per plate. After 72 hrs of incubation at 37°C colony counts were measured using a microscope. The mean number of CFUs in the 3 areas of bacterial growth on each plate was determined and the number of CFU/mL was calculated for each contact period and analysed stastically.

STATISTICAL ANALYSIS

Mean number of colony forming units (CFU) were evaluated for differences among the mouthwashes by using Kruskal Wallis test. Pairwise differences among the groups were evaluated by Post hoc tests i.e. Mann Whitney U test and Duncan's-test of multiple comparisons.

RESULTS

The CFU ml⁻¹ of Staphylococcus aureus, E.faecalis and Candida Albicans after the application of the tested mouthrinse solutions at different contact times i.e. t5, t8 and t10 were evaluated.

The number of CFUs dropped to zero after 5 minutes and remained zero after 8 minutes and 10 minutes contact time with Persica mouthwash and CFUs dropped to zero after 10 minutes and remained zero with Triphala mouthwash with E.faecalis and Candida Albicans as tested organism shown in Figure 1. Control i.e. Chlorhexidine (CHX) showed significant growth of bacteria with chlorhexidine (CHX) 0.2% mouthwash after 5 minutes and 8 minutes of contact period, reduced slightly after 10 minutes with Staphylococcus aureus as tested organism shown in Figure 2 whereas showed bacterial growth at all time intervals with E.faecalis and Candida Albicans. A Kruskal Wallis test was conducted to evaluate differences among the mouthwashes on mean number of CFU. The test was highly significant (P = 0.000). Post hoc test i.e. Mann Whitney U test were conducted to evaluate pair wise differences among the groups. The results of these tests indicated a significant difference between Persica and Triphala group.

Persica Mouthwash showed significantly high antibacterial activity against all the tested organisms whereas Triphala showed mild effects and Chlorhexidine were completely ineffective for Enterococcus faecalis (P < 0.05) and Candida Albicans (P < 0.05).

DISCUSSION

Persica mouthwash was also shown to have antibacterial activity in vitro. In another study with 1:32 dilution it has been shown that the mouthwash inhibited the bacterial growth. The main component of persica is Miswak herb extract. The reduction of micro-organisms could be related to the various agents available in which might release agents in saliva which could prevents their bindings to the tooth by interfering with bacterial function.^[25]

CHX is considered the gold standard because of its superior antiplaque effects. CHX rinsing can cause a number of local side effects, such as extrinsic tooth and tongue brown staining, taste disturbance, and enhanced supragingival calculus formation. CHX rinsing can also cause desquamation of the oral mucosa, but this is less common. On the other hand, because of its natural ingredients, Persica does not cause any side effects.

In this study, antibacterial effect of Persica on E.faecalis, Staphylococcus aureus and Candida Albicans was evaluated and compared with that of CHX. In the present study, all the tested solutions significantly reduced the microorganisms in oral cavity in a period of 5 minutes whereas 0.2% CHX didn't showed any reduction.

Noumi E et al, Khalessi AM et al, Sanei A et al in their studies investigated the effect of miswak or its extract on the microbial count of the mouth, a significant decrease in the level of different bacteria was seen.^[26,27,28] The study by Poorshahidi et al. reported more obvious antiviral effects of persica when compared to chlorhexidine and acyclovir.^[29] In 1999, Almas showed an antibacterial effect of Salvadora persica extract 50.0% on Streptococcus mutans and Enterococcus faecalis.^[30] However, laboratory studies by Yaghooti et al. reported a lack of sensitivity in enterococci and fungi when using persica; such results could be explained considering the in vitro situation of the study.^[31] Noumi et al. reported strong antifungal effects of persica against all species of candida.^[26] Furthermore, Mozaffari et al. demonstrated that although Persica mouthwash and chlorhexidine do not have similar antimicrobial effects, persica could be applied in case of chlorhexidine contraindications.^[32] Sanei et al. reported that siwak has antibacterial effect and is helpful for plaque control.^[28]

In general, the results of study done by Shafiei Bafti L et al suggested that persica significantly decreases the level of Enterococcus faecalis and Candida albicans.^[33]

In a study by Sadeghi et al., the effect of chlorhexidine on common oral bacteria was more prominent compared to persica.^[34]

In this regard, the study conducted by Salehi et al. could be mentioned where persica and chlorhexidine were compared, and it was mentioned that 13.0% and 86.0% showed discoloration in case of persica and chlorhexidine usage, respectively. Salehi et al. suggested the use of persica to limit bacterial count amongst orthodontic patients. They reported the better results of persica compared to chlorhexidine considering less tooth discoloration and a significant decrease in the microorganism level around the base of orthodontic braces.^[35] Regarding the direct and desirable antiviral effect of persica compared to acyclovir, its herbal origin, and fewer side effects in comparison with chlorhexidine, Poorshahidi et al. suggested the use of persica to decrease oral fluid contamination resulting from viral shedding and infections of intraoral wounds.^[29]

The study conducted by Atai et al. revealed very poor effects of Persica mouthwash on *Candida albicans*.^[36] However, Al-Bagieh et al. reported the fungistatic effects of Persica on *Candida albicans*.^[37] Although most studies have been conducted on the antimicrobial effects of Persica mouthwash are in-vitro, more in-vivo studies are needed due to the different and paradoxical presented results. There is a lack of sufficient evidence for antibacterial and antifungal effects of Persica mouthwash, especially on some microorganisms.

CONCLUSION

In all the tested mouthwashes, Persica Mouthwash showed significantly high antibacterial activity against all the tested organisms whereas Triphla mouthwash had mild effects on *Staphylococcus aureus*. Triphla and Chlorhexidine mouthwash were completely ineffective for *Enterococcus faecalis* and *Candida Albicans*.

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