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Research Article

Effects of Two Iranian Herbal Extracts of *Malva sylvestris* and *Salvadora persica* on Two Oral Streptococci

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Abstract

Background and aim. Gingivitis is a reversible inflammatory reaction seen with various degrees in most individuals aged 17 to 22 years. The aim of this study was to compare the effects of two herbal extracts on Streptococcus salivarius and Streptococcus sanguis.

Materials and methods. Aqueous alcoholic root extracts of *Malva sylvestris* and *Salvadora persica* were prepared and tested on *Pseudomonas aeruginosa*. The diluted extracts, 0.2% chlorhexidine mouthwash, Betadine and phenol were added to blood agar culture media with S. salivarius and S. sanguis in dilution degree of 5% Mc Farland (1.5×10 cfu/ml) and average diameter for 14 halo of no growth in each case was measured using a caliper. Data was analyzed using ANOVA.

Results. Average diameter of halo of no growth of bacterium 1449 showed no significant difference between *Salvadora persica* tree root extract with 16±0.21 mm and chlorhexidine mouthwash with 16.1±0.22 mm (p=0.00). Mean diameter of halo of no growth created by *Malva sylvestris* root extract (9.1±0.21 mm) was higher than that of both mouthwashes (7.1 ±0.23 mm; p=0.00) but lower than that of chlorhexidine mouthwash and Betadine (10±0.21mm; p=0.00). *M. sylvestris* showed a significant difference with the other three materials regarding mean diameter of no-growth (p=0.00). Mean diameter of halo of no growth of bacterium 1448 for *Malva parviflora* (15.6±3.63 mm) and *Salvadora persica* tree (16.1±4.2 mm) showed no significant difference (p>0.05). However, the mean diameter of *S. persica* was less than that of chlorhexidine (24±0.2 mm) and more than that of Irsha month wash (7.7±0.3 mm) & Betadine (5.5 ±0.6 mm) and showed significant differences with each of the three materials (p=0.00).

Conclusion. According to the results, the effect of evaluated aqueous-alcoholic herbal extracts on some of oral micro biota are comparable to chlorhexidine, Irsha and Betadine mouthwashes. More in vitro and in vivo studies are recommended to demonstrate practical approach of using herbal mouthwashes for the oral biofilms.

Introduction

Gingivitis is a reversible inflammatory reaction and a common form of periodontal disease[1], which can progress to other periodontal tissues and cause alveolar bone destruction, leading to tooth mobility, tooth loss and many social and psychological problems. It necessitates prosthodontic treatments, which are among expensive procedures all over the world.[2] Almost all individuals aged 17-22 years have gingivitis of different degrees.[3]

Mechanical procedures such as root planning are time-consuming and require more than one visit.[4] Chemical materials including chlorhexidine can cause staining of the teeth, toxic effects on connective tissues, and also dryness and soreness of oral cavity[4], and therefore, the use of a herbal agent can be a useful alternative.

The effects of different herbal extracts on periopathogen bacteria have been tested with controversial results.[3,5-9] This study was designed to evaluate the effects of root extracts of *Malva sylvestris* and *Salvadora persica* on two oral streptococci, *Streptococcus sanguis* and *Streptococcus salivarius* and compare them with chlorhexidine, one essential oil mouthwash (Irsha) and Betadine disinfectant.

Materials and Methods

In this experimental study, one year old Malva sylvestris and Salvadora persica were collected from north and south areas of Iran. The roots were prepared and their extracts were excavated by the succilate method.6 Briefly, 50% solution of 96% ethylic alcohol with deionized water was prepared and poured in the extracting machine.3 The powder of both plants was placed at the filter of extracting and then the heater was turned on in order to excavate the extracts of the roots of both plants for 72 hours. Then, the solution was carried on from balloon to the wide container. In order to evaporate its water and alcohol, it was placed in 38°C for 24 hours, after which both extracts seemed like solid tar-like material. S. Sanguis (ATCC 1449) and S. Salivarius (ATCC 1448) were prepared in the form of standard and lyophilized from the center of Iran's fungi collection and infectious, Industrial Bacteria, Tehran, IRAN.

At first the extracts of both plants with dilution of 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8 were prepared with aqua distillates and were sterilized with millipore filter with 0.2 hole diameters.10 Then solutions of 1, 2, 3, 4, 5, 6, 7, 8 and 9 percents (weight to volume) phenol and aqua distillates were prepared to be used as a standard.[10]

Anti bacterial effects of the extracts with the method of agar gel diffusion were evaluated.10 The material of the nutrient agar (NA) which was sterile in 11.5 ml volumes at capped tubes were cooled by placing them at the warm water bath up to 45°-50° C 10, then 100 ml of 18-hours culture of S. sanguis and S. salivarius, diluted at the mixture of tioglicolate to about 0.5 McFarland (1.5×108 Colony Forming Unit per milliliters: cfu/ml), was added to agar mixture and placed at circle shaped plates with 78-mm diameter.10 The plates of agar were kept at the temperature of 4°C for 24 hours before using. At the center, each plate was sterilized and cooled by means of the head of experiment tube that made wells with 5-mm diameters. Sixteen plates were determined for each bacterial strain, 14 plates for different dilutions, 1 plate for growth control, 1 plate for sterility control, 18 plates for phenolian standards from 1% to 9% and 6 plates for chlorohexidine, Irsha and Betadine. Also, 2 plates of NA which included CO_2 were used to compare the power of streptococci, in two context of O_2 and CO_2, by 1:5 dilution of the extract.[3] There were no differences in the halo of non-growth extract's powders. The plates of the bacteria in each well with extracts were placed in 37ºC incubator for 18 hours. By measuring 14 vertical directions, the diameters of 28 plates of different extract's dilution, 18 plates of phenol, 6 plates of the two mouthwashes and Betadine were calculated (Figure 1).

Figure 1. Making essence by Succilate method technique.

In order to evaluate the phenolian coefficient of each case, 13 plates of blood agar were prepared according to the method explained before. Eleven plates of them were used to make culture of Pseudomonas aeroginosa, one plate for growth control and one plate

for sterility control. Phenolian percentages of 1 to 9 were poured in 9 of 11 wells and in the two other plates, and the dilution of 1:5 extracts of two plants were also added (Figure 3).
The mean ± SD of 14 halo's diameter of non-growth of 9 phenol plates is shown in Figure 2.

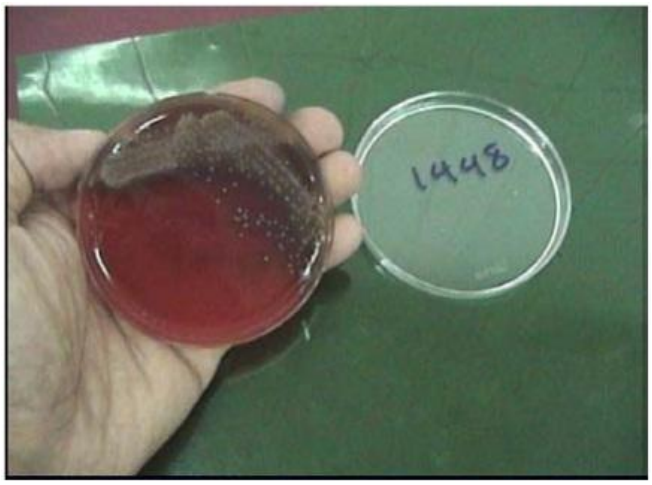

Figure 2. Plate of growth control for S. Salivarius (ATCC 1448)

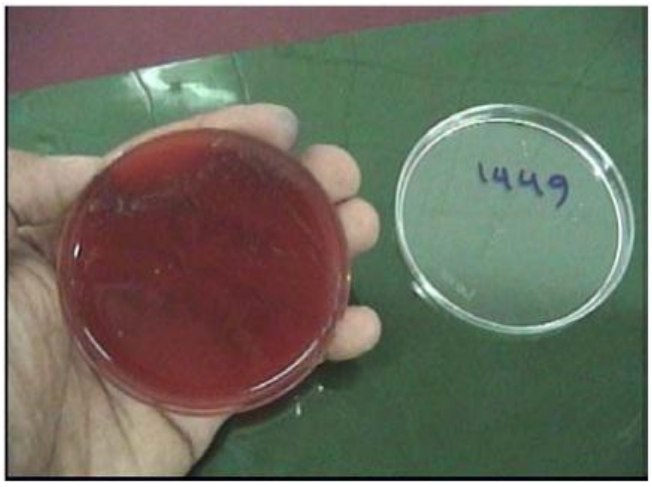

Figure 3. Plate of growth control for S. Sanguis (ATCC 1449)

The linear equation was Y = 78.485X – 129.64, where Y is the square of non-growth halo's mean diameters caused by the effect of both herbal extracts, and X is equal to phenol and percentage can be find that we can calculate Phenolian coefficient of each extract by means of this formula:

$$\text{Phenolian coefficient} = (\text{chart 1})\ \frac{\%\ \text{extracts dilution}}{\text{The equal amount of phenolia n percentage}}$$

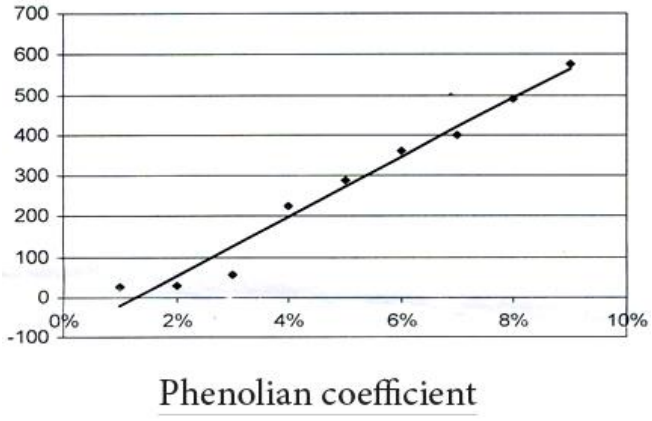

Chart 1. Linear chart of square of no growth halo diameter caused by Phenolian effect on Pseudomonas Aeroginosa ATCC 27853

Finally, the ANOVA (Duncan) test with an alpha error level less than 0.05 was determined as statistically significant.

Results

Following set up formation, stages affecting different dilution showed that 1:5 dilution or 20% of these extracts had the most non-growth halo diameter; hence, we performed the next stages with this dilution.
The phenolian coefficients of the two extracts according to explained method were found for the root of 0.14 M. sylvestris and also for the root of 0.19 S. persica. After the effects of 1-9 percent phenol, both extracts of the plants and under investigations cases on S. salivarius ATCC 1448 and S. sanguis ATCC 1449 and the means of 14 non-growth halo diameters, numerator was found that %1 phenol did not produce any non-growth determining mean and the diameter of that well was 5 mm. According to ANOVA test results, the difference of mean diameters was significant ($p<0.05$).

Bacteria 1449
There was no significant difference between the mean no-growth halo diameter (10 ± 0.21 mm) caused by Betadine with that of 3% phenol.
The mean no-growth halo diameter (7.1 ± 0.23 mm) of Irsha mouthwash was between those of 1% and 2% phenol and had significant difference with every dilution. The mean diameter of non-growth halo of the root of S. persica (16 ± 0.2 mm) was between those of 4% and 5% phenol and had significant difference with both dilutions. The mean diameter of no growth halo of the chlorhexidine mouthwash (16.1 ± 0.22 mm) was

between those from 4% and 5% phenol and had significant difference with both delusions; however, in this case there was no significant difference between these sizes with that of the extract of the root of S. persica. (chart 2)

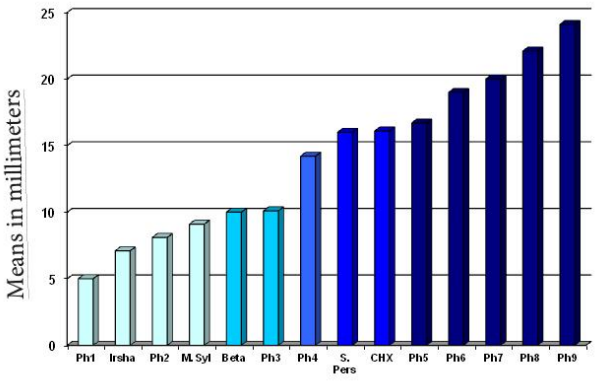

Chart 2. The mean of no growth halo diameter caused by 1% to 9% phenol, Irsha and chlorhexidine mouth washes, Betadine and extract of M. sylvesteris and S. persica root on S. Sanguis ATCC 1449.

Bacteria 1448

There was no significant difference between the mean of Betadine no growth halo diameter and those of 1% and 2% phenol.

There was no significant difference between the mean of no growth halo diameter of Irsha mouthwash (7.7 ± 0.29 mm) with those of 1% and 2% phenol, and therefore, it had no significant difference with Betadine.

There was no significant difference between the mean of non-growth halo diameter of S. persica root extract (16.1 ± 4.16 mm) with those of 4% and 5% phenol.

Also, there was no statistically significant difference between the mean of no growth halo diameter of M. sylvestris root extract (15.6 ± 3.63mm) with those of 4% and 5% phenol, and therefore, it had no significant difference with the extract of S. persica.

There was no significant difference in the mean of no growth halo diameter of chlorhexidine mouthwash (24 ± 0.19 mm) with those of 8% and 9% phenol. (chart 3)

Discussion

The results of this study showed that there were no significant differences among the effects of alcoholic-water root extract of S. persica with the dilution of 1:5 with 0.1 phenolian coefficients on S. sanguis ATCC 1449 or with that of chlorhexidine mouthwash, although it was less than those of Irsha and Betadine.

The effect of alcoholic-water root extract of M. Sylvestens on dilution of 1:5 with 0.14 phenolian coefficients

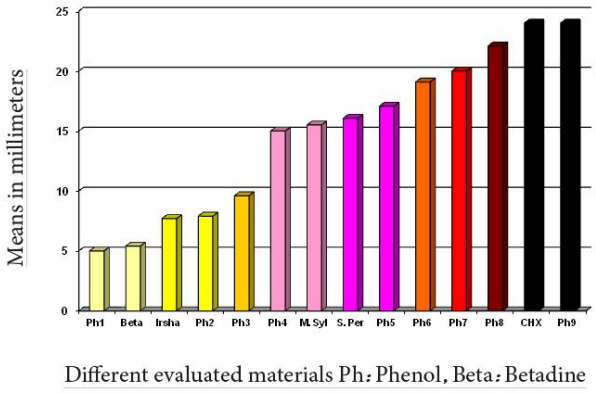

Chart 3. The Mean of nongrowth halo diameter caused by 1% to 9% phenol, Irsha and Chlorhexidine mouth wash, Betadine and extract of M. Sylvesteris and S. Persia's root on S. Sanguis ATCC 1448.

coefficients on S. Sanguis was less than that of chlorohexidine mouthwash and more than those of Irsha and Betadine. We investigated these herbal extracts for any antibacterial effects as these plants have been used routinely as brewing essences (M. sylvestris in the north part of Iran) or chewing materials (S. persica in the south provinces of Iran) for many years as part of traditional rural medicine. Since none of the previous studies have used our method to evaluate the effect on bacteria, comparison of the results have been done for the method, amount of extract's effect and bacteria. The results are in line with some of previous studies.[3,6] Other studies,[7,8,11] however, have not show positive results with the effect of herbal extract on gingival bacteria, perhaps because the use of water extract to get bacteria from saliva by Wolinsky & Shapiro.[7,11] In addition, the different dilutions of S. persica root extracts have been used previously.[8,11]

Tannin has shown antibacterial effects in most studies on plants since alcohol can resolve more tannin within itself. The use of alcoholic-water extract can be more useful than water extract alone; however, in an in vitro context, water extract is more useful than alcoholic extract according to many authors, as even little amounts of in vitro alcohol can be a competitive antagonist for tannin.[12]

Studies on dental plaque,[11] and saliva can yield different results, as these environments harbor different interfering bacteria. Extract diluting in saliva context that can change the essence of these extracts, pH of the saliva and its effects on the function of

bacteria and extracts, the amount of CO_2 in the mouth, interference of several bacteria to induce gingival disease and different bacterial resistance on oral context are all factors that prevents from extrapolating the results of this study to other in vitro studies.

Despite being a strong disinfectant, Betadine did not show a strong effect in our study. This weak effect can be attributed to its high concentration.

Increasing dilutions of the both extracts in the oral cavity with saliva and possible absorbance of the material by oral mucosa or dental tissue can cause differences with in vitro results.

Some dilutions lower than the control do not kill bacteria but rather interfere with bacterial functions like virulent factors,[13] their hydrophobic function for their stickiness together and to tooth surface, and the formation of the plaque.[13] These decreased dilutions in the oral cavity, thus, affect S. sanguis and S. salivarius hydrophobic function that hinders their production in the dental plaque.

Since different microorganisms are present in the oral cavity as normal flora, getting to main biofilm incluing S. sanguis and S. salivarius presented difficulties and this may be the reason why different results were found in the plaque model study of Shapiro et al.[11]

Positive effects of these two herbal extracts on two bacterial strains in this study paves the way for future studies to determine the effective dose and duration on other oral bacteria, to take the next step in order to recognize the effective usage of world's rich herbal resources.

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