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ORIGINAL RESEARCH

In vitro Antimicrobial Comparison of Chlorhexidine, Persica Mouthwash and Miswak Extract

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ABSTRACT

Aim: Herbal mouthwashes, such as persica (*Salvadora persica*, mint and yarrow extracts) and miswak extract have been shown to decrease gingival inflammation and plaque accumulation. The aim of this study was to compare the antimicrobial activities of persica and miswak extract with the conventional mouthwash chlorhexidine against *Streptococcus salivarius*, *Streptococcus sanguis*, *Lactobacillus vulgaris* and *Candida albicans*.

Materials and methods: In this *in vitro* study, blood-agar culture (Merk, Germany) was used to grow the streptococcus strains, saburd-dextrose culture (Merk, Germany) was used to grow *C. albicans* and MRS-agar was used to grow *L. vulgaris*. Various concentrations of these substances (0.1, 0.05 and 0.025% of miswak extract, 0.1, 0.05, 0.025 and 0.0125% of persica, 0.2, 0.1, 0.05 and 0.025% of chlorhexidine) were added to paper disks, separately, inserted into culture plates and transferred into the incubator. The inhibition zone around each disk was measured after 24 hours and the data was analyzed by the Kruskal-Wallis test.

Results: Chlorhexidine possessed antibacterial activity at all concentrations tested. It was more effective than persica and miswak at all concentrations on *S. salivarius* ($p = 0.022$ for 0.1%, 0.009 for 0.05 and 0.025%). It had greater effect than the other two tested material on *S.sanguis* only at concentration 0.01%. Chlorhexidine was the most effective against *S.salivarius*; persica was the most effective against *Lactobacillus* ($p = 0.005$) and the least effective against *S. salivarius*; and miswak extract was the most effective against *S. salivarius* and *S. sanguis* at concentrations 0.1 and 0.05% ($p = 0.005$) and ineffective against *L. vulgaris*. None of these mouthwashes were effective against *C. albicans*.

Conclusion: This study revealed that chlorhexidine remains the gold standard as an antimicrobial agent, although herbal-based mouthwashes do have marginal antimicrobial activities. It is necessary to conduct more clinical and microbiological studies focusing on periodontal pathogens and anaerobic microorganisms.

Clinical significance: Mechanical plaque control is the main way for periodontal disease prevention and mouthrinses are used to improve its efficacy. Based on the results of this study, chlorhexidine has the most antibacterial effect and although persica mouthwash and miswak are routinely used in some Asian countries their antibacterial efficacies are suspected.

Keywords: Persica mouthwash, Chlorhexidine, Miswak extract, Antimicrobial activity.

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INTRODUCTION

Dental plaque is known as the prime initiating factor for dental caries and periodontal diseases. Thus, several methods to reduce the amount of dental plaque have been reported, including mechanical (toothbrush, toothpaste and miswak chewing stick) and chemical methods.[1]

Chemical methods of plaque elimination involve the use of mouthwashes. Mouthwashes have a significant role in decreasing the amount of microbial plaque.[2] Among all available mouthwashes, chlorhexidine is highly capable of reducing plaque and pathogenic microorganisms, such as *Streptococcus mutans*, and is the gold standard.[2,3] Nonetheless, chlorhexidine has certain adverse effects, such as tooth discoloration, unpleasant taste alteration, xerostomia, and burning sensation, which may prevent its use.[4,5] Recently, there has been a rising trend toward the use of herbal mouthwashes like miswak extract and persica.[6-8] *Salvadora persica*, a member of Salvadoraceae family, is grew up in Africa, South America, Asia and the Middle East including Saudi Arabia. Its derived chewing sticks are widely used in these countries as toothbrush. This plant is locally named as miswak, siwak or arak in different countries.[9,10] Many studies have demonstrated the anti-microbial properties of aqueous extracts of chewing sticks. Miswak extract also acts on cariogenic bacteria and periodontal pathogens and disrupts plaque accumulation.[11,12]

Persica is a herbal mouthrinse made of *Salvadora persica*, mint and yarrow extracts (Poursina Company, Tehran, Iran).

It been shown that both of above-mentioned mouthwashes improve periodontal health,[13] reduce microbial plaque accumulation[13] and decrease bleeding on brushing.[14,15]

Studies that have compared the anti-inflammatory activities of chlorhexidine and persica showed that both of these mouthwashes reduce gingival inflammation without any significant difference.[12,16,17] However, only a few studies have compared the antimicrobial activity of chlorhexidine with herbal mouthwashes like persica and miswak extract on certain types of bacteria or fungi. The aim of this study was to compare the antimicrobial activities of chlorhexidine, persica and miswak extract against *Streptococcus salivarius*, *Streptococcus sanguis*, *Lactobacillus vulgaris* and *Candida albicans.*

MATERIALS AND METHODS

In this *in vitro* study, blood-agar culture (Merk, Germany) was used to grow the streptococcus strains, saburd-dextrose culture (Merk, Germany) was used to grow *C. albicans*, and MRS-agar was used to grow *L. vulgaris*. After culturing oral secretions of a patient infected with *C. albicans* in saburd-dextrose media and incubation for 1 week, tube test and colony sampling were done. Methylene-blue and Gram staining confirmed the existence of *Candida*. Each plate was divided into several parts on the basis of the applied solution's concentration as well as that of the extract. For each concentration, three plates were utilized. Then, under a class 3 hood, 1.8×10^8 CFU/ml concentration of each bacteria were prepared and cultured on appropriate media by means of a sterile loop. Concentration disks were placed on culture plates using sterile forceps. Following disk placement, the *Lactobacillus* plate was immediately moved to a 37°C anaerobic incubator while the other bacterial plates remained in an ordinary incubator.

After placing the bacterial cultures in an incubator for 24 hours, the inhibition zone was measured. Because of the slow growth of *Candida*, plates containing its samples were sealed all around using tape and were incubated in freezer bags for 5 days. Ultimately, after 5 days, the inhibition zone was measured.

Disk Preparation

The procedures were done on blank disks (Iran Daru, Iran) and solutions under a class 3 laminar hood. First, the hood was completely disinfected with 10% hypochlorite. Then, the UV lamp was turned on for 15 minutes. During the process, the air pumps, which pump the inside air out, were active. Samplers were completely cleaned using disinfectants and were transferred into the hood along with sampler caps, tubes, extract and solutions. One gram of miswak extract was dissolved in 10 ml of distilled water (0.1%); and in order to acquire total solubility, a few drops of methanol were added as well. Solutions of 0.05 and 0.025% were prepared in sterile tubes and were filtered through 10 µm filters and 10 ml syringes to assure sterility. Similarly, chlorhexidine solutions of 0.2, 0.1, 0.05 and 0.025% and persica (Pursina co, Iran) solutions of 0.1, 0.05, 0.025 and 0.0125% were prepared and filtered. Blank disks were distributed into sterile plates using sterile forceps and were sampled once by a 10 µl sampler. After drying, another 10 µl sample was added to the plates. The disks remained under the hood until the extract and the solutions were completely dried onto the disks. Thereafter, they were transferred into sterile capped glass boxes and placed in a refrigerator at 4°C.

The inhibition zone dimensions around various concentrations of the solutions were statistically analyzed using the Kruskal-Wallis nonparametric test.

RESULTS

As shown in Table 1, chlorhexidine inhibited the growth of *S. salivarius* better than miswak and persica, at the concentrations tested. In addition, 0.2, 0.1 and 0.05% of chlorhexidine all inhibited the growth of *S. salivarius* equally, while the lowest concentration tested had less of an effect. Miswak extract at 0.1% caused inhibition of

Table 1: Comparison between the effect of various concentrations of chlorhexidine, persica and miswak extract on *S. salivarius* growth

Antimicrobial agent	*Concentration (%)*	*Mean (inhibition zone) (mm)*	*SD*	*Median (mm)*	*p-value*
Chlorhexidine	0.2	2.97	0.1707	2.95	0.034
	0.1	2.97	0.1707	2.95	
	0.05	2.97	0.1707	2.95	
	0.025	2	0.0816	2	
Miswak	0.1	0.97	0.1707	0.95	0.009
	0.05	0.002	0.005	0	
	0.025	0	0	0	
Persica	0.1	1	0.0816	1	0.009
	0.05	0	0	0	
	0.025	0.002	0.005	0	
	0.0125	0.002	0.005	0	

S. salivarius that was significantly different than the lower concentrations of miswak extract tested ($p < 0.009$). Similar results were obtained for persica mouthwash.

The effects of chlorhexidine, miswak and persica on *S. sanguis* were also obtained (Table 2). Again, chlorhexidine was the most effective at inhibiting bacterial growth in this strain, while miswak extract was less potent and persica was ineffective at the concentrations tested.

Interestingly, both chlorhexidine and persica at 0.1% were able to inhibit *Lactobacillus* growth to a similar degree, while miswak did not cause an effect at any concentrations tested (Table 3). In fact, 0.05% of chlorhexidine was shown to be much less effective; while persica at this concentration and even 0.025% was just as effective as at the higher dose.

Using the same concentrations of chlorhexidine, persica and miswak extract to test their effects on *C. albicans*, it was found that these agents did not inhibit the growth of this fungus at all.

Chlorhexidine at 0.2% concentration showed the greatest effect against *S. salivarius*, after that against *S. sanguis* and *Lactobacillus*. It had no effect on *C. albicans* ($p = 0.002$).

The results showed that 0.1% concentration of chlorhexidine was the most effective against *S. salivarius* while less effective against *S. sanguis* and *Lactobacillus* ($p = 0.005$). Likewise, 0.1% of miswak extract was the most effective against *S. salivarius* and *S. sanguis* and ineffective against *Lactobacillus* ($p = 0.005$). However, 0.1% of persica was effective against *S. salivarius* and *Lactobacillus* and ineffective against *S. sanguis* ($p = 0.005$).

At concentration of 0.05%, chlorhexidine was the most effective on *S. salivarius*, less effective on *Lactobacillus* and no effective on *S. sanguis* ($p = 0.005$). This concentration of miswak extract had equal effect on *S. salivarius* and *S. sanguis* and no effect on *Lactobacillus* ($p = 0.005$). At this concentration persica was the most effective on *S. salivarius* and *Lactobacillus* respectively and no effective on *S. sanguis* ($p = 0.005$).

Chlorhexidine 0.025% was effective on *S. salivarius* and none effective on other microorganisms ($p = 0.025$). Miswak extract had no effect on all microorganisms and persica at this concentration had the most effect on *Lactobacillus*, less on *S. salivarius* and no effect on *S. sanguis* ($p = 0.005$).

At concentration of 0.0125%, persica had no effect on microorganisms.

Graphs 1 to 3 show antibacterial activity of different concentrations of chlorhexidine, persica and miswak extract against tested microorganisms.

DISCUSSION

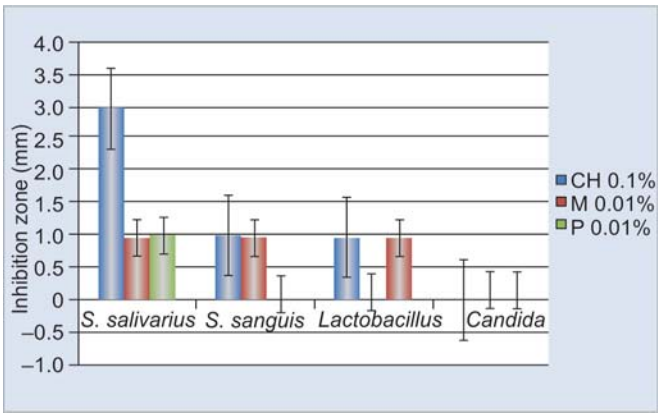
The application of antiseptic mouthwashes has been widely accepted as a complementary method for plaque control. As a cationic mouthwash, chlorhexidine is the most effective

Table 2: Comparison between the effects of various concentrations of chlorhexidine, persica and miswak extract on *S. sanguis* growth

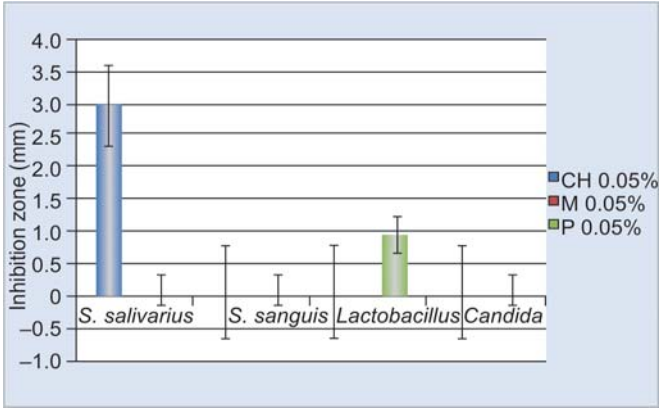
Antimicrobial agent	*Concentration (%)*	*Mean (inhibition zone) (mm)*	*SD*	*Median (mm)*	*p-value*
Chlorhexidine	0.2	1.97	0.1707	1.95	0.002
	0.1	1	0.0816	1	
	0.05	0	0	0	
	0.025	0	0	0	
Miswak	0.1	0.97	0.1707	0.95	0.009
	0.05	0.002	0.005	0	
	0.025	0	0	0	
Persica	0.1	0	0	0	1
	0.05	0	0	0	
	0.025	0	0	0	
	0.0125	0	0	0	

Table 3: Comparison between the effects of various concentrations of chlorhexidine, persica and miswak extract on *Lactobacillus* growth

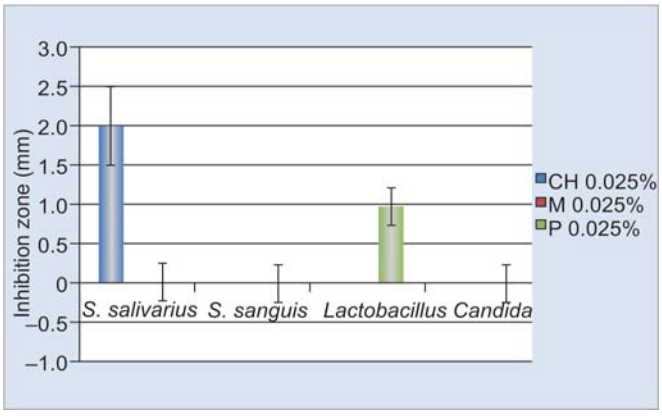
Antimicrobial agent	*Concentration (%)*	*Mean (inhibition zone) (mm)*	*SD*	*Median (mm)*	*p-value*
Chlorhexidine	0.2	0.97	0.1707	0.95	0.006
	0.1	0.97	0.1707	0.95	
	0.05	0.002	0.005	0	
	0.025	0	0	0	
Miswak	0.1	0	0	0	1
	0.05	0	0	0	
	0.025	0	0	0	
Persica	0.1	0.97	0.1707	0.95	0.033
	0.05	0.97	0.1707	0.95	
	0.025	0.97	0.1707	0.95	
	0.0125	0.002	0.005	0	



Graph 1: Comparison of inhibition zone measurements against *S. salivarius*, *S. sanguis*, and *L. vulgaris* for chlorhexidine, persica and miswak extract (0.1%)(mean and standard error)
*p-value for difference between effect of mouthwashes against *S. salivarius* was 0.022
**p-value for difference between effect of mouthwashes against *S. sanguis* was 0.019



Graph 2: Comparison of inhibition zone measurements against *S. salivarius*, *S. sanguis* and *L. vulgaris* for chlorhexidine, persica and miswak extract (0.05%) (mean and standard error)
*p-value for difference between effect of mouthwashes against *S. salivarius* was 0.009
**p-value for difference between effect of mouthwashes against *Lactobacillus* was 0.009



Graph 3: Comparison of inhibition zone measurements against *S. salivarius*, *S. sanguis* and *L. vulgaris* for chlorhexidine, persica and miswak extract (0.025%) (mean and standard error)
*p-value for difference between effect of mouthwashes against *S. salivarius* was 0.009
**p-value for difference between effect of mouthwashes against *Lactobacillus* was 0.005

among antiseptic mouthwashes and is commonly used.[2,3] In addition, chlorhexidine has been introduced as the gold standard of mouthwashes. Numerous clinical studies have substantiated the efficiency of persica mouthwash in plaque elimination as well as gingivitis.[15,17] However, data is scarce regarding the antibacterial activity of persica. Regarding miswak extract, some studies have reported that this extract has antiplaque characteristics and contains pharmaceutical agents.[12,18] It has been claimed that the mechanical effect of miswak chewing sticks in plaque elimination is similar to ordinary toothbrushes.[19] Additionally, epidemiological studies in Saudi Arabia have demonstrated that periodontal diseases are less common among people who habitually use miswak sticks.[20]

The antimicrobial tests in our study showed that chlorhexidine is more potent compared to persica mouthwash and miswak extract in both therapeutic and more dilute concentrations. The antimicrobial activity of chlorhexidine has been confirmed in a study which applied 125 times more dilute concentration than the 0.025% concentration used in this study.[21] In addition, our study showed persica mouthwash to have less antimicrobial activity than chlorhexidine, which is consistent with other studies.[22] Recently, it has been indicated that 50% miswak extract possesses antimicrobial characteristics against *Streptococcus mutans* and *Streptococcus faecalis*.[23] Similarly, in our study it was shown that miswak extract has antimicrobial characteristics at more dilute concentrations (0.1 and 0.05%). In addition, the finding that miswak extract possesses the least antimicrobial activity is similar to other studies.[24]

In the present study, miswak extract at 0.1% concentration produced an inhibition zone of 1 mm around *S. salivarius* and *S. sanguis*, while it was totally ineffective against *Candida*. This finding was inconsistent with that of other studies which reported the activity of miswak extract against *Candida*.[25,26] This inconsistency might be attributed to the fact that a higher concentration and an aqueous solution were used in that study. In addition, the inactivity of miswak extract against *Lactobacillus* at all concentrations tested was similar to the findings of other studies.[18,23,27]

The cationic structure of chlorhexidine accounts for its bacteriostatic and bactericidal characteristics, which result from penetration through the cell membrane and eventually leads to cytoplasm coagulation.[28] However, persica and miswak contain antimicrobial agents in their components. Chloride, sulfate, nitrate, and isothiocyanate have been detected in miswak extract.[29] Owing to its nitrate content, miswak extract has antimicrobial activity against *S. faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[30]

It has been shown that its aqueous and methanol extracts has antibacterial activity against other isolated oral pathogens , *Streptococcus mutans*, *Streptococcus pyogenes* and some dental plaque and cariogenic bacteria.[26,31,32]

Although the antimicrobial activity of persica mouthwash in culture media is far less than that of chlorhexidine, clinical studies have substantiated the positive effects of persica on the treatment of periodontal diseases.[33] It should be noted that the activity of all mouthwashes are attenuated in the oral cavity due to the presence of saliva glycoproteins which tend to bond to cationic radicals, perpetual cleansing effect of saliva, limitation of time microorganisms are exposed to mouthwash and ultimately the presence of microbial flora as well as diversity of food consumed.

Thus, considering the poor *in vitro* characteristics of persica mouthwash and miswak extract and their attenuation in the oral cavity, their application as effective antiseptics is still a matter of debate. Although miswak extract is a component of persica mouthwash, the increased antimicrobial activity of persica mouthwash might be due to the existence of other components, e.g. mint and yarrow extracts.

CONCLUSION

The present study clearly proves the antimicrobial activity of chlorhexidine, while demonstrating only marginal effects for persica and miswak extract. Therefore, conducting similar clinical and microbiological studies focusing on anaerobic and pathogenic microorganisms is strongly recommended.

CLINICAL SIGNIFICANCE

Mechanical plaque control is the main way for periodontal disease prevention and mouthrinses are used to improve its efficacy. Based on the results of this study, chlorhexidine has the most antibacterial effect and although persica mouthwash and miswak are routinely used in some Asian countries their antibacterial efficacies are suspected.

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