

Full Length Research Paper

Evaluation of the effect of Persian shallot (*Allium hirtifolium*, boiss) aqueous extract on mouth bacterial count compared with chlorhexidine mouth rinse

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This study attempted to compare Persian shallot aqueous extract with commercially available chlorhexidine mouth rinse with respect to their anti-microbial activity on salivary bacterial counts. Three groups of 10 volunteers with a healthy oral status, were randomly enrolled. Using sterile water as negative control, using 0.2% chlorhexidine as standard control and Persian shallot aqueous extract for test group. A single mouth rinse lasting 15 s of each mouth rinse was employed. Salivary bacterial counts were obtained by collecting unstimulated saliva samples at the beginning before rinsing for measurement of baseline count and 1, 5 and 24 h after rinsing with the assigned solution. Analysis of variance and Bonferroni *post hoc* tests were used to evaluate significant differences among groups. No significant differences among the allocated groups were detected at baseline. Chlorhexidine produced more significant reduction of salivary bacterial count relative to both shallot and distilled water control at 1 h. In addition, a significant difference at 1 h was also detected between both chlorhexidine and shallot extract with distilled water. Furthermore, at 5 h there was a significant difference in bacterial count between chlorhexidine and shallot extract. After 24 h, the level of bacterial count in shallot extract was still significantly lower than both chlorhexidine and distilled water control groups. The results of this study suggest that Persian shallot extract has more persistent inhibitory action than chlorhexidine mouth rinse lasting up to 24 h.

Key words: Antimicrobial mouth rinse, Persian shallot extract, oral bacteria.

INTRODUCTION

Dental caries and periodontal diseases are probably the most common chronic diseases in the world. Dental plaque is the etiological factor of gingivitis and dental caries. Daily removal of plaque by dental flossing, tooth

brushing and mouth rinsing with suitable mouth washing solutions are effective measures for prevention of caries and periodontal diseases (Addy and Moran, 1997). Chlorhexidine (CHX) is the most commonly used and effective mouthwash and is considered as the gold standard among dentists. It has been demonstrated that CHX has a higher and immediate *in vivo* activity against a wide range of bacteria, some fungi and viruses than other oral antiseptics (Netuschil et al., 1995). However, due to

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Figure 1. A photographic illustration of (A) fresh and (B) freeze-dried powder of Persian shallot.

side effects such as reversible staining of the teeth, tongue, silicate and resin restorations and transient impairment of taste perception, as well as its limited period of effectiveness and development of resistance, many researchers have sought to find an alternative agent that is devoid of these side effects and have a more persistent activity (Netuschil et al, 2003).

Shallot is among the medicinal plants that are available and have been used for centuries in many cultures to enhance the flavor and aroma of foods. The scientific name of Persian shallot is *Allium hirtifolium* (boiss) and it is a member of Liliaceae family. The genus *Allium* consists of more than three hundred different species. They have many nutritious and medicinal applications. According to some studies *Allium* has antibacterial, antifungal, antiviral, antiprotozoal and antihelmintic properties (Taran et al, 2006; Amin et al., 2009). Persian shallot (*A. hirtifolium*) (Figure 1A) is a native Iranian plant. There are ample evidences that this plant has favorable influence on improvement of some diseases such as diabetes, arthritis, colds and flu, stress, fever, cough, headache, hemorrhoids, asthma, arteriosclerosis and cancer (Jellin et al., 2002). More recent studies have demonstrated that aqueous extract of Persian shallot is effective antibacterial agent both under *in vitro* and *in vivo* (Amin et al., 2005) conditions. No previous work has been specifically conducted to test the effectiveness of Persian shallot as a mouth rinse. The aim of this study was therefore to assess the effect of aqueous extract of Persian shallot on reducing the number of oral bacteria in comparison with that of CHX.

MATERIALS AND METHODS

Thirty healthy volunteers among dentistry students in age range of 20 to 30 years, randomly assigned into three groups (5 boys and 5 girls in each group) were candidate for this study. The permeation of ethical committee was issued with number ETH 122. The candidates had no infection in their mouth and tooth. They were non-smokers and were not taking any antibiotics during study and one month before.

Preparation of Persian shallot aqueous extract

The Persian shallot bulbs were collected from the Zagros Mountains in Dezful area, and were authenticated by botanists in the Agriculture Department of Shahid Chamran University, Ahvaz, Iran. The water extracts of underground bulbs of Persian shallot was prepared by suspending 300 g of crashed bulbs of shallot in 200 ml distilled water and was homogenized in a mixer. After 24 h of incubation, the mixture was filtered through Whatman filter paper (No. 1). The filtrate was freeze dried (Figure 1B) and re-dissolved in distilled water at concentrations of 1, 5, and 10%.

Experimental protocol

The candidates were volunteered dental students who were divided in 3 groups (n = 10 in each group, comprising 5 males and 5 females). Assessment of baseline salivary bacterial count was made in the morning of the experiments. All groups were asked to wash their mouth with 15 ml sterile distilled water for 30 s and turn back washed water in sterilized test tubes before using the allocated mouth rinse. The first group was considered as test group who were asked to rinse their mouths with 15 ml of Persian shallot aqueous extract for 30 s. The second and third groups were allocated as standard group and negative control group who rinsed their mouths for the same period with 15 ml of 0.2% chlorhexidine mouth rinse solution (Shahr Daro, Iran) and sterilized distilled water, respectively.

Microbial culture and colony count

At the allocated times of 1, 5 and 24 h after the use of mouth rinse, unstimulated saliva samples were obtained by requesting the subjects to spit into a graded test tube, and 1 ml was collected. All samples were immediately transferred to microbiology laboratory. Normal bacteriological procedures of dispersion were done by vortexing, serial dilution was done in phosphate-buffered saline (PBS), and finally, 0.01 ml of each tube was cultured on Mueller-Hinton agar media (Merck, Germany). After 24 h incubation at 37°C under 10% CO₂ condition, counting of the colonies was carried out. All tubes were tested in triplicates.

Statistic methods

The collected data were analyzed by one way analysis of variance and Bonferroni *post hoc* tests.

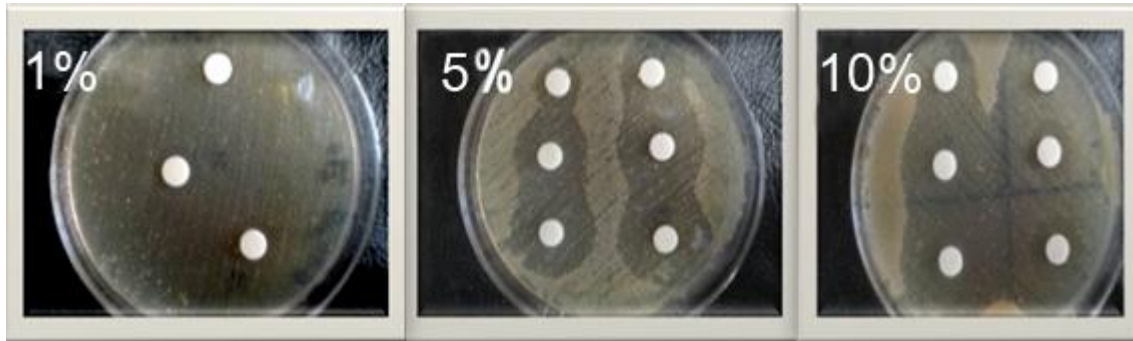


Figure 2. The effectiveness of various concentrations (1, 5 and 10%) of aqueous Persian shallot in inhibiting the growth of bacteria taken from oral swap after 24 h incubation in media. From these preliminary tests, the optimal concentration (10%) aqueous Persian shallot was selected for preparation of the mouth rinse.

RESULTS

Selection of the optimal concentration of Persian shallot aqueous extract

The results of the preliminary experiments for selection of optimal concentration of the Persian shallot extract that produced maximum inhibition, the growth of bacteria samples taken from oral swap in the plates was found at 10% (Figure 2) which was used for subsequent experiments.

Salivary bacterial count following a single mouth rinse with distilled water, CHX and Persian shallot extract

Changes in the mean log bacterial counts between baseline and at the allocated time intervals of 1, 5, and 24 h were significantly different among the groups. The mean logarithmic colony count of growth bacteria for Persian shallot mouthwash before use, 1, 5, and 24 h after use were 4.9 ± 0.022 , 4.8 ± 0.3 , 3.63 ± 0.13 , and 4.85 ± 0.04 , respectively (Figure 3). While these results for chlorhexidine were 5 ± 0 , 4.2 ± 0.44 , 4.7 ± 0.54 , and 5 ± 0 , respectively (Figure 3) and those for the negative control were 5 ± 0 at all the allocated times (Figure 3). Overall, the mean log of salivary bacterial counts over 24 h of the study period were 4.57 ± 0.58 , 4.73 ± 0.4 , and 5 ± 0 for Persian shallot, CHX, and distilled water groups, respectively.

No significant differences among the allocated groups were detected at baseline. CHX produced more significant reduction of salivary bacterial count relative to both shallot and distilled water control at 1 h (mean log count 4.2 versus 4.8 and 5, $p < 0.01$, respectively). In addition, a significant difference at 1 h was also detected between both CHX and shallot extract (log count 4.8) with distilled water. Maximum effect of reduction in salivary count with Persian shallot was observed at 5 h which was significantly lower than both CHX and distilled water groups (mean log count 3.6 versus 4.7 and 5, $p < 0.01$,

respectively). After 24 h, the level of bacterial count in Persian shallot extract was still significantly lower than both CHX and distilled water control groups (mean log count 4.8 versus 5 for both CHX and distilled water, $p < 0.01$).

DISCUSSION

Dental product manufactures around the world are seeking to find a suitable alternative mouth rinse of plant origin to chlorhexidine. Among the most recent studies are the use of grape seed extract (Haffajee et al., 2008), essential oils (Pan et al., 1999), goldenseal (Hwang et al., 2003), and *Aloe vera* (Langmead et al., 2004). This study aimed at testing the efficacy of a novel mouth rinse of plant origin, namely Persian shallot aqueous extract with regard to its capacity to reduce the number of salivary bacterial count in comparison with a standard commercially available chlorhexidine mouth rinse. The results showed that this mouth is more effective and has a more persistent antibacterial effects on oral micro-flora when tested under similar experimental conditions on healthy volunteers.

Previous *in vitro* and *in vivo* studies have demonstrated that Persian shallot to inhibit a wide range of bacterial (both Gram-positive and Gram-negative) and fungi (Amin et al., 2009; Amin et al., 2005; Zarei and Nasery 2009). These findings suggest that a suitable formulation containing the active constituents from this ancient medicinal plant may have a potential to be used as an effective mouth rinse. The results of this study confirmed this notion. It was found not only to be equally effective as CHX, but it also had a more persistent effect, that makes it suitable for single daily use in persons who cannot or do not like to use CHX mouth rinse.

For a mouth rinse to be effective, it should have the ability to deliver therapeutic ingredients and benefits to all accessible surfaces in the mouth, including interproximal hard and soft tissues and remain active for extended periods. This vital criterion poses an important question:

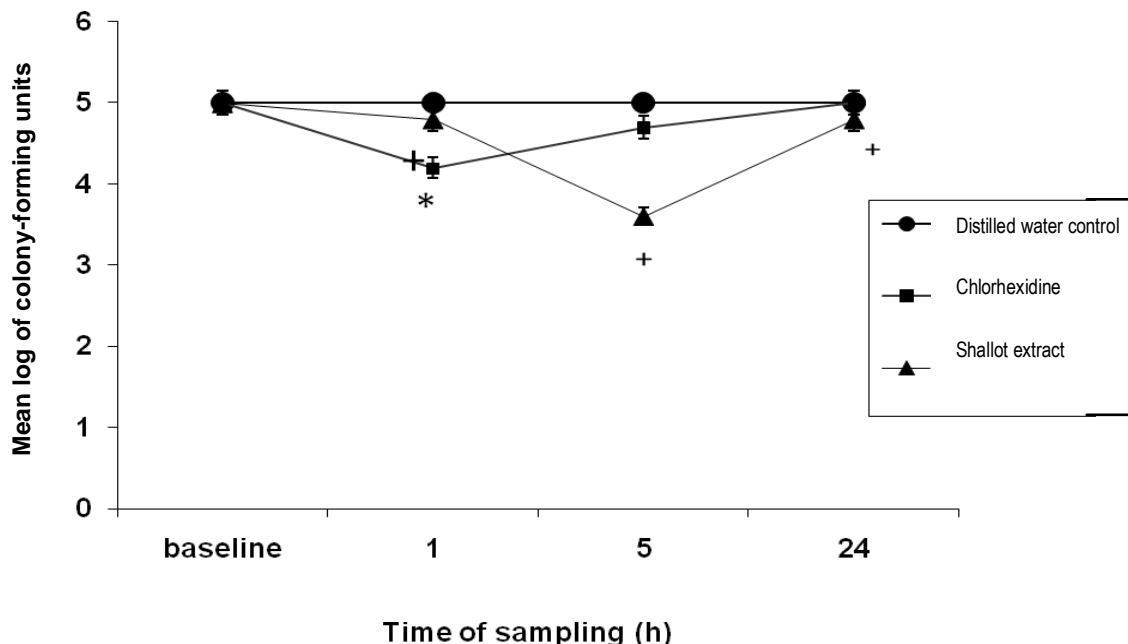


Figure 3. The effect of Shallot extract and chlorhexidine as mouth rinse against colony forming units of salivary samples in comparison with distilled water as control.

Does our new mouth rinse meet this criterion? It is well recognized that interpretation of results of this type study need to be guarded, since these results were collected from a limited number of subjects over a short period of study, and was focused upon the vitality indices which are not directly related to the anti-plaque indices (that is, the anti-adherent on dental biofilm).

Previous studies showed that Persian shallot has significant antioxidant properties and may have a potential to be utilized in the treatment and prevention of gingivitis (Leelarungrayub et al., 2006). Since the protocol of this study could not be extended to assess the anti-gingival properties of this new preparation. These facts dictate a separate study in which special experimental protocols of at least 2 weeks duration need to be planned for (Maruniak et al., 1992). If this notion is proven to be correct, the use of a mouth rinse of Persian shallot can have a wider clinical use, than suggested from this study.

The results of this *in vivo* study indicated that Persian shallot aqueous extract mouth rinse has a slower antibacterial effect than CHX. However, this plant-derived mouth rinse compared favorably, particularly in inhibiting the growth of salivary bacterial count that was maximal at 5 h after use as compared with 1 h CHX. Compared with CHX, Persian shallot extract was found to be effective, at low MIC range, against a wide range of salivary pathogens under *in vitro* conditions including *Actinomyces* species; the periodontal pathogens, *Eubacterium nodatum*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Prevotella nigrescens*, and *Tannerella forsythia*; and the caries pathogen *Streptococcus mutans*

and *Fusobacterium nucleatum* (Jahhangirnejad and Amin, 2012).

What are the clinical implications of the findings of this study? Although, it is early to come to a generalized conclusion for recommending the use this new agent in clinical practice, mainly due to the shortness of the period of this study, as well as due to the type of the adopted protocol of the study which was limited to salivary bacterial count. However, this study has raised several important questions that need to be answered, and the answer which may pave the way for better understanding of the potential for wider use in clinical settings. Among these, are the degrees of effectiveness of this agent as an anti-gingival and anti-halitosis agent and more importantly the adverse side effects associated with its prolonged use. Furthermore, Persian shallot aqueous extract has proved to be an effective natural antimicrobial agent against a wide range of bacteria and yeasts; these qualities support the notion that this agent has a favorable profile, especially due to its persistent activity (Elworthy et al., 1996), that makes it an additional candidate for use as a new mouth rinse in controlling plaque and gingivitis, especially in patients who cannot use CHX or wish to avoid alcohol, artificial preservatives, flavors and colors.

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