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The Effect of Vinegar, Rose Water and Ethanolic Extract Green Tea Against Oral *Streptococci*, an *In Vitro* Study

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Abstract

Objective: Dental caries is the most common infectious diseases that are involved all groups, ages and classes of society people. Among the oral bacteria, *streptococci* especially *Streptococcus mutans* and *Streptococcus sobrinus* are known as the most important microbial agents in dental caries and dental plaque. Dental caries treatment imposes heavy costs in all countries. Although there are different chemical antimicrobial agents for the prevention of dental caries, but their important side effects have reported. Therefore, many attempts have done for finding alternative safe medications specially natural ingredients. The aim of this research is determination of antimicrobial effects of vinegar, rose water and green tea against four cariogenic bacteria.

Methods: The antimicrobial effects of different concentrations of vinegar, rose water and green tea against *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguis* and Streptococcus *salivarius* are evaluated with disc diffusion, well plate and microtitre plate methods, also the effects of them against biofilm formation were studied.

Results: The results showed vinegar, rose water and green tea significantly reduced biofilm formation of the *streptococci*. Vinegar and green tea decreased more then 70% the adhesion of *streptococci* but rose water decreased more than 70% of *S. salivarius*, more than 60% of *S. sobrinus*, *S. sanguis* and more than 50% of *S. mutans* adhesion. The diameter of highest inhibition zone was 24.2 mm for *Streptococcus salivarius* and 22 mm for *Streptococcus sobrinus* against vinegar.

The MIC of vinegar was for *S. salivarius* 0.0312 and MBC was 0.0625 ($P<0.05$), but the MIC of green tea for *Streptococcus mutans* and *Streptococcus sobrinus* was 7.81 mg/ml and its MBC was 31.25 mg/ml which was significantly less than that for *Streptococcus sanguis* and Streptococcus salivarius which was 15.625 and 62.5 mg/ml, respectively ($P<0.05$). The MIC of rose water for *Streptococcus sobrinus*, *Streptococcus sanguis*, *Streptococcus salivarius* was 1.

Conclusions: This results indicated the potential capacity of vinegar, rose water and green tea for prevention or control of cariogenic *streptococci* proliferation.

Keywords: Dental caries; Mutans *streptococci*; Vinegar; Green tea; Rose water

Introduction

Dental caries is one of the most major oral health problems in the world [1]. The people are susceptible to dental caries throughout their lifetime and approximately 36% of world population are experiencing tooth decay in their permanent teeth [2]. Microorganisms especially mutans *streptococci* have a major role in the initiation of dental caries. In early stage of dental plaque formation, *Streptococcus sanguis* is colonized on the tooth surface [3].

Due to increased resistance to antibiotics and chemotherapeutics, it is needed to safe and effective products for prevention and management dental caries [4]. Natural products with herbal, animal or microbial origin have good potential in this area. Healing herbs has long history for use in gum and tooth problems [5].

Vinegar is a sour liquid [6] and includs acetic acid, vitamins, mineral salts, amino acids, polyphenolic compounds, nonvolatile organic acids. Vinegar with anti-infective properties, antitumor activity, antiglycemic effect [7] has various medicinal uses such as prevention of hypertension, reduction serum total cholesterol and triacylglycerol [8]. Vinegar in the Iranian traditional medicine used in prevention of dental caries [9].

Rose water is major product of *Rosa damascena* in Iran. It contains 10-50% rose oil [10]. In folk medicine of Iran rose water has been used for various skin problems [11] and as antispasmodic for abdominal pain [12] and is important ingredient in cosmetic industries [11]. Rose water have traditionally been used in to prevent tooth decay [9]. It is reported that rose water has antimicrobial effects and can inhibit

Candida albicans mycelial growth and has bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) [13].

Green tea (*Camellia sinensis*) contains proteins, amino acids, carbohydrates, minerals, trace elements, lipids, vitamins B, C and E, caffeine, theophylline, polyphenolic compounds. It has various effects such as antiviral, antibacterial and antidiabetic effects, and may be used for skin treatment, also recommended for prevention of dental caries [14].

The aim of this study was to determine antibacterial effects of vinegar, rose water and green tea against oral streptococci including *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguis* and *Streptococcus salivarius*.

Materials and Methods

Test organisms

The antibacterial activity of the vinegar, rose water and green tea was evaluated against four strains of bacteria which was purchased from Iranian Research Organization for Science and Technology (IROST) including: *Streptococcus mutans* (ATCC 35668), *Streptococcus sobrinus* (ATCC 27607), *Streptococcus sanguis* (PTCC1449) and *Streptococcus salivarius* (PTCC 1448).

All The bacterial strains were cultured in Tryptic Soy Broth (TSB), Blood Agar and Mitis Salivarius Agar (MSA) at 37°C in a atmosphere containing 5% CO_2. Then, these bacteria were confirmed by Gram staining, catalase test and bacitracin tests. The bacteria kept in freezer at -80°C until used in the study.

Material preparation

For this study, homemade grape vinegar was prepared from Isfahan, Rose water purchased from Kashan and Green tea was purchased from Medicinal Plants Market of Isfahan, Iran.

The dried powder of green tea was mixed with 50% ethanol solution. After 48 hours it was filtered through Whatman (No.1) filter paper. Then extract was placed in the rotary evaporator (Heidolph Company, Germany) and finaly put in the oven at 40°C to dry completely.

Determination of antibacterial activity

Bacterial suspensions with 1×10^8 bacteria, were prepared from tryptic soy agar; (TSA; Quelab, Montreal, Canada). They were cultured on the Muller-Hinton agar medium with 5% defibrinated sheep blood using sterile swabs. Then, 50 µL of the vinegar, rose water and green tea solutions were poured into the wells (6 mm in diameter). These plates were kept at 4°C until the materials in the wells were completely diffused into the agar, and the plates were incubated anaerobically at 37°C for 24 h. The diameter of the inhibition zone was measured [15].

Determination of MIC and MBC

The MIC (minimum inhibitory concentration) of vinegar, rose water and green tea solutions determined by micro-dilution broth method. Overnight culture of bacteria in tryptic soy broth (TSB; Quelab, Montreal, Canada) were adjusted to 10^6 colony-forming units (CFU/mL). Two-fold serial dilutions of test compounds were prepared in broth. In 96-well plates, 100 µL of each dilution of test solutions were placed into the well containing 100 µL of bacterial suspension. Triplicate samples were performed for each test concentration [3]. Negative control wells were containing culture medium and bacteria. After 24 h anaerobically incubation at 37°C the turbidity was measured by micro-plate reader (AWARENESS, Technology INC, Stat fax 2100).

MBC (minimum bactericidal concentration) was considered as the lowest concentration of test solutions that did not seen any visible bacterial growth on the tryptic soy agar (TSA) plates after 24 h incubation under anaerobic condition at 37°C [16].

Determination of anti-adhesion effect

Anti-adhesion effect of test solutions measured by the micro-titer plate method as described previously by Di et al. with some modifications [17]. Briefly, the overnight grown Streptococci in TSB with 1% sucrose, were adjusted to reach a concentration of 106 CFU/mL. Then 10 µL serial dilutions of test solutions were added to 1 mL of each bacterial suspension. Then 200 µL of them were transferred into each well. Blank wells only contained buffer and control wells contained bacteria without treatment. After 24 h of incubation at 37°C at 5% CO_2, wells were washed 3 times with 200 µL of sterile phosphate-buffered saline (PBS), to remove unattached cells. Then the adherent bacteria were stained with 200 µL of 2% crystal violet for 5 min, then the excess stain was rinsed off using placing the plates under running tap water.

Then 200 µl of 33% (v/v) glacial acetic acid was added and the level of biofilm formation was evaluated via measuring the absorbance of the solution at 492 nm by an ELISA reader of the adherence reduction was calculated using equation:

$$\text{Percentage reduction} = \frac{(C-B)-(T-B)}{(C-B)} \times 100$$

B=absorbance of blank, C=absorbance of control and T=absorbance of test [18].

Statistical analysis

The data were statistically analyzed using Graf Pad Prism5 software (GraphPad Software Inc., CA, USA) by one way ANOVA and Tukey's post hoc tests.

Results

Measurement of inhibition zone diameter

The means of three separate measurements of inhibition zone diameter of vinegar, rose water and green tea against *Streptococcus mutans*, *S. salivarius*, *S. sobrinus* and *S. sanguis* are summarized in Table 1.

Vinegar compare to rose water and green tea showed the highest antibacterial activity against all test bacteria ($P<0.05$). Rose water did not showed zone of inhibition against mentioned bacteria.

The largest zones of inhibition were measured as 24.2 and 22 mm as diameters of zones of inhibition of vinegar against *Streptococcus salivarius* and *Streptococcus sobrinus* respectively.

The largest zones of inhibition by green tea were found at the first dose (500 mg/ml) and a decrease in concentration resulted in a decrease in the zone of growth inhibition. Green tea in concentration of 62.5 mg/ml did not express zone of inhibition.

Rose water did not show antibacterial activity against *S. mutans*, *S. sobrinus*, *S. sanguis* and *S. salivarius*.

Zone of inhibition (mm)												**Concentration**
Streptococcus salivarius			*Streptococcus sanguis*			*Streptococcus sobrinus*			*Streptococcus mutans*			**mg/ml**
Green tea	Rose water	vinegar	Green tea	Rose water	vinegar	Green tea	Rose water	vinegar	Green tea*	Rose water	vinegar	
12.5	-	24.2	11.5	-	17.5	11.5	-	22	13.67	-	18	1
9.5	-	21	9	-	14.5	9.5	-	17	9	-	15	0.5
7	-	16	-	-	12	6.5	-	13	6.83	-	12.5	0.25
-	-	12.5	-		9.3	-		10	-		9.5	0.125

Table 1: The mean of diameters of inhibition zones of vinegar, rose water and green tea against four oral streptococci *Green tea 500-62.5 mg/ml.

Determination of MIC and MBC

The MIC and MBC of vinegar, rose water and green tea against *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguis*, *Streptococcus salivarius*, are presented in Table 2. The MIC and MBC of vinegar against *S. salivarius* was 0.0312 and 0.0625 respectively which was significantly less than that for the other three bacteria (P<0.05), indicating higher antibacterial activity of vinegar against *S. salivarius*.

The MIC and MBC of green tea against *Streptococcus mutans* and *Streptococcus sobrinus* was 7.81 and 31.25 mg/ml respectively which was significantly less than that for *Streptococcus sanguis* and *Streptococcus salivarius*: 15.625 and 62.5 mg/ml, respectively (P<0.05).

The MIC of rose water against *Streptococcus sobrinus*, *Streptococcus sanguis*, *Streptococcus salivarius* was 1 and for *Streptococcus mutans* was not seen any inhibition zone, Also, the rose water did not express MBC against all test bacteria (Figure 1).

MBC			**MIC**			
Green tea*	**Rose water**	**vinegar**	**Green tea***	**Rose water**	**vinegar**	
31.25	-	0.5	7.81	-	0.25	*Streptococcus mutans*
31.25	-	0.25	7.81	1	0.0625	*Streptococcus sobrinus*
62.5	-	0.025	15.625	1	0.125	*Streptococcus sanguis*
62.5	-	0.0625	15.625	1	0.0312	*Streptococcus salivarius*

Table 2: Values of MIC and MBC for the four groups of oral streptocci bacteria. *Green tea: mg/ml.

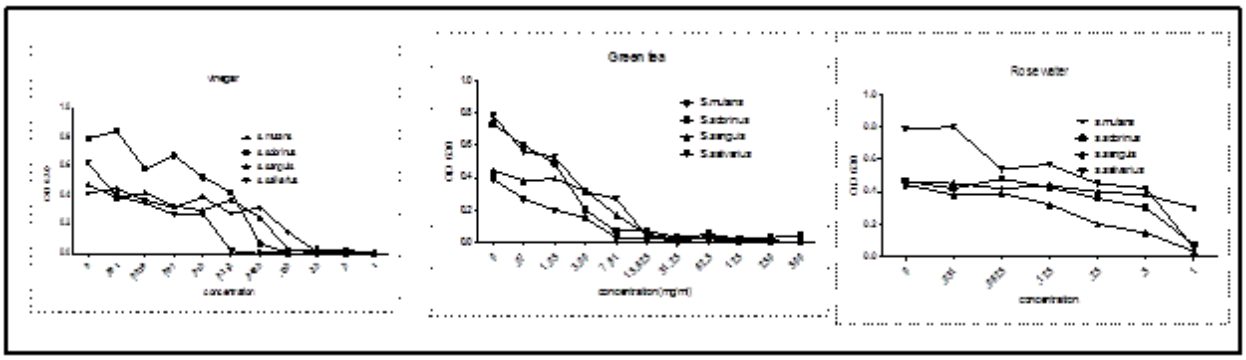

Figure 1: The effect of various concentrations of vinegar, rose water and green tea on the growth of four oral *streptococci*.

Determination of anti-adhesion effect

The anti-adhesion effect of vinegar, rose water and green tea was evaluated for each streptococci species and the results are presented in Table 3 and Figure 2.

All three materials significantly decreased the adhesion of test streptococci compared to the control group (P<0.05).

Vinegar and green tea showed highest antibacterial activity against test streptococci. Rose water showed lowest adherence reduction (53.27%, 60.75% and 65.90%) for *Streptococcus mutans*, *Streptococcus sobrinus* and *Streptococcus sanguis* respectively, compare with green tea and vinegar.

Percentage of adherence reduction												**Various concentrations of material mg/ml**
Streptococcus salivarius			*Streptococcus sanguis*			*Streptococcus Sobrinus*			*Streptococcus mutans*			
Green tea*	Rose water	vinegar	Green tea*	Rose water	vinegar	Green tea*	Rose water	vinegar	Green tea*	Rose water	vinegar	
74.35	75.38	76.92	79.31	65.9	74.13	70.88	60.75	73.68	79.09	53.27	76.63	1
64.102	22.2	55.12	68.96	25.22	72.41	77.21	28.3	65.78	87.27	12.3	51.04	0.5
48.71	13	51.28	50	9.12	56.89	69.62	15	60.52	67.27	8.66	37.38	0.25

Table 3: Percentage of adherence reduction of various concentrations of vinegar, rose water and green tea on oral streptococci *Green tea 500-125 mg/ml.

Discussion

In this study, antimicrobial activity of vinegar, rose water and green tea was evaluated on four oral streptococci, including *S. mutans*, *S. sobrinus*, *S. sanguis* and *S. salivarius*. Various concentrations of vinegar have expressed maximum inhibition zone against *Streptococci* compare to rose water and green tea.

Maruyama et al. evaluated the effects of rose water against *Candida albicans* and methicillin resistant *Staphylococcus aureus* (MRSA) and showed rose water decreased MRSA 99.99% within 1 h, and inhibited *Candida albicans* mycelial growth 50% [13].

Ismael showed that types of Date, Apple, and Grape vinegars eradicated biofilm of Streptococcus pyogenes (100%), (95.5%), and (90.9%), respectively [6].

Komiyama et al. evaluated the antimicrobial and disinfecting effects of 0.12% chlorhexidine, 0.50% white vinegar and two other materials on *S. mutans*, *S. pyogenes*, *Staphylococcus aureus* and *Candida albicans* on toothbrush and showed that vinegar decreased the count of *S. mutans*, *S. pyogenes* and *S. aureus* [19].

Ramezanalizadeh et al. studied effect of pomegranate vinegar and rose water in comparison with Persica mouthwash on *Streptococcus mutans* and *Streptococcus sobrinus* and showed reduced plaque formation by *S. mutans* 93%, 80% and 68%, and for *S. sobrinus* were 92%, 57% and 48% respectively, and reported pomegranate vinegar was more effective than the other two materials.

And showed inhibition zone for *S. mutans* and *S. sobrinus* was respectively and dont seen inhibition zone for rose water [9].

The result of this study shows that vinegar and green tea decreased >70% that adhesion of *S. mutans*, *S. sobrinus*, *S. sanguis* and *S. salivarius*. And rose water decreased >70% that adhesion of *S. salivarius*, >60% of *S. sobrinus*, *S. sanguis* and >50% *S. mutans*.

El-Shamy et al. was evaluate the antimicrobial effectiveness of vinegar against *Streptococcus mutans*, *Lactobacillus salivarius*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans* and showed vinegar was effective against *S. mutans*, *S. aureus*, *E. faecalis*, and *C. albicans* as compared to oradex mouthrinse. The mean inhibition zones of vinegar for *Streptococcus mutans* was 31.60 ± 0.5 [20]. In our study inhibition zone diameter vinegar for *Streptococcus mutans* was 18 mm.

Our results revealed that rose water had a effect on decreasing the adhesion of bacteria but had no effect on their growth and had not bactericidal effect, that these findings are consistent with the results of previous study.

Further studies are required to determine bioavailability active these compounds and understand of their mechanism.

Grover et al. studied effect of antimicrobial activity green tea at varying concentrations against oral bacteria and showed with increased the concentration inhibitory effect increased and highest mean zone of inhibition was 2.83 mm at 80 mg/ml [21].

The results of the present study showed that zone of inhibition green tea decreased with decrease in concentration and the highest zone of inhibition green tea was for *S. mutans* at 500 mg/ml that these findings are in agreement with the results of previous study.

Naderi et al. evaluated antibacterial effect Iranian green and black tea on *Streptococcus mutans* and showed they have an antibacterial effect on 100 to 400 mg/ml. And MIC green tea was 150 mg/ml, mean diameter of inhibition zone was 9.5 mm [22].

In this study MIC for green tea for *Streptococcus mutans* was 7.81 mg/ml and zone of inhibition was 13.67 mm at 500 mg/ml. And for *S. sobrinus*, *S. sanguis* and *S. salivarius* were 11.5, 11.5, 12.5 mm.

Comparison of vinegar, green tea and rose water showed that effect of vinegar on growth bacteria was significantly higher than that of green tea and rose water (P<0.05). In summary, the present study shows that these substances have anti-adhesion effects *in vitro*.

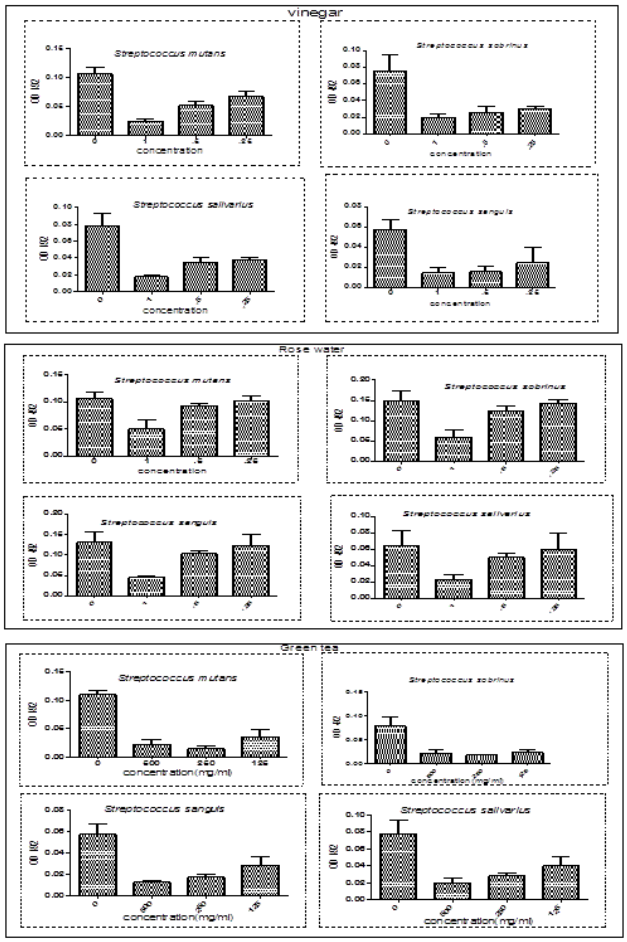

Figure 2: The effect of various concentrations of vinegar, rose water and green tea on adherence of four studied oral *Streptococci*.

Conclusion

The current study showed that vinegar and green tea expressed inhibitory effect against oral *Streptococci* and vinegar, rose water and green tea decreased adhesion of oral *Streptococci*.

Although this materials are regarded as safe and affordable, but additional qualitative and quantitative efforts are needed to get the most effective and safe protocol for using of them for prevention of dental caries.

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