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Original Article

Antibacterial Effects of Ethanolic Extractions of Aloe Vera, Black Tea, Pomegranate and Orange on *Streptococcus mutans*: An *In-vitro* Study

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ABSTRACT

Background and Objectives: This study was designed to assess antibacterial effects of herbal alternatives such as aloe vera, black tea, pomegranate and orange extractions.

Materials and Methods: In this *in-vitro* study, extractions of *Aloe barbadensis miller* (aloe vera), *Camellia sinensis assamica* (black tea), *Punica granatum* (pomegranate) and *Citrus* spp. (orange) were prepared. The antibacterial activity of the extractions was assessed against *Streptococcus mutans* ATCC 35668 using well-diffusion technique. The microbial inhibition zones were assessed using antibiotic zone reader. The antibacterial activity was assessed using chromatography to report minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). To analyze data, SPSS v.26.0 (IBM, Armonk, NY, USA) and ANOVA were used at the significance level of 0.05.

Results: All four extractions included a significant antibacterial effect against *Streptococcus mutans*. Pomegranate included the highest antibacterial effect, compared to black tea, aloe vera, orange and positive control. (P = 0.042, P = 0.025, P = 0.003 and P < 0.001, respectively) Following pomegranate, black tea included the highest antibacterial effect, compared to aloe vera, orange and positive control (P = 0.039, P = 0011 and P = 0.007, respectively). Aloe vera included a higher antibacterial effect than those orange and positive control did (P = 0.033 and P = 0.026, respectively). Orange included a higher antibacterial effect than that the positive control did (P = 0.048).

Conclusions: The studied herbal medicines were effective against one of the most important microorganisms of oral cavity, *Streptococcus mutans*, which is involved in tooth decay and gingival inflammation.

Keywords: Aloe, Black tea, Citrus, Herbal Medicine, Pomegranate, *Streptococcus mutans*

Highlights

- The primary goal of periodontal treatment is to stop the progression of the disease by decreasing the microbial biofilm formation, which contains a variety of bacteria.
- The ethanolic extractions of pomegranate, black tea, aloe vera and orange included significant antibacterial effects against *S. mutans*.
- Pomegranate included the strongest inhibition toward *S. mutans* followed by black tea, aloe vera and orange, respectively.
- These herbal medicines, pomegranate, black tea, aloe vera and orange, can be considered as sufficient treatments in addition to conventional dental treatments, especially in the management of dental caries, gingivitis and periodontitis.

Introduction

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Periodontal diseases, including gingivitis and periodontitis, are the result of inflammation in periodontal tissues (1). Untreated periodontal diseases often lead to the loss of periodontal attachment, supporting bone and involved teeth (2). Based on the Global Burden of Disease Study, severe periodontal disease was the 11th most prevalent condition worldwide (3,4). The prevalence of periodontal disease was reported as 20–50% globally (5,6). Worldwide loss of productivity due to severe periodontitis was estimated as US$ 54 billion per year (7). The primary goal of periodontal treatment is to stop the progression of the disease by decreasing the microbial biofilm formation, which contains a variety of bacteria (8). Methods such as using hand instruments, ultrasonic devices and lasers are effective approaches to decrease the bacterial load in periodontium (9). Experiments were carried out to increase the efficacy and efficiency of subgingival debridement and bacterial elimination; within which, use of local antibiotics or antimicrobials can be addressed (10).

Chlorhexidine (0.2%, CHX) mouthwash, as an adjunct to oral hygiene and non-surgical periodontal therapy, is one of the most widely prescribed antimicrobial agents by oral health care practitioners (11–13). Currently, CHX mouthwash is the most potent chemotherapeutic agent and gold standard in decreasing *Streptococcus mutans* and oral biofilms (13–15). However, this mouthwash included disadvantages such as dysgeusia, changes in taste, restoration discoloration, tooth staining and parotid gland swelling (11–15). To overcome problems of CHX mouthwash, herbal medicine was introduced. Nowadays, herbal extractions are becoming popularity worldwide. Of these, aloe vera, black tea, pomegranate and orange draw more attentions (16–18).

In dentistry, aloe vera gel has been tried for healing of aphthous ulcers, alveolar osteitis and lichen planus lesions. This ingredient includes strong bactericidal activity against cariogenic and periodontopathic bacteria (19–22). Black tea, the second most used beverage, is a non-alcoholic caffeinated beverages prepared by the infusion of *Camellia sinensis* plant. The inhibitory effect of Japanese and Chinese teas on dental caries has been verified in previous studies (23,24).

Pomegranate is a shrub of the Lythraceae family and its parts include various medicinal importance with various active ingredients and therapeutic indications. Thus, this is addressed as a great potential plant for preventing and fighting various diseases with antioxidant, hypoglycemic, cholesterol decreasing, antiviral, antiparasitic, antifungal and antidiarrhoeal characteristics as well as cancer preventive, cell differentiation promoting, estrogen enhancing and antimicrobial (against *Staphylococcus aureus* and *Salmonella typhi*), anti-inflammatory, healing and antiseptic characteristics for the large quantity of tannin in the fruit skin (25–29).

Orange, a tasty juicy fruit, belonging to Rutaceae family, is botanically known as *Citrus sinensis. Citrus senensis* peel includes several medicinal characteristics and is widely used against various diseases such as colic, upset stomach, cancers and is diuretic, cormunative, immune enhancing, stomachic and tonic to digestive system, immune system and skin. Dubey et al. showed potent antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella flexineri*, *Bacillus subtilis* and *Escherichia coli* of extract from orange peels using disk diffusion method (30–32). Therefore, this study was designed to assess the antibacterial effect of herbal alternatives such as aloe vera, black tea, pomegranate and orange extractions.

Materials and Methods

In this *in-vitro* study, extractions of *Aloe barbadensis miller* (aloe vera), *Camellia sinensis assamica* (black tea), *Punica granatum* (pomegranate) and *Citrus* spp. (orange) were prepared. The aloe vera leaves were collected, washed with distilled water (DW) and cut open and fresh aloe latex was achieved for aloe vera extraction. The aloe latex was dried at 80 °C for 48 h using oven and then ground into fine powder using electric grinder. A 10% ethanol extract of aloe vera was prepared by dissolving 100 g of the powder in 95% ethanol. Paper with 150-µm diameter was used for liquor filtration. The ethanol soluble fraction was evaporated under decreased pressure to achieve a 10% aloe vera ethanol extract. The extraction was stored at 4 °C.

The black tea leaves were collected and washed with DW for black tea extraction. The achieved black tea leaves were dried in an oven at 80 °C for 48 h and then ground into fine powder using electric grinder. A 10% ethanol extract of black tea was achieved by dissolving 100 g of the powder in 95% ethanol. Paper with 150-µm diameter was used for liquor filtration. The ethanol soluble fraction was evaporated under decreased pressure to achieve a 10% black tea ethanol extract. The extraction was stored at 4 °C.

The pomegranate was collected and washed with DW for pomegranate extraction. The achieved pomegranate peel was dried at 80 °C for 48 h using oven and then ground into fine powder using electric grinder. A 10% ethanol extract of pomegranate was achieved by dissolving 100 g of the powder in 95% ethanol. Paper with 150-µm diameter was used for liquor filtration. The ethanol soluble fraction was evaporated under decreased pressure to achieve 10% pomegranate ethanol extract. The extraction was stored at 4 °C.

Oranges were collected and washed with DW for orange extraction. The achieved orange peel was dried at 80 °C for 48 h using oven and then ground into fine powder using electric grinder. A 10% ethanol extract of orange was achieved by dissolving 100 g of the powder in 95% ethanol. Paper with 150-µm diameter was used for liquor filtration. The ethanol soluble fraction was evaporated under decreased pressure to achieved 10% orange ethanol extract. The extraction was stored at 4 °C

The antibacterial activity of the extractions was assessed against *S.mutans* ATCC 35668 using well-diffusion technique. Hence, four blood agar plates (Merck, Germany) were inoculated using sterile swab-dipped into the culture in columns adjusted to 1.5×10^8 bacteria/ml (0.5 McFarland turbidity standard). In the media surface of the first blood agar plate, 0.1 ml of aloe vera extraction was transferred into each well using micropipette. This procedure was repeated for the second, third and fourth blood agar plates using black tea, pomegranate or orange extractions, respectively.

Plates were stored at room temperature (RT) for 30 min and then microorganisms were incubated under aerobic conditions at 37 °C. After 24 h of incubation time, the microbial inhibition zones were measured using antibiotic zone reader. The diameter of the plates was 88 mm; therefore, 44-mm microbial inhibition zone meant no bacterial growth. Penicillin disc was used as positive control. The inhibition zone diameter of the penicillin disc was 28 mm.

The antibacterial activity was assessed using chromatography to assess MIC and MBC. The MIC assesses the lowest concentration of an antibacterial agent necessary to inhibit visible growth and MBC assesses the minimum concentration of an antibacterial agent that results in bacterial death. To assess MIC and MBC, concentrations of 10, 5, 2.5 and 1.25 mg/ml were prepared for each extraction. To analyze data, SPSS v.26.0 (IBM, Armonk, NY, USA) was used and ANOVA was used at the significance level of 0.05.

Results

Based on the results, the microbial inhibition zone is presented in Chart 1. All four extractions included a significant antibacterial effect against *S. mutans*. Pomegranate included the highest antibacterial effect, compared to black tea, aloe vera, orange and positive control (P=0.042, P=0.025, P = 0.003 and P < 0.001, respectively). Following pomegranate, black tea included the highest antibacterial effect than those aloe vera, orange and positive control did (P = 0.039, P = 0011 and P = 0.007, respectively). Aloe vera included a higher antibacterial effect than those orange and positive control did (P = 0.033 and P = 0.026, respectively). Orange included a higher antibacterial effect than that the positive control did (P = 0.048).

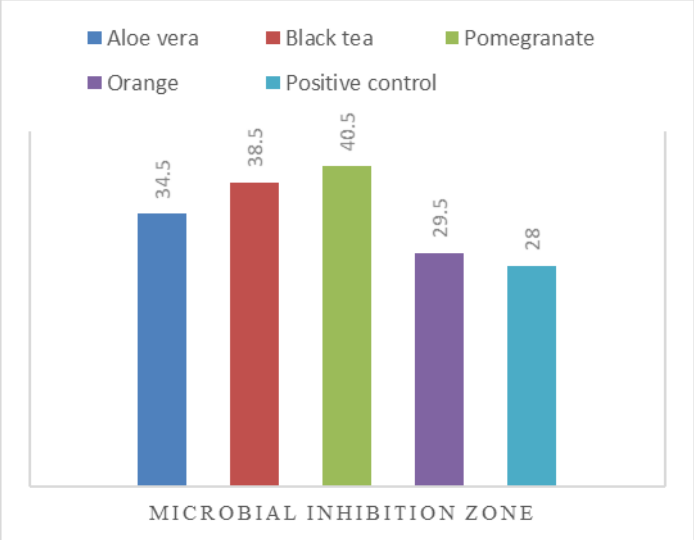

Chart 1. The means of microbial inhibition zones of aloe vera, black tea, pomegranate and orange extractions against *Streptococcus mutans* (mm)

The MIC and MBC of the aloe vera, black tea, pomegranate and orange extractions against *S. mutans* are present in Table 1. The MIC of pomegranate and black tea extractions included no significant differences (P= 0.998). The MIC of pomegranate and black tea extractions were significantly lower than that of aloe vera and orange extractions (P = 0.022 and P = 0.041, respectively). The MIC of aloe vera was significantly lower than that of orange extraction (P = 0.01). The MBC of pomegranate and black tea extractions included no significant differences (P = 0.998). In addition, the MBC of aloe vera and orange extractions included no significant differences (P = 0.998). The MBC of pomegranate and black tea extractions were significantly lower than that of aloe vera and orange extractions (P = 0.01).

Table 1. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of the aloe vera, black tea, pomegranate and orange extractions against *Streptococcus mutans* (mg/ml)

Extractions	MIC	MBC
Aloe vera	2.5	5
Black tea	1.25	2.5
Pomegranate	1.25	2.5
Orange	5	5

Discussion

Results of this study showed that all the ethanolic extractions included significant antibacterial effects against *S. mutans*. Pomegranate included the strongest inhibition toward *S. mutans* followed by black tea, aloe

vera and orange, respectively. Bioactive components of pomegranate are phenolic compounds, minerals, potassium, nitrogen, calcium, phosphorus, magnesium, sodium and polysaccharide complexes. Of these components, phenolic compounds such as Gallic acid, ellagic acid, anthocyanins, ascorbic acid, quercetin, catechin or epicatechin demonstrate antimicrobial characteristics against a broad spectrum of microorganisms. Black tea includes numerous beneficial compounds such as phenolic compounds, pigments, tea polysaccharides, alkaloids, amino acids and saponins. The phenolic compounds of black tea include quinic acid, epicatechin gallate, epigallocatechin gallate, epicatechin, epigallocatechin, Gallic acid, nicotiflorine and isoquercitrin. Aloe vera contains phenolic compounds, which are responsible for the antibacterial characteristic of this plant. Phenolic compounds of aloe vera include aloesin, hydroxaloin, aloin and aloinoside. Orange showed antibacterial effects as it is a major source of phenolic compounds. The phenolic compounds of orange include quercitrin, rutin, hydroxycinnamic acids caffeic, p-coumaric, ferulic and sinapic acid (29–32).

The presence of phenolic compounds in pomegranate, black tea, aloe vera and orange explained why all the extractions included antibacterial effect against *S. mutans*. Studies have clarified that how phenolic compounds inhibit the growth of *S. mutans*. The mechanisms of phenolic compounds antibacterial activity are linked to the strong changes in the membrane fluidity of *S. mutans* and to the interaction of phenolic compounds with the membrane proteins of *S. mutans* that can result in great disturbances of bacterial physiology. In addition, phenolic compounds of pomegranate, black tea, aloe vera and orange bind to the lipid bilayer cell membrane of *S. mutans* causing membrane damages. Ultimately, these mechanisms inhibit bacterial growth and trigger their death.

Setiawan et al. assessed the antibacterial effect of green tea and pomegranate ethanolic extracts against *S. mutans* and *Veillonella parvula*. Based on the results, the inhibition zones of pomegranate against *S. mutans* and *V. parvula* were 7.25 and 7.03, respectively, and the inhibition zones of green tea against the bacterial were 8.03 and 10.5 mm, respectively. The MBC of pomegranate and green tea was 12.5 and 3.125% against *S. mutans* and 25 and 12.5% against *V. parvula*. They concluded that green tea and pomegranate included antibacterial characteristics. The results of Setiawan et al. regarding the antibacterial effect of pomegranate ethanolic extract on *S. mutans* were similar to those of the current study (33).

In another study, Asmin et al. assessed the antibacterial effect of pomegranate, apple and guava aqueous extractions against *S. mutans*. The inhibitory zones of pomegranate, apple and guava extractions against *S. mutans* were 4.56, 8.9 and 11.26 mm, respectively. They concluded that all three studied extractions included significant antibacterial chracteristics; of which, the pomegranate extraction included the least inhibition zone. In the current study, the antibacterial effect of apple and guava extractions was not assessed. However, the results of this study supported those of Asmin et al. regarding the inhibitory effect of pomegranate juice. However, the extraction base was ethanolic in the current study and aqueous in the study of Asmin et al (34).

Al Qaralusi et al. studied the antibacterial effect of black tea and green tea aqueous extractions against *S. mutans*. The means of inhibition zones for 50 mg/ml black tea and 50 mg/ml green tea were 8.5 and 11.45 mm, respectively. Although extractions in the study of Al Qaralusi et al. included an aqueous base and in the current study included an ethanolic base, results were similar verifying that the black tea included antibacterial characteristics against *S. mutans* (35).

Shakeriana et al. reported the antibacterial effect of aloe vera extraction against *S. mutans*, *S. Sanguinis* and *S. Salivarius.* They claimed that hydroalcoholic extract of aloe vera included significant inhibitory effect against the three microorganisms. The results of this study verified findings of Shakeriana et al. regarding the antibacterial effect of aloe vera extraction. However in the current study, Aloe vera was extracted using ethanol and in the study of Shakeriana et al., aloe vera was extracted using hydroalcoholic base (36). Katuromunda et al. assessed the antibacterial effect of orange ethanolic extraction against *S. mutans*. Based on their results, orange extract and orange pulp extract included inhibitory zones of 18.67 and 18.33 mm against *S. mutans*, respectively. However in the study of Katuromunda et al., extraction was prepared from orange juice and pulp and in the current study, extraction was prepared from orange peel. Results showed that orange included antibacterial effects against *S. mutans*. Similar to the current study, previous studies by Dubey et al. and Jabuk et al. demonstrated that the antibacterial effect of orange peel extraction was stronger than that of orange juice and pulp extractions (37).

Conclusion

Pomegranate, black tea, aloe vera and orange ethanolic extractions included significant antibacterial effects against *S. mutans*. Pomegranate included the strongest inhibition effect against *S. mutans* followed by black tea and aloe vera, respectively. Orange included the least inhibitory effect against *S. mutans*, compared to the current extraction. It can be concluded that the highlighted herbal medicines are effective against one of the most important microorganisms of oral cavity, *S. mutans*, which is involved in tooth decays and gingival inflammation.

These herbal medicines, pomegranate, black tea, aloe vera and orange, can further be investigated as sufficient treatments in addition to conventional dental treatments, especially in the management of dental caries, gingivitis and periodontitis.

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