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The Antibacterial Effects of the Hydroalcoholic Extracts of Aloe Vera and Glycyrrhiza Glabra against Cariogenic Bacteria InVitro

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ABSTRACT

BACKGROUND AND OBJECTIVE: Medical treatment of tooth decay is associated with the possibility of allergic reactions and increased bacterial resistance to antibiotics. This study aimed to evaluate the phenolic compounds and antimicrobial effects of the hydroalcoholic extracts of Aloe vera and *Glycyrrhiza glabra* against four cariogenic bacteria in vitro.

METHODS: In this empirical study, hydroalcoholic extracts of *Aloe vera* and *Glycyrrhiza glabra* were obtained using the percolation method. Then preparing standard strains of *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Actinomyces viscosus*. Antibacterial activity of extracts were determined by micro broth dilution method. Concentration of phenolic compounds, flavonols and flavonoid were determined using the optical density (OD) method.

FINDINGS: In this study, total phenolic content and concentrations of flavonols and flavonoids were 3, 37 and 10 mg/g in the Aloe vera extract, respectively, while they were 36, 78 and 14 mg/g, respectively in the extract of *Glycyrrhiza glabra*. Regarding the frequency of cariogenic bacteria, MIC and MBC of the *Glycyrrhiza glabra* extract for *Streptococcus mutans* were 0.5 and 1 mg/ml, respectively, while they were 0.25 and 0.5 mg/ml for *Streptococcus salivarius*, 0.125 and 0.5 mg/ml for *Streptococcus sanguinis*, and 0.25 and 0.5 mg/ml for *Actinomyces viscosus*, respectively. Moreover, MIC and MBC of the Aloe vera extract were 4 and 16 mg/ml for *Streptococcus mutans*, 0.5 and 2 mg/ml for *Streptococcus salivarius*, 1 and 4 mg/ml for *Streptococcus sanguinis*, and 1 and 2 mg/ml for *Actinomyces viscosus*, respectively. MIC and MBC of *Aloe Vera* extract (4 and 16 mg/ml) was significantly higher than the *Glycyrrhiza glabra* extract (0.5 and 1 mg / ml) ($p<0.05$).

CONCLUSION: According to the results of this study, the hydroalcoholic extract of *Glycyrrhiza glabra* exerted greater antibacterial effects against the studied bacteria compared to the Aloe vera extract due to the higher concentration of phenolic compounds. In addition, *Streptococcus mutans* showed higher resistance against the herbal extracts compared to the other bacteria.

KEY WORDS: *Tooth decay, Glycyrrhiza glabra, Aloe vera, Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguinis.*

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Introduction

Tooth decay is a microbial infectious disease in nature, which leads to the dissolution and destruction of calcareous tissues. Regardless of the causes, symptomatic and restorative treatments are not effective for tooth decay (1). In this disease, enamel and dentin lose calcium and phosphorus minerals due to the acid secretion from cariogenic bacteria, which will gradually destroy these hard tissues (2). Treatment and prevention of tooth decay using antibiotics and steroids may affect the oxidation-reduction potentials of saliva, diminish lysozyme activity, facilitate allergic reactions, and reduce resistance to pathogenic agents. The Iranian traditional medicine recommends various applications for medicinal herbs in the treatment of many diseases.

As such, maintenance of oral health and hygiene is a major concern in the Iranian traditional medicine, and reliable references have elaborated on this health issue in detail. Considering the diversity of treatment methods in the references of Iranian traditional medicine, researchers have been attempting to discover new approaches for the treatment of oral and dental diseases (3). In recent years, extensive research has been conducted in Iran and other countries aiming to evaluate different compounds in medicinal herbs and their effects on microbial pathogens (4).

Among traditional medicinal herbs, the roots and stems of *Glycyrrhiza glabra* and Aloe vera leaves have often been associated with special antibacterial properties, which are widely used for therapeutic interventions. *Glycyrrhiza glabra* grows abundantly in different regions of Iran, especially in Eqlid city (Fars province), eastern and northeastern Iran, and Azerbaijan province. Underground stems and roots of *Glycyrrhiza glabra* are rich sources of various compounds, which were used traditionally for the treatment of muscle spasms, swelling, bronchitis, rheumatism, and arthritis. In modern medicine, *Glycyrrhiza glabra* is one of the key ingredients of cough syrup.

This medicinal herb is known to be effective in the treatment of mouth sores and gastric ulcers. Furthermore, *Glycyrrhiza glabra* has diuretic properties and laxative qualities and could be used as a topical antiviral agent for the inflammation of shingles, eyes, mouth, and genitals. In particular, *Glycyrrhiza glabra* exerts positive effects on the digestive system. This medicinal herb could also be used for the treatment of swelling, peptic ulcers and duodenum. Moreover, *Glycyrrhiza glabra* could improve stomach cancer, alleviate indigestion, and eliminate tympanites (5, 6). Aloe vera is a sustainable herb with yellow flowers, and the leaves of this plant are the only part with therapeutic properties. This medicinal herb is widely used for the treatment of conditions such as arthritis, asthma, chronic fatigue syndrome, indigestion, gastrointestinal disorders, skin diseases, epilepsy, and migraine. In addition, Aloe vera topical gel is beneficial in the treatment of mild burns, skin injuries, acnes, and stomatitis (7, 8).

Viridans streptococci, especially Streptococcus mutans, Streptococcus sanguinis, Streptococcus salivarius, and Actinomyces viscosus, are the main causes of dental problems, such as tooth decay and gum infections, which may ultimately lead to cardiovascular diseases. Given the importance of dental hygiene in maintaining overall health and owing to the efforts of health care officials and policymakers, many countries across the world have turned to the mass production of different types of mouthwash, toothpaste and herbal medicines for the prevention and treatment of oral disorders, such as tooth decay, gum infections, and systematic complications, especially cardiovascular diseases.

Aloe vera and *Glycyrrhiza glabra* grow abundantly in our country and are considered as cost-efficient, available medicinal herbs. The essence of these plants could be used in toothpastes in order to maintain oral health and prevent the complications caused by the associated infections. This study aimed to evaluate the antibacterial effects of the hydroalcoholic extracts of Aloe vera and *Glycyrrhiza glabra* against four cariogenic bacteria, including *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, and *Actinomyces viscosus* in vitro. Furthermore, we determined the concentrations of phenolic compounds, flavonols and flavonoids in the obtained herbal extracts.

Methods

This empirical study was conducted after providing the herbal extracts of Aloe vera leaves and *Glycyrrhiza glabra* roots, which were evaluated and approved by an agricultural engineer. Extraction was performed using the percolation method. Initially, 100 grams of each herb was separately dried, grinded and powdered. Afterwards, 100 grams of each dried powder was mixed with 500 cc of 70% ethanol, and the mixtures

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were incubated for 24 hours at room temperature. In the next step, the mixtures were filtered using a paper and entered into the Rotary device, and the obtained extracts were dried at temperature of 50°C. At this point, each of the extracts with concentration of 32 mg/ml was separately solved in 20% dimethyl sulfoxide mixed with distilled water. After sterilization using a 0.45-micron Millipore filter paper, the extracts were used for the experiment. In order to determine the concentration of total phenolic content based on the method proposed by Sherafati et al. (10), 0.5 milliliter of Folin-Ciocalteu solution was added to 0.1 milliliter of the diluted extract (0.01 grams in 10 milliliters of 60°C methanol). After 3-5 minutes, 0.4 milliliter of 7.5% sodium carbonate was added to the obtained extract. After 30 minutes of incubation at room temperature, sample absorbance was measured against distilled water blank. Simultaneously, different dilutions of gallic acid were produced, and a standard curve was prepared in accordance with the aforementioned method.

Absorbance of the samples was compared using the standard curve, and total phenolic content was calculated for each herbal extract (milligram per each gram of the dried extract) (11, 12). Concentration of flavonoids was determined based on the altered version of the method proposed by Asadi et al. In addition, different routine dilutions were provided and evaluated using the standard curve. Absorbance of the samples was compared based on the standard curve, and flavonoid concentrations were calculated for each extract (milligram per each gram of the dried extract) (13). In order to measure the flavonol compounds, 0.5 milliliter of each soluble extract (0.01 gram in 10 milliliters of 60°C methanol) was mixed with 0.5 milliliter of 2% aluminum chloride, and three milliliters of 5% sodium acetate was added to this mixture. After 2.5 hours, sample absorbance was measured against distilled water blank at wavelength of 440 nm. Simultaneously, different routine dilutions were provided and evaluated using the standard curve. Absorbance of the samples was compared based on the standard curve, and flavonol contents were calculated for each extract (milligram per each gram of the dried extract) (14). For this study, cariogenic bacteria, including Streptococcus mutans PTCC 1683, *Streptococcus sanguis* PTCC 1449, *Streptococcus salivarius* PTCC 1448, and *Actinomyces viscosus* PTCC 1202 were purchased from the Iranian Research Organization for Science and Technology. A set of 12 microplate reducers and test tubes (from 16 mg/ml to 8 µg/ml of the extract) was prepared in Mueller-Hinton broth (MHB) (Merck, Germany). In addition, two other solutions as positive and negative control were prepared in each set. After the initial culture of the selected bacteria in sheep blood and brain-heart infusion agar (Clinical and Laboratory Standards Institute, 2006, M7-A4, USA) (15), the herbal extracts were separately cultured on each of the studied bacteria twice using the microbroth dilution method. Moreover, the extracts were cultured once in 12 test tubes, and the obtained results were recorded. For this process, bacterial suspension with McFarland standard opacity of 0.5 was provided from the colonies of the selected bacteria.

Afterwards, 200 microliters of the obtained bacterial suspension was added to each of the ELISA microplate wells. After 48 hours of incubation at 37°C, dilution series of each extract were cultured in MHB in candle jar conditions, and 100 microliters of the suspension of each bacterium was added to the compound. Finally, incubation was carried out for 48 hours at 37°C in candle jar conditions. Optical density of microplate wells or test tubes was measured using the ELISA Microplate Reader (model: STAT FAX 2100, USA) at wavelength of 630 nm. In this process, concentration in the first well with no signs of bacterial growth was recorded as the minimal inhibitory concentration (MIC) of each herbal extract. Afterwards, contents in each well were cultured for the bacteria and herbal extracts separately. After 48 hours of incubation, the first concentration of the extract with no signs of bacterial growth in the microplates was considered as the minimal bactericidal concentration (MBC) of the extract for each bacterium. By Kruskal-Wallis test the difference between bacterial groups was evaluated and measured.

Results

According to the results of this study, concentrations of total phenolic content and flavonols in the hydroalcoholic extract of Glycyrrhiza glabra were significantly higher compared to the Aloe vera extract. Moreover, flavonoids had a higher concentration in the extract of Glycyrrhiza glabra compared to Aloe vera. In this study, concentrations of total phenolic content, flavonols and flavonoids in each gram of the Aloe vera extract were 37, 3 and 10 milligrams, respectively, while these values were

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estimated at 78, 36 and 14 milligrams in the extract of Glycyrrhiza glabra, respectively (fig 1). In this study, MIC and MBC of Glycyrrhiza glabra extract were 0.5 and 1 mg/ml for Streptococcus mutans, 0.25 and 0.5 mg/ml for Streptococcus salivarius, 0.125 and 0.5 mg/ml for Streptococcus sanguinis, and 0.25 and 0.5 mg/ml for Actinomyces viscosus, respectively. In addition, MIC and MBC of Aloe vera extract were 4 and 16 mg/ml for Streptococcus mutans, 0.5 and 2 mg/ml for Streptococcus salivarius, 1 and 4 mg/ml for Streptococcus sanguinis, and 1 and 2 mg/ml for Actinomyces viscosus, respectively (fig 2). MIC and MBC of Aloe Vera extract (4 and 16 mg / ml) was significantly higher than the MIC and MBC Glycyrrhiza glabra extract (0.5 and 1 mg/ml) ($p<0.05$). The MIC of Glycyrrhiza glabra extract(4 mg/ml) on Streptococcus mutans was the lowest and MIC of Aloe Vera extract (0.5 mg/ml) on Streptococcus salivarius was the lowest.

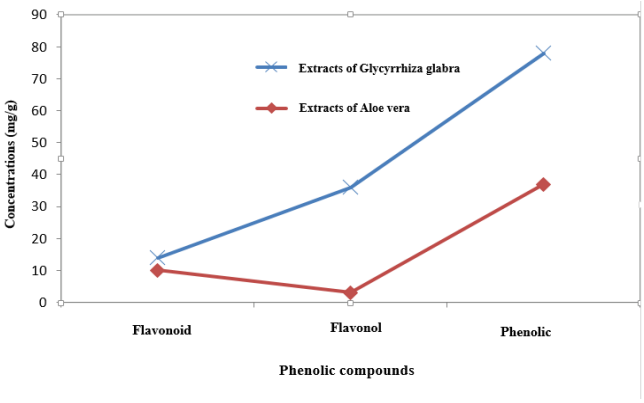

Figure 1. Concentrations of phenolic compounds (total phenolic content, flavonoids and flavonols) in the extracts of *Glycyrrhiza glabra* and Aloe vera (mg/g of each dried extract)

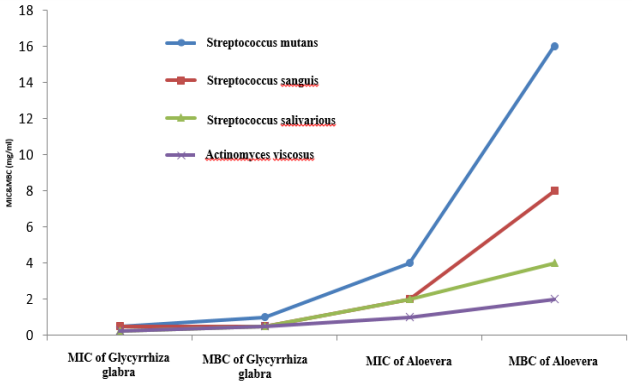

Figure 2. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the extracts of *Glycyrrhiza glabra* and Aloe vera for studied bacteria (mg/g of each dried extract)

Discussion

According to the results of the present study, concentrations of total phenolic content and flavonols in the extract of the roots and stems of Glycyrrhiza glabra were significantly higher compared to the Aloe vera extract. In addition, evaluation of MIC and MBC of these herbal extracts for cariogenic bacteria indicated that both extracts exerted inhibitory and destructive effects against the growth of these bacteria. On the other hand, comparison of the herbal extracts revealed that the antibacterial effects of Glycyrrhiza glabra against the studied bacteria were more significant compared to Aloe vera, especially in case of Streptococcus mutans, which could be attributed to the high concentration of antibacterial compounds in these herbal extracts.

Among the studied bacteria, Streptococcus mutans showed higher resistance towards the antibacterial properties of Glycyrrhiza glabra compared to Streptococcus salivarius, Streptococcus sanguinis and Actinomyces viscosus. In a similar study, concentrations of 25 micrograms per disk for Escherichia coli, 100 micrograms per disk for Staphylococcus aureus, and 50 micrograms per disk for Bacillus subtilis and Pseudomonas aeruginosa had the highest levels of antibacterial properties (16). In another study, Jothi reported the antibacterial properties of 1-5 g/L of the extract of Aloe vera leaves to be 97-99.1% against gram-positive bacteria (17). Furthermore, the results of another study by Etusim et al. indicated that the aqueous and alcoholic extracts of the leaves, stems and roots of Aloe vera could exert inhibitory effects against the bacterial growth of Pseudomonas, Salmonella and Proteus (lack of bacterial growth on medium: 14.5, 16 and 17.5 millimeters, respectively) (18).

According to the findings of Keithwas et al., the extract of Aloe vera exhibited inhibitory effects against the growth of several bacteria, including Escherichia coli, Staphylococcus epidermidis, Salmonella typhimurium, Aureus, Bacillus subtilis, Enterococcus faecalis, Proteus vulgaris, and Pseudomonas aeruginosa (15). In another research conducted by Jebashree et al., the ethanol, methanol, hexane and ethyl acetate extracts of Guava, Myrobalan, Mimusops elengi, and *Achyranthes aspera* were reported to have significant antibacterial properties (19). Moreover, the results of one study by Shirazi et al. indicated that the extract of Glycyrrhiza glabra exerted antibacterial effects against the growth of Salmonella typhi,

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Salmonella paratyphi B, Shigella sonnei, Shigella flexneri, and Enterotoxigenic Escherichia coli (20). According to the findings of Sedighniya et al., MIC of the extract of Glycyrrhiza glabra for Streptococcus mutans, Streptococcus sanguis and Actinomyces viscosus were 12.5, 12.5 and 50 mg/ml, respectively, while MBC values were reported to be 50, 12.5 and 12.5 mg/ml, respectively (21). In another research, Dilip George et al. compared the antimicrobial effects of commercial toothpastes and those containing Aloe vera extract against bacteria such as Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis, Prevotella intermedia, and Candida albicans using the disk-diffusion method.

According to the results, the extract of Aloe vera increased the antibacterial effects against the aforementioned bacteria. In another research, Bertolini et al. investigated the antibacterial effects of a toothbrush containing Aloe vera against Streptococcus mutans and concluded that this herbal extract decreased the contamination of the toothbrush with these bacteria (22). The results of the present study were indicative of the antibacterial properties of different concentrations of Glycyrrhiza glabra and Aloe vera extracts against various gram-positive and gram-negative bacteria. Variations in the effects of different concentrations of these herbal extracts could be due to the differences in the physiological features of the bacteria. However, the mechanisms of the antibacterial activities of these medicinal herbs remain unclear, while their antibacterial and antifungal effects could be attributed to the presence of phenolic compounds (23-26). Since Glycyrrhiza glabra and Aloe vera were observed to contain large proportions of phenolic compounds in the current study, the antimicrobial properties of these medicinal plants could be associated with the presence of these compounds. Therefore, further investigation of other medicinal herbs with similar antimicrobial agents seems necessary (27-30). Given the abundance of these plants in our country, especially Glycyrrhiza glabra, which mostly grows in the slopes of Zagros Mountains, using these herbal extracts is considerably more cost-efficient compared to other antimicrobial drugs. In conclusion, findings of the present study regarding the features and properties of Glycyrrhiza glabra and Aloe vera could be beneficial for researchers and pharmaceutical manufacturers, and it is recommended that these compounds be used in toothpastes and antimicrobial drugs in order to prevent various bacterial infections.

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