



Comparison of the Antibacterial Effects of Jaftex Herbal Mouthwash with Matrica and Persica on *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus salivarius* and *Lactobacillus casei*

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ABSTRACT

Introduction: Using herbal mouthwashes improve oral hygiene because of their antimicrobial activity and dental plaque control. The objective of this experimental study was to compare the antibacterial effects between Persica and Matrica mouthwashes, and Jaftex herbal mouthwash.

Materials and Methods: The standard strains of four pathogenic bacteria involved in the formation of dental plaque were used in the present experimental study, including *S. mutans*, *S. Salivarius*, *S. sanguis*, and *Lactobacillus casei*. The minimum inhibitory concentration (MIC) was evaluated by the tube dilution method for each mouthwash, and the growth and non-growth were determined in each tube. The minimum bactericidal concentration (MBC) was determined for each mouthwash using culturing in tubes, then on Muller Hinton Agar specific gel medium, and incubation in a specific temperature and period. In each case, the lowest concentration with no growth was recorded as MBC. The data were analyzed by using ANOVA test ($p < 0.05$ were considered significant).

Results: The MIC value of Persica mouthwash was 1 micrograms/ml for all bacteria. This value was higher than Jaftex mouthwash MIC for all four bacteria and Matrica mouthwash MIC for *S. mutans* and *S. saliviris*. The MIC value for Matrica and Persica mouthwashes was the same for *S. sanguinis* and *L. casei*. Although the MIC and MBC values of Matrica mouthwash for *S. sanguinis*, *S. salivirus* and *L. casei* were higher than Jaftex, they showed the same MIC and MBC value for *S. mutans* compared to Jaftex mouthwash.

Discussion and Conclusion: Jaftex mouthwash was more effective in inhibiting bacterial growth compared to Matrica and Persica. The antibacterial effects of Matrica the antibacterial effects of Matrica on *Streptococcus mutans* were similar to Jaftex, and Persica showed the lowest antibacterial effect compared to the two other types of mouthwash. Therefore, its utilization in controlling the dental plaque is recommended.

Key words: Persica, Jaftex, Mouthwash, Matrica, Oral microorganisms

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INTRODUCTION

Dentistry comes with a huge sense of attraction, and at the same time, it has specific dangers. Dentistry has a

close relationship with other fields of science, especially medicine [1-6]. The microbial flora of the mouth has a large diversity, as up to now, an estimated number of over 500 species of them are recognized in the mouth. Today, we know that dental plaque has a major role in tooth decay and periodontal diseases [7]. The dental plaque is a complex of developing and reproducing microorganisms, epithelial cells, leukocytes, and

macrophages. The bacteria are responsible for 80-70 percent of the plaque [8,9]. Mouthwashes have a major role as in controlling plaque along with mechanical methods such as brushing and using dental floss, which leads to a reduce in the number of bacteria in the mouth, and eventually reduces the occurrence of the cavity [10]. The desirable mouthwash should have a proper antimicrobial spectrum, and minor drug resistance at the same time. As well, it should damage the natural microflora of the mouth at the minimum level [11,12]. Chlorhexidine gluconate (CHX) is the most effective chemical antimicrobial mouthwash approved by the food and drug administration, which is used as a golden standard for controlling the microbial plaque [7-9]. Despite that, it has a number of undesirable effects which include alternations in the sense of taste, dryness and burning in the mucosa, negative systemic effects on swallowing, and change of color in restorations having the same color as the tooth [13-16]. There has been more tendency to herbal medicines over the past years due to their antimicrobial, antifungal, and anticancer effects, as well as their fewer side effects [8,12,17]. Even students of the schools learn about using natural and organic products in their textbooks [18]. Herbal mouthwashes are more proper than chlorhexidine because they contain organic compounds, fit more with body physiology, and they have lower toxicity; therefore, they are recommended for people who cannot use chemical mouthwashes [15,19]. Oak is one of these medicinal plants for which several therapeutic properties have been mentioned. Persian oak is a tree with 2 meters' height, large spherical corona, being in *Fagaceae* family. The thin exocarp of the fruit, which covers all around the oak fruit, is called "Jaft", which has medical and industrial uses. Jaft has a huge impact on treating bacterial and viral diseases [14]. The antimicrobial properties of different oak species are discussed in the literature. Oak fruits are known for having a disinfectant effect [20]. *Matrica* is an herbal mouthwash, which is produced under the name of "Cami Sel" in Iran. The main bioactive ingredient of this mouthwash is the chamomile extract, which interrupts microbial growth [16]. Its antimicrobial properties are mostly because of bisabolol flavonoids, which facilitate mucosal wound healing. Despite that, this mouthwash has a number of disadvantages including the formation of pigmentation the surface of the teeth and an unpleasant smell, which has limited its use despite its proper antimicrobial effects [15,21-23]. *Persica* is an herbal mouthwash containing extract of *Salvadora persica*, mint, and yarrow. *Salvadora persica* is a rich source of fluoride, isothiocyanate, vitamin C, silica, resin, calcium salt, chloride, tannin, and tannic acid. In contact with the saliva, the isothiocyanate releases cyanide, which can prevent the growth of bacteria in the mouth [13]. However, results from the antibacterial effect of this mouthwash on some bacteria of the oral area such as *Streptococcus mutans* are controversial [15]. Jaftex is a

new herbal mouthwash that is consisted of oak jaft (Oak Fruit) aquatic extract as a base, thyme (*Zataria multifio*) aquatic extract, and aquatic extract of *Saturej bachtiarica*. The plant extracts were obtained from pharmaceutical plants growth center of Ahvaz Jundishapur University of Medical Sciences, southwest of Iran. The base plant for this herbal mouthwash was the oak jaft [24,25]. Several epidemiological studies have reported that streptococci are the main cause of tooth cavity. Therefore, the study samples consisted of four groups including *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus salivaris*, and *Lactobacillus casei* [12,19]. The objective of this study was to compare the antibacterial effects of Jaftex herbal mouthwash with *Matrica* and *Persica* on some common oral microorganisms.

MATERIALS AND METHODS

This laboratory study was conducted in the Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, southwest of Iran, in 2017. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Jaftex, *Matrica* (Barijessence pharmaceutical) and *Persica* (Barijessence pharmaceutical) mouthwashes on common oral cavity bacteria were investigated using serial dilution method.

For this purpose, the studied bacteria were obtained from the Institute of fungi and bacteria collection of Iran as lyophilized vials. The bacteria included *Streptococcus mutans* (PTCC=1683), *Streptococcus sanguinis* (PTCC=1449), *Streptococcus salivaris* (PTCC=1448), and *Lactobacillus casei* (PTCC=1608). In the next step, the contents of the vials of these bacteria were mixed by a sterile pipette and transferred to a test tube containing broth medium by the same tool. One drop of the bacteria suspension was dropped on a plate containing Agar medium (Merck/Germany) and then it was spread using the mixed method. Then, both solid and liquid culture media were incubated at 37°C.

To determine the minimum inhibitory concentration of growth, four rows of 12 test tubes (totally 48) were prepared and labeled for each pathogen specimen. In the next step, except for the tubes in the first column, all tubes (containing 2 ml of each mouthwash, and 0.1 ml suspension of each pathogen) were filled with 1 ml of the culture medium by a sterile sampler. Then, 1 ml of each mouthwash was injected into all tubes in the second column to obtain a dilution of 1:2. After that, 1 ml of the tube solution was removed by the sampler from the second column and was transferred to the tubes of the third column, and the dilution series was done.

Therefore, tubes number 1 in each row contained a 100% dilution of mouthwash [1], the tubes of the second column contained 50% dilution of mouthwash (1:2), the third column tubes contained 25% dilution of the mouthwash (1:4), and 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, and 1:512 dilutions were achieved [24].

Separate microbial suspensions were prepared from the microorganisms, and 100 µl (0.1 ml) of that was transferred to all test tubes using a sterilized sampler.

Each tube was mixed with a vibrator immediately after transferring so that the bacteria were uniformly spread in the liquid culture medium. Tubes in column 11 in all rows were selected as a negative control, so they contained only 2 ml of culture medium. As well, tubes in column 12 in all rows were selected as a positive control, so they contained 2 ml of culture medium and 0.1 ml of each pathogen's suspension.

All stages of mouthwash dilution preparation and transferring to tubes were separately implemented under a laminar hood and under aseptic conditions for each pathogen. Tubes containing bacterial pathogens were incubated at 37°C. The results of each tube were recorded in the next step, no need to mention that most of them were visually visible. It should be noted that each completely clear environment in terms of transparency was recorded as a negative (no growth), and any environment showing a clear turbidity in terms of microbial growth, was considered as a positive (bacteria growth). MIC results were inspected and recorded for each tube (by eye), and the tube with the lowest mouthwash concentration and no growth of bacteria was considered as the MIC.

In order to determine the MBC of mouthwash for each of the study's pathogens, tubes in which no turbidity was observed, were selected, and they were cultured on a specific solid culture medium (MH-A). All pellets were incubated at the proper temperature and period for each pathogen. The lowest concentration in which no growth of pathogen was seen was considered as the minimum bactericidal concentration.

The incubation setting for bacteria was 37°C for 24 hours in all the experiments. This procedure was performed for all bacterial strains. This test was performed for all four target bacteria. The data were analyzed with ANOVA test using SPSS software version 13.0. P value less than 0.05 was considered significant.

RESULTS

The results of measuring the effect of MIC and MBC for Jaftex, Matrica, and Persica on *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus Salivarius* and *Lactobacillus casei* are reported in Table 1.

MIC and MBC of Jaftex herbal mouthwash were determined 0.5 and 0.5 for *Streptococcus mutans*, 0.125 and 0.25 for *Streptococcus sanguinis*, 0.125 and 0.125 for *Streptococcus Salivarius* and 0.0625 and 0.25 for *Lactobacillus casei*, respectively.

Both MIC and MBC results of Matrica mouthwash was 0.5 for *Streptococcus mutans*, 1 for *Streptococcus sanguinis*, 0.5 for *Streptococcus Salivarius*, and 1 for *Lactobacillus casei*.

The results of Persica mouthwash MIC for all 4 bacteria were 1, while the MBC of Persica mouthwash had no effect on the mentioned bacteria.

As stated, the MIC of Persica mouthwash on all 4 bacteria was 1, which was higher than the Jaftex MIC for all four bacteria, and the Matrica MIC for *Streptococcus mutans* and *Streptococcus saliviris*. This difference was statistically significant (p-value=0.005). In contrast, Matrica herbal mouthwash MBC effect on *Streptococcus sanguinis*, *S. saliviris*, and *Lactobacillus casei* were more than the two other types of mouthwash. This difference was statistically significant as well (p-value=0.005). Jaftex and Matrica MBC effect on *S. mutans* were observed to be the same.

DISCUSSION

The results of the present study showed that Jaftex mouthwash has more effect on bacterial growth inhibition compared with Matrica and Persica, though, the antibacterial effect of Matrica on *Streptococcus mutans* was similar to Jaftex. Jaftex is a complex herbal mouthwash that contains aquatic extract of the oak jaft as a base, *Saturej bachtiarica*, and thyme. In this study, the antibacterial effects of Jaftex on *S. mutans*, *S. sanguinis*, *S. Salivarius*, and *Lactobacillus casei* were proven. These are known as the most common bacterial plaque of the teeth.

Jaft is the basic ingredient of Jaftex mouthwash [24,25]. The exocarp of the oak fruit is called Jaft. In several studies, the antibacterial effect of Jaft is concluded [20]. The antibacterial properties of this mouthwash are probably due to the tannin, which have proven to be effective on microbes in several studies. The findings of the present study are similar to other studies that have been done in this field.

Babadi et al. compared the antibacterial effects of chlorhexidine mouthwash with Jaftex on some common oral microorganisms. They concluded that Jaftex has

Table 1: Comparison of the levels of MIC and MBC (µg/ml) for Jaftex, Matrica and Persica mouthwashes on oral microorganisms

Mouthwash	Concentration	Bacteria			
		<i>Lactobacillus casei</i>	<i>Streptococcus Salivarius</i>	<i>Streptococcus mutans</i>	<i>Streptococcus sanguinis</i>
Jaftex	MIC	0.0625	0.125	0.5	0.125
	MBC	0.25	0.125	0.5	0.25
Matrica	MIC	1	0.5	0.5	1
	MBC	1	0.5	0.5	1
Persica	MIC	1	1	1	1
	MBC	0	0	0	0

antibacterial effects on *S. mutans*, *S. sanguinis*, and *S. salivaris*, but it is less effective for oral bacteria growth inhibition compared to CHX [24].

Ebrahimi et al. investigated the antimicrobial activity of oak extract on *Staphylococcus aureus*, *Staphylococcus epidermis*, *Staphylococcus saprophyticus*, and *Escherichia coli*. They concluded that the antimicrobial effect of oak is concentration-dependent, and Persian oak has antibacterial properties [26].

Khosravi et al. proved that the oak seed Jaft extract has a greater or similar effect than Nalidixic acid and antibiotics in some concentrations [27].

They also concluded in their study that Matrica has a greater antibacterial effect than Persica. The antimicrobial effect of Matrica is known to be due to bisabolol flavonoids, which accelerates the healing of mucosal wounds [22]. The following studies have investigated the antibacterial effect of Matrica.

Sadeghi et al. investigated the antibacterial effect of Persica and Matrica mouthwashes on common bacteria of the oral area in an experimental study. They concluded that Matrica had an antimicrobial effect on the studied bacteria. In contrast, Persica had no inhibitory effect on the growth of bacteria except for *Klebsiella pneumoniae*. It should be mentioned that their antibacterial properties were lower than chlorhexidine. They concluded that the herbal mouthwashes have less power in bacteria growth inhibition than chlorhexidine [28].

Paknejad et al. showed that Matrica mouthwash is statistically less effective than chlorhexidine as an antibacterial agent. They concluded that herbal compounds still are not properly competitive with chlorhexidine in changing microbial flora [15].

Salehi et al. reported that using Persica and Matrica herbal mouthwashes in orthodontic patients led to a significant decrease in the level of microorganisms, suggesting that these mouthwashes could be used in these patients to control the number of bacteria [29]. It is believed that the antimicrobial effect of Persica is due to isothiocyanate, that in contact with saliva, releases cyanide. Cyanide prevents the growth of bacteria in the oral area. Another result of this study was that Persica has a less antibacterial effect than Jaftex and Matrica [13]. Review of literature show conflicting results about the antibacterial effects persica. The results listed below are similar to our results. Jajarm et al. studied patients with cleft lip and cleft palate, concluding that Persica mouthwash does not significantly change the level of oral microbes, including *Streptococcus mutans*, *Staphylococci*, and Gram-positive bacteria. In addition, the level of *Candida albicans* increased significantly in these patients [30].

Sadeghi et al. reported that Persica is not able to inhibit bacteria growth except for *Klebsiella pneumoniae* [28].

Mozaffari et al. showed that Persica mouthwash does not have similar antimicrobial effects to chlorhexidine, but

in case of contraindication with chlorhexidine (such as pregnant women), Persica can be used [7].

The studies listed below are different to our results.

Sofrata et al. reported that washing mouth with *Salvadora persica* extract instead of water increases the dental plaque pH, and stimulation of saliva secretion from the parotid gland, which plays an important role in the prevention of dental cavity [31].

Hammad et al. concluded that washing the oral area with different concentrations of *Salvadora persica* aquatic extract mouthwash significantly reduces the binding of *Streptococcus mutans* to buccal epithelium cells [32].

The results of this study showed that the Jaftex mouth wash has antibacterial effects on microorganisms *S. mutans*, *S. sanguis*, *S. Salivarius* and *Lactobacillus casei*.

The use of herbal medicines in recent years due to antibacterial and antifungal effects and less side effects for oral health has been common. Herbal mouth washes due to having natural compounds in terms of compatibility with the body's physiology and less poisoning has better condition than CHX and is recommended for people who do not have the possibility of using chemical mouth rinses [24].

CONCLUSION

According to the results of the present study, Jaftex mouthwash has a higher potency in growth inhibition of *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus Salivarius* and *Lactobacillus casei* compared to Matrica and Persica mouthwashes.

It should also be noted that the antibacterial effects of Matrica on *Streptococcus mutans* are similar to Jaftex, and Persica showed the least antibacterial effectiveness compared to the other two types of mouthwash.

We recommend using these herbal mouthwashes for controlling dental plaque due to their antimicrobial activity.

ETHICAL CONSIDERATIONS

Ethical issues have been completely observed by the authors.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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