

## An in vivo evaluation of antimicrobial effects of Persica herbal mouthwash on *Candida albicans* and *Enterococcus faecalis*

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### Original Article

#### Abstract

**BACKGROUND AND AIM:** Due to their antimicrobial and dental plaque control activity, mouthwashes lead to an improvement in oral health. Although chemical mouthwashes have demonstrated the greatest antimicrobial and anti-inflammatory effects, their usage has been limited because of their numerous side effects. This study was conducted in vivo to determine the antibacterial and antifungal effects of Persica herbal mouthwash containing *Salvadora persica*, mint, and yarrow in comparison with a placebo.

**METHODS:** In this experimental, single-blind study, 80 dentistry students, who were eager to participate in the study, were randomly allocated into two groups of forty. One group was given Persica while the other group received a placebo. They were asked to apply the mouthwash twice a day for four weeks. The participants were unaware of the mouthwash type. Saliva sampling was conducted in all cases before and after mouthwash application and the samples were dispatched to a laboratory for microbial culture (Sabouraud and Clark-Kenner media culture). After two weeks, the washout times in groups were swapped with each other and the sampling was conducted just like before. Finally, the data were analyzed using independent and paired t-test. P values of less than 0.05 were considered significant.

**RESULTS:** The mean age of participants was  $23.20 \pm 4.14$ . Persica mouthwash significantly decreased the count of *Candida albicans* ( $1.43 \pm 0.15$  to  $0.80 \pm 0.35$ ) ( $P < 0.001$ ) and *Enterococcus faecalis* ( $0.93 \pm 1.76$  to  $0.71 \pm 2.10$ ) ( $P = 0.008$ ).

**CONCLUSION:** The statistical tests revealed that applying Persica mouthwash would result in a significant decrease in the *Candida albicans* and *Enterococcus faecalis* count. Regarding the significant and desirable effect of Persica on *Candida albicans* and *Enterococcus faecalis*, and its herbal origin, its application could be suggested to reduce oral microbes and infections from fungi and enterococci.

**KEYWORDS:** Herbal Mouthwash, Persica, *Candida Albicans*, *Enterococcus Faecalis*, Oral Microbes, Saliva

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There are around 500 types of microbes in the oral cavity, of which some can cause infectious oral diseases. Reduction of pathogen microbes is very important in wound healing and infections. In addition, considering the worldwide prevalence of AIDS and high prevalence of different types of cancers, the incidence of

opportunistic infections, such as fungal infections, has increased amongst these patients due to suppressed immune system or immunosuppressive drugs. In this regard, one should seek a solution to solve these problems.<sup>1-3</sup>

Enterococci are regarded as a part of oral microbial flora and form most of the primary

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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.<sup>4,5</sup>

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.<sup>5,6</sup> 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.<sup>7</sup>

Some mouthwashes such as chlorhexidine have very good antimicrobial effects, but they accompany side effects such as tooth and filling discoloration, taste changes, mouth dryness, and burning sensation in the mouth mucosa.<sup>8</sup> Over recent years, application of herbal medicines to maintain oral health has been more common due to their antimicrobial, antifungal, and anticarcinogenic effects together with fewer side effects.<sup>1,9-11</sup> Because of their natural components, which are compatible with the body physiology and have lower probability of poisoning, herbal mouthwashes are of better status; hence, they are suggested to those who cannot take chemical ones.<sup>11,12</sup>

Persica herbal mouthwash contains the extracts of *Salvadora persica*, mint, and yarrow. *Salvadora persica* is a rich source of fluoride, isothiocyanate, vitamin C, Selika, resin, calcium, tannin, chloride, and tanic acid. The isothiocyanate, present in this herb, releases cyanide in the presence of saliva which can prevent oral bacterial growth. Not having the complications of chemical mouthwashes, Persica is not contraindicated in pregnant women due to lack of alcohol among its ingredients; even its digestion is safe.<sup>2,3,11,13</sup> Several studies have reported that Persica mouthwash has antiplaque, antihemorrhagic, anti ulcer, analgesic, and

antimicrobial effects, and can be effective in prevention and treatment of gingival and periodontal diseases and prevention of dental caries. In addition, it does not have the side effects of chemical mouthwashes.<sup>3,11,14,15</sup>

Laboratory studies have reported the various antimicrobial effects of persica. In a study by Sadeghi et al., the effect of chlorhexidine on common oral bacteria was more prominent compared to persica.<sup>11</sup>

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.<sup>8</sup>

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.<sup>16</sup>

The study conducted by Atai et al. revealed very poor effects of Persica mouthwash on *Candida albicans*.<sup>17</sup> However, al-Bagieh et al. reported the fungistatic effects of Persica on *Candida albicans*.<sup>18</sup>

Although some studies have been conducted on the antimicrobial effects of Persica mouthwash, more in vivo studies are needed due to the different and paradoxical presented results. There was a lack of sufficient evidence for antibacterial and antifungal effects of Persica mouthwash, especially on some microorganisms. Moreover, considering the controversial results of its antimicrobial effects and fewer side effects because of its herbal components, this study was conducted to evaluate the effect of Persica herbal mouthwash on *Candida albicans* and *Enterococcus faecalis* count in the saliva.

## Methods

In this experimental single-blind study, 80

dentistry students, who were inclined to take part in this study, were selected using nonrandom convenient sampling. The inclusion criteria consisted of absence of any type of systemic disease, lack of usage of any type of antibiotics over the past six months, appropriate oral health (at least twice brushing a day), absence of rapidly progressing dental caries, orthodontic plaques, and removable and/or fixed prosthesis in the mouth. These participants were completely aware of the study's process and a written consent was taken from them before enrolment in the study.<sup>17</sup>

A saliva sample was taken from all participants at the beginning of the study. They were asked to wash their mouth with physiologic serum before breakfast, brush their teeth, and then spit the content. Then, 1cc of their saliva was collected in a sterile tube using a non-stimulatory method and the sample was then dispatched to the microbiology laboratory.

Then, all the participants were randomly divided into two groups of forty. Group 1 was given Persica (Poorsina company-Iran) and group 2 was given a placebo containing stiller water, propylene glycol, and some additives for color and taste. They were all asked not to change their prior oral hygiene and to apply mouthwash twice a day for two weeks (in the morning before brushing and breakfast, and at night before brushing). At each use, they had to gargle 15cc of the mouthwash for 30 seconds and avoid eating, drinking, and brushing for 30 minutes after that. The participants were also asked not to change their oral health habits, brushing method, the type of their toothbrush and toothpaste, and not to use any other type of mouth rinse.<sup>17-20</sup>

After a period of two weeks, a similar sampling was conducted and the samples were dispatched to the laboratory. After two weeks of wash out period, the sampling was repeated again. Group 1 was advised to use placebo, whereas group 2 was told to apply persica mouth wash for another two weeks.

At the end of this period, sampling was conducted again and the samples were sent to the laboratory within an hour.

Sabouraud culture media was used for *Candida albicans*. To prepare Sabouraud 4.0% dextrose agar, 65 grams of media was dissolved in one liter of water and then it was autoclaved.<sup>21</sup>

To determine and count the enterococci, Clark-Kenner media was used. To prepare this media, 71.5 grams of media was dissolved in one liter of water and was autoclaved in mild condition (10 minutes in 121°C). After cooling to 50°C, 10cc of TTC 1% (Triphenol Tetrazolium Chloride) was added to the media. In cases of suspicion to enterococci colonies, the following methods were applied: 1) direct sampling from the colony and microscopic evaluation, and 2) taking an amount from the colony and culture that in NaCl 6.5%.

The media were kept in an incubator for 48 to 72 hours under aerobic condition in 37°C. After this stage, colony count was conducted and registered. Then, the participants were asked some questions about dental interventions and any other diseases during this period. In case the response was positive, the participant was excluded from the study.<sup>17-20</sup> Finally, the data were analyzed using SPSS for Windows (version 16; SPSS Inc., Chicago, IL., USA) with a p-value of less than 0.05 considered as significant. Moreover, independent and paired t-tests were applied.

## Results

The present study was conducted on 80 dentistry students. During this period, 22 of them could not complete the study due to various reasons such as drug or antibiotic intake, complaints about the taste of the mouthwash, and feelings of discoloration.

Distribution of participants in the first stage of the study according to age and sex is shown in table 1. The mean and standard deviation of *Candida albicans* and *Enterococcus faecalis* count in 1cc of saliva taken at different times

of sampling is shown in table 2.

**Table 1.** Distribution of participants according to age and sex

Sex	Number (%)	The mean age and standard deviation
Male	44 (55)	23.18 ± 4.88
Female	36 (45)	23.22 ± 3.07
Total	80 (100)	23.20 ± 4.14

A declining trend after usage of the mouthwash was evident and the relationship between start point and 6 weeks later was significant. There were no significant differences between other stages of the study; however, persica showed a declining trend after start point, while the trend for placebo was not stable during consumption.

### Discussion

Regarding the infectious origin of dental caries and periodontal diseases, which stand amongst the most common diseases of the century, the need to conduct investigations and research to find a solution for these diseases is clear.<sup>17</sup>

Applying antiseptic disinfectant materials, such as mouthwash, is one way to prevent infectious diseases. Hence, the antibacterial and antifungal properties of these materials have been evaluated in several studies and researches. However, the number of in vivo studies is limited. In the present study, we evaluated the antimicrobial effect of Persica herbal mouthwash on *Candida albicans* and *Enterococcus faecalis*.

In the present study, the antimicrobial effect of Persica herbal mouthwash was clear. Such a finding is in accordance with the results of a laboratory study done by al-Bagieh

et al. in 1994 about the effect of Persica on *Candida albicans*.<sup>18</sup> Taking this point into consideration that the main component of persica is siwak extract, such a decrease in the level of microorganisms could be referred to different components of miswak. In other words, siwak results in the release of antibody-like materials in the saliva, which prevent bacterial adhesion to teeth by interfering in the bacterial action. However, considering the fact that persica is a combination of three herbs, its observed effects could be referred to mint and yarrow that have been added to the effect of miswak.<sup>8</sup>

In other studies which were investigating 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.<sup>3,13,19</sup> The study by Poorshahidi et al. reported more obvious antiviral effects of persica when compared to chlorhexidine and acyclovir.<sup>16</sup> In 1999, Almas showed an antibacterial effect of *Salvadora persica* extract 50.0% on *Streptococcus mutans* and *Enterococcus faecalis*.<sup>12</sup> 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.<sup>5</sup>

Noumi et al. reported strong antifungal effects of persica against all species of candida.<sup>3</sup> 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.<sup>1</sup> Sanei et al. reported that siwak has antibacterial effect and is helpful for plaque control.<sup>19</sup>

**Table 2.** The mean and standard deviation of *Candida albicans* and *Enterococcus faecalis* count in 1cc of saliva taken at different times

(Group A first consumed placebo then persica and group B consumed persica and then placebo)

Microorganism	Group	2 weeks after start point	4 weeks later	6 weeks later	P*
<i>Candida albicans</i>	A placebo	2.36 ± 4.76	1.45 ± 0.34	0.61 ± 3.48	0.003
	B persica	0.80 ± 1.15	1.05 ± 0.26	0.80 ± 0.35	0.001
<i>Enterococcus faecalis</i>	A placebo	1.00 ± 2.86	0.63 ± 2.35	0.18 ± 0.64	0.001
	persica	0.96 ± 2.09	0.71 ± 1.14	0.71 ± 2.10	0.008

\*P-value is related to paired t-test between start point and 6 weeks later

In the present study, 22 of the participants were not able to complete the study due to various factors such as diseases, and not being satisfied with taste or smell of the mouthwash, which is a common problem in vivo studies. The results of this present study revealed that the participants were satisfied with the taste of persica and were inclined to continue its usage. Some other studies have reported that patients are reluctant to take chlorhexidine mouthwash due to its side effects such as unpleasant taste and smell and tooth discoloration. 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.<sup>8</sup>

In general, the results of this study suggest that persica significantly decreases the level

of *Enterococcus faecalis* and *Candida albicans* and the participants consented to take this mouthwash.

### Conclusion

The results of this study revealed that Persica herbal mouthwash would decrease the level of *Enterococcus faecalis* and *Candida albicans* in the oral cavity; hence, its application is suggested as an aid besides the common methods of oral hygiene.

### Conflict of Interests

Authors have no conflict of interest.

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