

In Vitro Effect of Hydroalcoholic Extract of Aloe Vera and 0.2% Chlorhexidine Mouthwash on Streptococcus Sanguinis, Streptococcus Salivarius and Streptococcus Mutans

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Objectives This study aimed to assess the antimicrobial effects of aloe vera hydroalcoholic extract and 0.2% chlorhexidine (CHX) mouthwash on *Streptococcus sanguinis* (*S. sanguinis*), *Streptococcus salivarius* (*S. salivarius*) and *Streptococcus mutans* (*S. mutans*) in vitro.

Methods Four concentrations (25%, 50%, 75% and 100%) of hydroalcoholic extract of aloe vera were prepared. *S. sanguinis*, *S. salivarius* and *S. mutans* were cultured on tryptic soy agar, and a single colony was removed from each microorganism and the opacity of pure microbial suspension was set at 0.5 McFarland standard concentration. Brain heart infusion broth culture medium, different concentrations of aloe vera extract, 0.2% CHX mouthwash, negative (distilled water) and positive (penicillin) controls and 0.5 McFarland bacterial suspension were added to the tubes and incubated at 37°C for 5, 10, and 15 minutes. Then, the samples were cultured on tryptic soy agar and incubated at 37°C for 48 hours. Number of colony forming units (CFUs) in the groups was compared with two-way repeated measures ANOVA. For paired comparisons, Tukey's multiple comparisons test was used ($P < 0.05$).

Results The results showed significant effect of hydroalcoholic extract of aloe vera (25, 50, 75, and 100%) on the tested microorganisms but the mean CFUs following the use of 0.2% CHX was significantly less than that after using different concentrations aloe vera extract ($P < 0.001$). Regardless of the type of the material, the number of CFUs decreased with increased exposure time.

Conclusion Aloe vera can be used alone or with CHX mouthwash to eliminate cariogenic bacteria.

Keywords Aloe vera, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus mutans*

Introduction

At present, bacterial resistance to antibiotics and chemical drugs is a growing dilemma worldwide. Use of herbal medications has attracted recent attention due to their easier accessibility and fewer complications.¹ Considering the natural resources for medicinal plants and their abundance, research is ongoing to find plants with suitable medicinal usage.²

Dental caries is a multi-factorial disease and is currently an important public health problem in many societies. Epidemiological studies have shown that tooth decay and periodontal disease are among the most common oral afflictions.^{3,4} Oral bacterial species such as *Streptococcus mutans* (*S. mutans*) and lactobacilli have a major effect on initiation and progression of oral diseases.⁵ Mechanical removal of dental plaque is the best approach for prevention of dental caries; however some individuals especially the handicapped or the elderly may not be able to accomplish mechanical plaque removal sufficiently; use of antimicrobial mouthwashes may be beneficial for such individuals.⁶

For many years, synthetic chemical agents have been used because of their antimicrobial effect on the oral environment. Chlorhexidine (CHX) is the benchmark mouthwash but it should not be used for a long period of time because of its side effects such as taste alteration, tooth

staining, development of hypersensitivity reactions and bacterial resistance.⁵

Aloe vera is a cactus-like plant and a member of the Liliaceae family. It is famous because of its curative historic reputation. Once the outer green cuticle is removed, the preserved inner gel can be used as a health and beauty product. Water comprises about 98 to 99% of the aloe vera inner gel; the remaining 1 to 2% include its active components.⁷⁻⁹ Over 75 active compounds have been identified in the leaf gel, such as aloin, aloe-emodin, aloemannan, acemannan and flavonoids that have pharmacological actions, including anti-inflammatory, anti-oxidative, anti-arthritis, anti-bacterial and anti-fungal effects. Aloe vera is used in dentistry for treatment of periodontal disease and for its cariostatic activity.^{9, 10} The aloe vera pulp contains some nutritional components such as carbohydrates and vitamins that promote the growth of probiotic microorganisms and reduce the activity of acidogenic bacteria; thus, different concentrations of aloe vera extract can alter the pH.¹¹ Considering the scarcity of studies that have been carried out on the effects of aloe vera on oral cariogenic bacteria, this study was conducted to evaluate the effect of different concentrations of hydroalcoholic extract of aloe vera on three common oral cariogenic bacteria in comparison with 0.2% CHX mouthwash.

Materials and Methods

In this *in vitro* study, aloe vera was obtained from the Herbarium Center of Kerman Pharmacy School and its scientific name was approved. To prepare the extract, 80 g of dried inner gel of aloe vera leaves was ground in solvent containing 200 mL of ethanol (100%) and 200 mL of distilled water for 72 hours and its extract was obtained using a Soxhlet extractor (Behdad, Tehran, Iran). In order to remove the solvent, it was placed on a rotary device (Behdad, Tehran, Iran) to extract the added solvent and to dry it completely. Then, the extract was pulverized in a mortar and the desired concentrations were prepared by adding distilled water (9,12) (Figure 1).



Figure 1- Hydroalcoholic extract of aloe vera

Streptococcus sanguinis (*S. sanguinis*; PTCC=1449), *Streptococcus salivarius* (*S. salivarius*, PTCC=1448) and *S. mutans* (PTCC=1683) were obtained from the Industrial and Infectious Collection of Bacteria and Fungi of Iran and incubated at 37°C for 24 hours to grow in brain heart infusion broth liquid medium (Merck, Darmstadt, Germany)]. Then, they were streak-cultured on tryptic soy agar solid medium (Merck, Darmstadt, Germany) and incubated at 37°C for 72 hours. The colony forming units (CFUs) were identified and separated. After incubation, the isolated bacterial colonies of the stock culture were removed and transferred to sterile saline, and the opacity of pure microorganisms was set to 0.5 McFarland standard concentration [1.5×10^8 CFUs/mL].^{13,14}

All samples and microorganisms were coded by an observer, and a skilled laboratory technician implemented the tests. The sensitivity of bacteria to antimicrobial agents was tested by the serial dilution method. In this experiment, 25, 50, 75, and 100% concentrations of aloe vera extract and 0.2% CHX mouthwash were used by special codes. Next, 2 mL of brain heart infusion broth, 250 µL of bacterial suspension with 0.5 McFarland standard concentration, 100 mL of each of the 25, 50, 75, and 100% concentrations of hydroalcoholic extract of aloe vera and 0.2% CHX mouthwash were added to the tubes and incubated at 37°C for 5, 10, and 15 minutes. Distilled water and penicillin were used as negative and positive control, respectively. The total number of tubes except for the positive and negative controls was 45 tubes. Next, 0.01 mL of the liquid inside each tube was cultured on tryptic soy agar and stored at 37°C for 48 hours. The amount of

bacterial proliferation was reported as CFUs/mL, according to the number of colonies grown on the medium (Figure 2).



Figure 2- Colonies grown on the culture medium

The colonies were counted manually using a counting pen and a counting grid. In order to assess the significance of differences in colony counts, each experiment was repeated three times and the mean values were used for statistical analysis. The plate count had to be within the range of 30-300 CFUs on a standard size Petri dish.^{9,15}

Data were analyzed by SPSS software version 21 (SPSS Inc., IL, USA). The number of colonies grown was reported as mean \pm standard deviation (SD). To compare the mean number of streptococcal colonies grown in presence of 25, 50, 75, and 100% concentrations of the alcoholic extract of aloe vera at 5, 10, and 15 minutes, two-way repeated measure ANOVA and for paired comparisons, Tukey's multiple comparisons test were used. The level of significance was set at 0.05.

Results

Table 1 shows the mean (\pm SD) number of *S. sanguinis*, *S. salivarius* and *S. mutans* colonies grown in presence of different concentrations of hydroalcoholic extract of aloe vera and 0.2% CHX at different time points. The results showed that the antibacterial effect of 0.2% CHX at all the studied time points was significantly greater than that of all different concentrations of hydroalcoholic extract of aloe vera on all three types of microorganisms. The minimum number of colonies of the three types of microorganisms grew at the three time points in presence of 0.2% CHX (Table 1 and Figures 3, 4 and 5).

The results showed that the effect of different concentrations of hydroalcoholic extract of aloe vera (25, 50, 75, and 100%) was significant relative to each other and the antibacterial effect increased with increasing the concentrations, with the greatest impact noted in presence of 100% concentration. It was also found that the effect of time was significant ($P < 0.05$). Regardless of the type of the studied material, the number of grown colonies decreased over time, with the greatest effect at 15 minutes.

The results of one-way ANOVA revealed that 0.2% CHX was almost equally effective on the three types of microorganisms but regarding the hydroalcoholic extract of aloe vera (concentrations of 25, 50, 75, and 100%) the results were different. The hydroalcoholic extract of aloe

vera at each time point had the greatest effect on *S. sanguinis*, *S. mutans* and *S. salivarius*, respectively so that the maximum number of colonies grown were *S. salivarius*

colonies counted at 5 minutes in presence of 25% hydroalcoholic extract of aloe vera ($2.81 \times 10^7 \pm 0.025 \times 10^7$).

Table 1- Mean (\pm SD) number of colonies in presence of different concentrations of aloe vera and 0.2% CHX at different time points

Time/Bacteria Sample Groups	5 min	10 min	15 min
Streptococcus mutans			
25% Aloe vera	$2.56 \times 10^7 \pm 0.045 \times 10^7$	$2.31 \times 10^7 \pm 0.020 \times 10^7$	$1.92 \times 10^7 \pm 0.049 \times 10^7$
50% Aloe vera	$1.89 \times 10^7 \pm 0.028 \times 10^7$	$1.59 \times 10^7 \pm 0.019 \times 10^7$	$1.26 \times 10^7 \pm 0.035 \times 10^7$
75% Aloe vera	$1.39 \times 10^7 \pm 0.030 \times 10^7$	$1.08 \times 10^7 \pm 0.055 \times 10^7$	$0.74 \times 10^7 \pm 0.042 \times 10^7$
100% Aloe vera	$0.92 \times 10^7 \pm 0.017 \times 10^7$	$0.64 \times 10^7 \pm 0.099 \times 10^7$	$0.27 \times 10^7 \pm 0.015 \times 10^7$
0.2% CHX	$0.10 \times 10^7 \pm 0.012 \times 10^7$	$0.08 \times 10^7 \pm 0.001 \times 10^7$	$0.04 \times 10^7 \pm 0.003 \times 10^7$
p-value	0.001	0.001	0.001
Streptococcus sanguis			
25% Aloe vera	$2.32 \times 10^7 \pm 0.056 \times 10^7$	$1.91 \times 10^7 \pm 0.082 \times 10^7$	$1.52 \times 10^7 \pm 0.013 \times 10^7$
50% Aloe vera	$1.61 \times 10^7 \pm 0.051 \times 10^7$	$1.19 \times 10^7 \pm 0.023 \times 10^7$	$0.75 \times 10^7 \pm 0.046 \times 10^7$
75% Aloe vera	$1.15 \times 10^7 \pm 0.043 \times 10^7$	$1.05 \times 10^7 \pm 0.042 \times 10^7$	$0.46 \times 10^7 \pm 0.052 \times 10^7$
100% Aloe vera	$0.78 \times 10^7 \pm 0.038 \times 10^7$	$0.49 \times 10^7 \pm 0.020 \times 10^7$	$0.19 \times 10^7 \pm 0.020 \times 10^7$
0.2% CHX	$0.12 \times 10^7 \pm 0.003 \times 10^7$	$0.07 \times 10^7 \pm 0.004 \times 10^7$	$0.03 \times 10^7 \pm 0.003 \times 10^7$
p-value	0.001	0.001	0.001
Streptococcus salivarius			
25% Aloe vera	$2.81 \times 10^7 \pm 0.025 \times 10^7$	$2.51 \times 10^7 \pm 0.026 \times 10^7$	$2.04 \times 10^7 \pm 0.039 \times 10^7$
50% Aloe vera	$2.23 \times 10^7 \pm 0.025 \times 10^7$	$1.86 \times 10^7 \pm 0.035 \times 10^7$	$1.40 \times 10^7 \pm 0.058 \times 10^7$
75% Aloe vera	$1.70 \times 10^7 \pm 0.017 \times 10^7$	$1.41 \times 10^7 \pm 0.034 \times 10^7$	$1.07 \times 10^7 \pm 0.040 \times 10^7$
100% Aloe vera	$1.16 \times 10^7 \pm 0.063 \times 10^7$	$0.83 \times 10^7 \pm 0.042 \times 10^7$	$0.47 \times 10^7 \pm 0.050 \times 10^7$
0.2% CHX	$0.10 \times 10^7 \pm 0.002 \times 10^7$	$0.06 \times 10^7 \pm 0.056 \times 10^7$	$0.03 \times 10^7 \pm 0.003 \times 10^7$
p-value	0.001	0.001	0.001

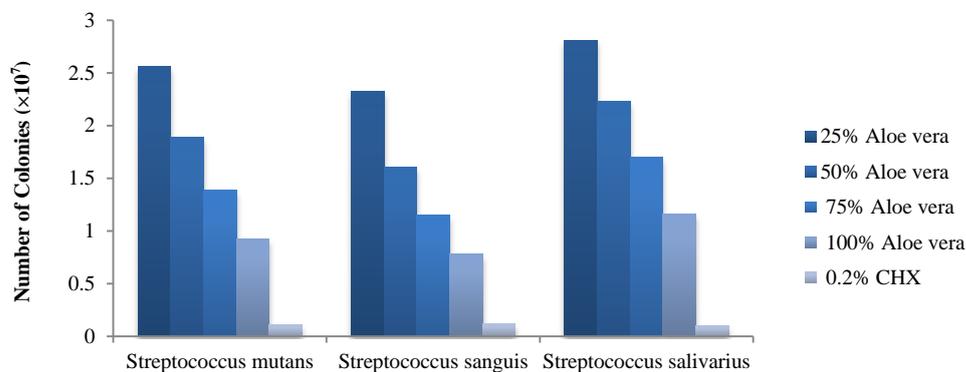


Figure 3- Mean number of colonies in presence of four different concentrations of Aloe vera and 0.2% CHX at 5 minutes

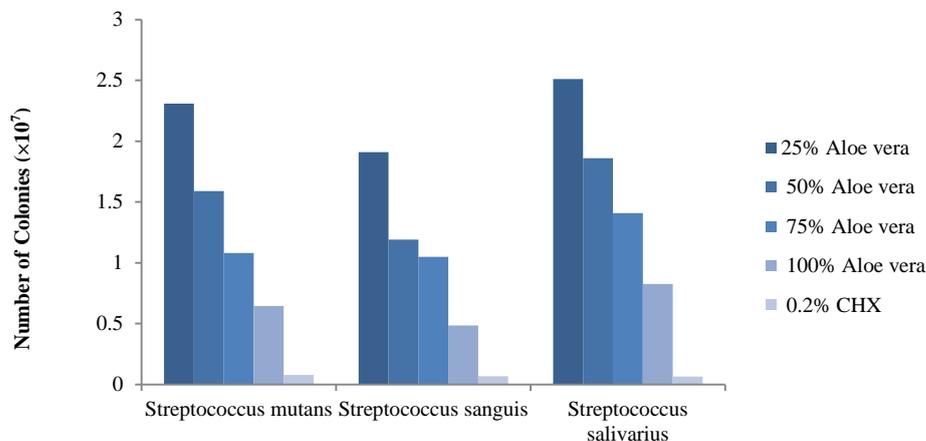


Figure 4- Mean number of colonies in presence of four different concentrations of Aloe vera and 0.2% CHX at 10 minutes

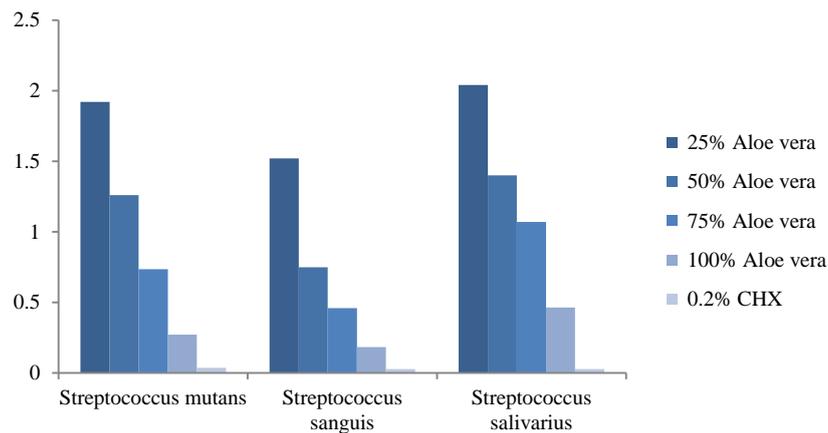


Figure 5- Mean number of colonies in presence of four different concentrations of Aloe vera and 0.2% CHX at 15 minutes

Discussion

Use of synthetic drugs and antimicrobial agents has caused some serious problems such as emergence of bacterial resistance and auto-immune conditions. Thus, there has been a great interest in use of essences and extracts of herbs and medicinal plants, which show antibacterial activities. The main techniques to prevent dental caries and periodontal disease include regular tooth brushing, flossing and rinsing mouthwashes containing antibacterial agents such as CHX. CHX is widely used as a disinfectant in the oral cavity but immediate hypersensitivity reactions, toxicity and tooth staining are among its common side effects.¹⁶ Considering the possible emergence of multidrug resistant oral bacteria and the afore-mentioned side effects, application of CHX is not recommended for a long period of time, and medicinal plants have been considered as useful alternatives to synthetic drugs for this purpose.⁵

In the present study, aloe vera hydroalcoholic extract effectively inhibited the three types of streptococci especially *S. mutans*, but its effect was significantly lower than that of 0.2% CHX. The disinfecting efficacy of CHX depends on the mechanism by which it destroys the cell walls of microorganisms and causes the leakage of intracellular components and irreversible damage characterized by precipitation and coagulation of cytoplasmic contents due to protein cross-linking.⁸ The antibacterial activity of aloe vera has been attributed to its various pharmacologically active constituents such as aloemodin, aloin, anthraquinones and acemannan. One factor that impels the antibacterial activity of aloe vera might be related to the cell wall structure of bacteria. Aloin and aloemodin have strong antibacterial and antiviral activities. They contain polyphenolic compounds in their structure that inhibit protein synthesis by bacterial cells. Anthraquinones and saponin present in aloe vera gel have direct antibacterial effects. Some other components such as acemannan have been considered to act indirectly by instigating the phagocytosis phenomenon.^{5, 13, 17}

George *et al.*¹³ concluded that aloe vera tooth gel was as

equally effective as two commercial toothpastes against some common oral bacteria. In another study by Mahabala *et al.*⁸ it was specified that the highest diameter of growth inhibition zone of *S. mutans* was created by CHX followed by propolis and then aloe vera, but there was no statistically significant difference. Our result was also in agreement with that of Fani *et al.*⁵ who concluded that use of aloe vera gel at optimal concentrations in mouthwashes could be useful for prevention of dental caries and periodontal disease. Rajendra kumar *et al.*¹⁶ in their study concluded that there was no significant difference between 0.2% CHX gluconate mouthwash and aloe vera mouthwash showing that aloe vera was potentially similar to CHX as an anti-plaque agent. They used 100% concentration of aloe vera. Karim *et al.*¹⁸ used 100% concentration of aloe vera mouthwash and concluded that aloe vera was similar to CHX in terms of efficacy. Based on our findings, 100% concentration of hydroalcoholic extract of aloe vera was not as effective as CHX and there was a statistically significant difference between them. This difference could be attributed to the use of plants from different geographical areas with variations in their chemical composition and different extraction techniques that were used to make products from the aloe vera leaf gel.⁸

Ehsani *et al.*¹⁴ concluded that aloe vera gel had mild antibacterial effect against *S. mutans* and it had low antibacterial potency compared to propolis. In another study, de Oliveira *et al.*¹⁹ assessed the effect of a dentifrice containing aloe vera on the reduction of plaque and gingivitis and reported that the dentifrice containing aloe vera did not show any additional effect on plaque and gingivitis compared to a fluoridated dentifrice. This result may be due to different study design and different concentrations of aloe vera used in the dentifrice.

Two factors that could be effective in antimicrobial activity of a material are the concentration and duration of exposure. In the present study, we considered both of these parameters. We tested different exposure times and the maximum exposure time with microorganisms was 15 minutes. In the study by Nagpal *et al.*¹¹ 15 to 25%

concentrations of aloe vera were not effective, but concentrations above 25% caused growth inhibition in *Lactobacillus*. They measured the pH of each sample using a pH meter and concluded that in higher concentrations, probiotic bacteria could be properly fed and increase the pH. In contrast, the growth of acidogenic bacteria in concentrations above 25% was restricted. With respect to the afore-mentioned studies, it is obvious that aloe vera has antibacterial activity, and use of different concentrations of aloe vera confirms that by increasing the concentration and duration of exposure, its antibacterial activity increases and the highest efficacy belongs to its 100% concentration.

In a study conducted by Subramaniam *et al.*²⁰ hydroalcoholic extract of aloe vera had significant antibacterial effect on *S. mutans* but only at 100% concentration. However, in our study, aloe vera inhibited bacterial growth at 25, 50, 75 and 100% concentrations, but none of these concentrations had antibacterial activity significantly higher than that of 0.2% CHX. This could be due to the inherently strong antibacterial efficacy of CHX.

CHX is a liquid solution that can diffuse more easily than aloe vera, which is a semisolid.^{7, 12} The authors of this paper suggest that it would be useful to prepare aloe vera extract in the form of a solution. Future studies are recommended to assess the efficacy of aloe vera for cavity disinfection and its effect on dentin bonding.

Conclusion

Based on the results of the present study, hydroalcoholic extract of aloe vera had antibacterial activity against cariogenic bacteria especially *S. mutans*, and with an increase in its concentration and duration of exposure, its efficacy increased; however, its effect was significantly lower than that of 0.2% CHX mouthwash.

Conflict of Interests

None Declared ■

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