

Evaluation of the antimicrobial effects of *Glycyrrhiza glabra L.* on some gram positive and gram negative pathogenic bacteria in laboratory conditions

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Abstract

Background and objectives: Today, due to the increasing antibiotic resistance of bacteria, the use of medicinal plants as a suitable alternative to antibiotics has increased significantly; therefore, in this study, the antibacterial effects of methanolic extracts of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli* were evaluated.

Methods: In this laboratory research, after collecting plants and confirming its scientific name, extract of *Glycyrrhiza glabra L.* Root was prepared by Soxhlet extractor method at concentrations of 20 mg / ml to 400 mg / ml. Then the antimicrobial effects of this extract were investigated using Agar well diffusion and Dilution test methods.

Results: The results showed that the methanolic extracts of *G.glabra L.* in both Agar well diffusion and Dilution test methods had antibacterial effects on the tested bacteria. The highest effect was observed on *S.aureus* and the lowest effect was observed in *P.aeruginosa*.

Conclusion: According to the above results, it can be expected that the *G.glabra L.* extract can be used to treat bacterial infections and is a suitable alternative to commonly used chemical treatments for the treatment of infections.

Keywords: Antimicrobial Effects; Extract; Medicinal Plants; Pathogenic Bacteria

Introduction

Medicinal herbs have always been closely related to humans and plants throughout the development of all civilizations. Although most plant species are known to date, there is still a long way to discover new and valuable

herbal resources (1, 2). In this way, plants can be considered as a useful source of chemicals, only a part of which has been identified. These potentially useful chemicals can be used not only as a medicine but as an unrivaled model as the starting point for making pharmaceutical analogues, as well as

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an interesting tool for better understanding of biological phenomena(3-6). One of the most important therapeutic challenges is coping with infectious diseases due to their high prevalence. After recognizing penicillin and expanding its use in treatment, new antibiotics were introduced every day for the treatment of infections. The result was the expansion of clinical use of natural and synthetic antibiotics in the treatment of infections. The excessive use of these antimicrobials has led to increased resistance to different antibiotics in most bacteria (7). This is one of the reasons for the growing use of plants as low risk natural substances, affordable, Relative to synthetic antibiotics in the treatment of bacterial infections. Also, these herbal medicines are more popular with people (8,9). *G. glabra L* is one of the traditional medicinal herbs growing in different parts of the world. This plant is native to Southeast Europe and Southwest Asia, including Iran. The roots of this plant have beneficial medicinal properties, such as anti-inflammatory, antiviral, antimicrobial and anticancer activity, along with the effects of strengthening the immune system, controlling cough and liver detoxification. It is also used in Addison disease, asthma, bronchitis, coughing, peptic ulcer and arthritis (10,11). *G. glabra L* is used to treat gastrointestinal disorders and oral ulcers (12). In traditional Chinese medicine, *G. glabra L* is used to treat hepatitis and heart disease (13,14). *G. glabra L* extract is suitable as an adjuvant for inhibiting the growth of colon cancer cells (15). It also reduces the growth of cancer cells such as prostate cancer (16), gastric cancer (17), and breast cancer (18). *G. glabra L* extract can inhibit the growth of bacteria involved in dental caries (19,20), *Helicobacter pylori* (21), *Aspergillus niger* and *Candida albicans* (22). *G. glabra L* extract inhibits the replication of HIV virus in

patients with AIDS(23) The root of the *G. glabra L* contains numerous compounds such as flavonoids, sterols, amino acids and saponins. The antimicrobial activity of saponin has been proven against a large number of pathogenic pathogens (24, 25). Therefore, this study intends to investigate the antibacterial effects of methanolic extracts of *G. glabra L* on some of the standard pathogenic bacteria.

Materials and Methods

In this laboratory study, plant samples were collected from natural areas around Marand city in East Azarbaijan province, Eish-Abad village. Samples were cleaned after collection and transfer, and dried in a large and convenient area. After complete drying of the specimens, Root parts were prepared for milling. Soxhlet extractor method was used for extraction, So that 60 grams of *G. glabra L* powder with 300 ml of methanol as a solvent was placed in a Soxhlet Extractor for 8 hours, this solvent was evaporated at 40 ° C using a rotary machine and evaporated slowly(26). The extracts were concentrated with 5% DMSO solvent, Concentrations of 20, 30, 50 and 400 mg / ml were prepared for use in Minimum inhibitory concentration (MIC), Minimum Bactericidal Concentration (MBC) , and Agar Well Diffusion. The microorganisms used in this study were: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 1052, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (the microbial collections of the University of Tehran). To achieve a concentration of 1.5×10^6 cfu / ml, a microbial suspension with a McFarland Turbidity Standard 0.5 was diluted to 0.01. In this study, the antimicrobial activity of methanolic extract was investigated using Agar Well Diffusion and Dilution Test. In

Agar Well Diffusion method, 500 ml of microbial suspensions of 1.5×10^6 cfu / ml was transferred onto agar medium and cultured in a 3-way sterile swab. Then the wells with a diameter of 6 mm and a distance of 2.5 cm were created at the agar level. Subsequently, 100 μ l of concentrations of 20, 30, 50 and 400 mg / ml were injected from methanolic extract into each well. The negative control was obtained using a solution that was used to dissolve the extracts (5% DMSO) and also used as a positive control for chloramphenicol antibiotics. Then the plates were incubated for 24 hours at 37 ° C and after a certain time, in terms of forming or not forming a non-growth zone in millimeters was measured. Using the Dilution Test method, the minimum inhibitory concentration and minimum bactericidal concentration of methanolic extract were determined. In this method, in order to determine the MIC, methanolic extracts from dilutions of 25.6, 12.5, 25, 50, 100 and 200 mg / ml were obtained in Mueller Hinton Broth medium. Then, to each dilution, 1 ml of active bacterial suspension was added. Beside the tubes, positive control (The culture medium containing bacteria, without extracts) and negative control (non-bacterial culture) were used. Finally, the tubes were incubated for 24 hours at 37 ° C. After incubation, the tubes were examined for turbidity induced by the inoculated bacterial growth and the last dilution in which no turbidity was observed (no growth) as MIC was considered. Subsequently, all tubes in which no bacterial

growth was observed were sampled and determined by cultivating the minimum concentration of MBC in the plate. To reduce the error of the test, each of the above experiments was repeated five times. SPSS software version 18 was used to analyze the data. In order to study the significant difference was found between the results of ANOVA and Chi-square and the difference between the groups was significant at the significance level of $p < 0.05$.

Results

According to Table 1, the antibacterial activity of methanolic extract of *G.glabra L.* quantitatively and qualitatively by determining the diameter of the inhibition zone and MIC showed that this extract showed a significant inhibitory effect on *S.aureus* and *B.cereus* bacteria. *E.coli* is inhibited at higher concentrations with this extract, but the *G.glabra L.* extract at high concentrations showed the least effect on *P.aeruginosa* bacteria. The values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanolic extract of *G.glabra L.* plant against tested bacteria showed that the *G.glabra L.* extract on gram-positive bacteria has a higher bactericidal potency than gram negative bacteria. These results indicate that there is a significant difference in the susceptibility of *G.glabra L.* among the bacteria ($p < 0.05$).

Table 1: Mean diameter of inhibition zone and Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Glycyrrhiza glabra L.* Methanolic extract on bacteria tested

Extract concentration (mg/ml)	Agar Well Diffusion method (mean ± SD) (mm)						Dilution Macro Method (mg/ml)	
	20	30	50	400	Negative control	Positive control	MIC	MBC
<i>S.aureus</i>	10.04 ± 1.34	14 ± 1.22	19.4 ± 1.14	±0.54 25.6	--	20	6.25	12.5
<i>B. cereus</i>	7 ± 1	12.2 ± 0.83	15 ± 0.83	±2.28 18.8	--	19	12.5	25
<i>E.coli</i>	6 ± 1.22	10.8 ± 0.83	13.6 ± 1.14	±0.83 15.8	--	26	50	100
<i>P. aeruginosa</i>	0	0	7.2 ± 1.30	±1.14 10.6	--	22	100	200

Discussion

Increasing pathogen microorganisms and their resistance to a wide range of antibiotics, along with the economic and social problems resulting from it, have led to the development of studies on the production of herbal medicines. As a result, there is a critical need for research into new antimicrobial agents with promising natural activities to provide alternatives to common antibiotics (27). Ates et al. (2003) showed that ethanolic extract *G. glabra L.* inhibited *B. cereus* bacteria, *K. pneumoniae* and *S. aureus*, as well as acetone extracts *G. glabra L.* inhibited *B. cereus*, *B. subtilis*, *K. pneumoniae* and *S. aureus*. Chloroform extract *G. glabra L.* has an inhibitory effect on *B. cereus*, *B. subtilis*, *K. pneumoniae* and *E. faecalis*(28). Irani et al. (2016) concluded that the *G. glabra L.* extract had the most inhibitory effect on gram-positive bacteria by studying the antibacterial properties of the extract, That effect on *S. aureus* is significant(29). Sedighinia and Afshar (2012) showed antibacterial effects of *G. glabra L.* extract on oral pathogens that showed an antibacterial effect against *S. mutans*, *E. faecalis* and *A. viscosa* bacteria(30). Several studies have reported the

antimicrobial activity of the *G. glabra L.* extract on *S. aureus*, *E. faecalis* and *E. coli*(31). It has been shown in one study that Glabridin, one of the most important substances in this plant, has antibacterial activity against some species and is more active in gram-positive bacteria than gram-negative bacteria(30). The results of Kriker et al. (2014) Show that *G. glabra L.* extract has an inhibitory effect on standard bacteria of *S. aureus* and *P. aeruginosa*, but has no effect on the *E. coli* strain(32). Erdogan in 2002(33), Nitaliker et al. in 2010(34), and Sultana et al. in 2010(35) showed that the *G. glabra L.* extract can inhibit the bacterial growth of most gram-positive bacteria, such as *S. aureus*, which is consistent with the results of this study. In the study of Shirazi et al. (2007), the antibacterial effect of the *G. glabra L.* extract of *S. typhimurium*, *S. paratyphi B*, *S. sonnei*, *S. flexneri* and Enterotoxigenic *E. coli* was reported (36). Gupta et al (2013) by investigating the antibacterial activity of *G. glabra L.* root in gram positive and gram negative bacteria strains, Reported that the plant had the greatest impact on *B. subtilis*, *E. coli* and *S. aureus*, in this study, *E. coli* was more sensitive than *S. aureus*(37), Which is not

consistent with the findings of this study. Quercetin also has antibacterial effects on the majority of bacteria involved in Respiratory disorders, digestive system, skin and Urinary tract infection(38). The differences between the findings of this study and the studies of other researchers may be due to the effect of different methods of Preparation and extraction the extract. Also, the severity and extent of the antibacterial effects of the G.glabra L extract on the environment and climatic conditions of the cultivation of this plant is also directly related.

Conclusion

The results of this study indicate that the methanolic extract of the G.glabra L has a very large and diverse antimicrobial activity. Due to the cheapness, high availability and also the significant effects of methanolic extract of G.glabra L on the bacteria studied, this extract can be considered as an appropriate alternative to the production of new plant products after further studies on

laboratory animals with the least side effects due to non-chemical use are against the above bacteria.

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Declarations

Conflict of interest

We declare that we have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the article.

Authors' contributions

All authors contributed equally to this work.

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