ORIGINAL ARTICLE

Antibacterial Activity of *Glycyrrhiza Glabra* Roots against food-borne bacterial pathogens

N Eshghi[1], SO Ghasemian[2], AA Malekirad[3], F Dehnamaki[4], S Abdi[5], E Shiri Malekabad[6], M Rezaei[7, 8]

ABSTRACT

Objective: *The antibacterial activity of* Glycyrrhiza glabra *(Liquorice) roots was evaluated against several food-borne bacterial pathogens.*
Methods: *The in vitro anti-bacterial activity was evaluated by determining the zone diameter of inhibition (ZDI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) using the aqueous. Ethanolic and methanolic extracts of the roots of* Glycyrrhiza glabra.
Results: *Therefore, significant increase in inhibitory feature was observed because of increase in extracts concentration. In addition, the aqueous extract was more effective than the others; while, among the tested bacteria,* Staphylococcus aureus *and* Pseudomonas aeruginosa *were the most sensitive and the most resistant, respectively.*
Conclusion: *Extracts of* Glycyrrhiza glabra *roots can potentially be used in the pharmaceutical and food industries as preservatives or antimicrobial agents.*

Keywords: Antimicrobial activity, food-borne bacterial pathogens, *Glycyrrhiza glabra*, *Liquorice*

Actividad Antibacteriana de la Raíz de *Glycyrrhiza glabra* Contra las Bacterias Patógenas Transmitidas por los Alimentos

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RESUMEN

Objetivo: *La actividad antibacteriana del extracto de raíces de* Glycyrrhiza glabra *(regaliz) fue evaluada frente a varias bacterias patógenas trasmitidas por los alimentos.*
Métodos: *La actividad antimicrobiana se evalúa determinando el diámetro de la zona de inhibición (DZI), y la concentración bactericida mínima (CBM). Extractos acuosos, etanólicos y metanólicos de la raíz de* Glycyrrhiza glabra *fueron analizados en su actividad antibacteriana* in vitro.

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Resultados: *Por lo tanto, se observó un aumento significativo en la característica inhibitoria debido al aumento en la concentración de extractos. Además, el extracto acuoso fue más eficaz que los otros, en tanto que, entre las bacterias probadas,* Staphylococcus aureus *y* Pseudomonas aeruginosa *fueron las más sensibles y las más resistentes, respectivamente.*
Conclusión: *Los resultados sugieren que los extractos de raíz de* Glycyrrhiza glabra *tienen un uso potencial en la industria farmacéutica y alimentaria, y pueden ser útiles como conservantes o agentes antimicrobianos.*

Palabras clave: Actividad antimicrobiana, patógenos bacterianos transmitidos por alimentos, *Glycyrrhiza glabra*, *regaliz*

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INTRODUCTION

Drug resistance and the side effects due to overuse of antibiotics have become major problems in therapeutic medicine. On the other hand, many studies have shown the beneficial effects of using medicinal plants in improving food safety and reducing economic losses due to food-borne pathogens (1). Among the medicinal plants, *Glycyrrhiza glabra* (*Liquorice*) is a well-known native plant of South-east Europe and South-west Asia, including Iran (2). The treatment of gastric and peptic ulcers was the first reported medicinal usage of this plant (3). *Liquorice* has been used for treatment of psoriasis, eye diseases, throat infections, arthritic conditions, liver diseases, sex-hormone imbalances, menopausal symptoms and *helicobacter pylori infection* (4–6). The crude extract of *Liquorice* has also found commercial use as a food additive in Japan, as it contains the sweetening agent glycyrrhizin (7). Other studies have also suggested that the volatile oils found in the root of the *liquorice* plant may have anti-microbial activity against food-borne pathogens such as *Bacillus subtilis* and *Staphylococcus aureus* [*S aureus*] (8). The antimicrobial activity of the extract of *liquorice* has been reported against *Mycobacterium tuberculosis* (ATCC 27294) and *Mycobacterium tuberculosis* [ATCC 25177] (9), methicillin-resistant *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis* and *Escherichia coli* (10). Ethanolic extract of *Glycyrrhiza glabra*, the *Liquorice* of the family *Papilionaceae*, has showed antifungal activity against *Candida albicans*, *Candida krusei*, *Candida pseudotropicalis*, *Cryptococcus neoformans* and six filamentous fungi namely *Aspergillus niger*, *Aspergillus flavus*, *Sporothrix schenckii*, *Trichophyton rubrum*, *Microsporum gypseumand* and *Histoplasma capsulatum* (11). Moreover, the antibacterial activity of *liquorice* has been evaluated in several studies in Iran (2, 12). However, only a limited number of strains have been studied. To complete the aforementioned studies, the antimicrobial activities of the methanolic, ethanolic, and aqueous extracts of *Glycyrrhiza glabra* roots were evaluated against main food-borne bacterial pathogens including various gram-positive and gram-negative bacteria.

SUBJECTS AND METHODS

Plant materials

The roots of *Glycyrrhiza glabra* were collected during May 2013 from Arak, Iran. The specimens were identified by herbarium of medicinal plants Faculty, Arak University.

Plant extracts preparation

Collected roots were shade dried at room temperature. The dried and powdered roots (200 g) were extracted using 1 L of methanol and 1 L ethanol in a Soxhlet extractor for 72 hours at temperatures not exceeding the boiling point of the solvent (13). The aqueous extract was prepared by adding 1 L of boiling water to 200 g of the powdered plant materials and incubated at room temperature on a rotating shaker for three hours (200 rpm). The aqueous extracts were filtered using Whatman No. 1 filter paper and then concentrated at 40 °C in an oven.

Bacterial strains

The bacterial strains tested included: *Staphylococcus aureus* (PTCC 1431), *Salmonella enterica* (PTCC 1709), *Escherichia coli* (PTCC 1763), *Pseudomonas aeruginosa* (PTCC 1707), *Yersinia enterocolitica* (PTCC 1477), *Shigella dysenteriae* (PTCC 1188), *Streptococcus pneumonia* (PTCC 1240), *Streptococcus pyogenes* (PTCC 1447), *Listeria monocytogenes* (PTCC 1166) and *Bacillus cereus* (PTCC 1015) were obtained from the Iranian Research Organization for Science and Technology (IROST).

Antimicrobial activity assay

Disk diffusion method

Following the agar disk diffusion method as described by CLSI (14), the bacteria were cultured in BHI medium (brain heart infusion) for 18 hours at 37 °C. The suspension was compared to that of the 0.5 McFarland Standard [1.5×10^8 CFU/mL] (15). The suspension was inoculated on the surface of Mueller-Hinton agar medium by cotton swab (Merck). After the inoculation of each microorganism, the agar diffusion method was used by putting 10 µL of each extract on paper disks (6 mm of diameter) and incubated at 35 ± 2 °C for 18 to 24 hours. At the end of the incubation, the zone diameter of inhibition was measured (16).

Determination of MICs and MBCs on culture media

A macrodilution method was used in order to determine the MICs of the extracts. The different concentrations of the extracts of *Glycyrrhiza glabra* were prepared. At first, 180 µL of sterile broth was added to each well of a 96-well microtiter-plate. Then, 20 µL of the microbial suspension and 20 µL of each extract concentrations were added to the designed wells. A well consisting of BHI with no bacteria and a well containing BHI and suspension of bacteria were considered as negative and positive controls, respectively. Moreover, one sterility control containing essential oil was run in each plate.

The plates finally were incubated at 35 ± 2 °C for 24 hours. The lowest concentration of the extract resulting in perfect inhibition of visible growth in the broth medium were chosen as MICs. To evaluate the MBCs of the extract, 0.1 mL from no turbid wells were sub-cultured on BHI agar and incubated at 35 ± 2 °C for 24-hour. Then, the lowest concentrations of extract that allowed less than 0.1% of the original inoculum to survive was considered as MBCs (17).

Phytochemical analysis by high performance liquid chromatography (HPLC)

High performance liquid chromatography has ability to separate and identify the compounds present in trace concentrations as low as parts per trillion. Due to this versatility, it is being used in pharmaceutical industry. Therefore, in the present investigation, phytochemical analysis of *Glycyrrhiza glabra* of the aqueous extract was investigated by HPLC, an advanced form of column chromatography that pumps sample mixture/analyses in a solvent at high pressure through a column with chromatographic packing material. The column of HPLC used was made of stainless steel.

Statistical analyses

All experiments were done in triplicate. Statistical analysis was performed using SAS (Ver. 9.1). The results showing $p < 0.05$ were considered significant.

RESULTS

The mean ± SD of the ZDI of different extracts used in this study are presented in Tables (1–3), respectively. The extracts had antibacterial activity against both gram-negative and gram-positive bacteria. Other studies have reported that *Liquorice* extract and its components exhibited widespread antimicrobial activity (6, 8, 18–20). The ZDI of aqueous, methanolic and ethanolic extracts were in the range of 7.17–21, 7–16.7 and 7–18.3 mm, respectively.

Table 1: Comparison of mean ± SD of ZDI (mm) of pathogenic bacterial strains for *Glycyrrhiza glabra* root ethanolic extract (mg/mL).

Bacteria	600	400	200	100	50
S aureus	16.7 ± 0.58	13.3 ± 0.58	10.3 ± 0.58	–	–
S enterica	15.2 ± 0.29	12.3 ± 0.58	9.5 ± 0.5	–	–
E *coli*	16.3 ± 0.58	13.5 ± 0.87	10.3 ± 0.58	–	–
P aeruginosa	11.8 ± 0.29	9.67 ± 1.15	–	–	–
Y enterocolitica	14.3 ± 0.87	12.2 ± 0.58	11	–	–
S dysenteriae	15	12.7 ± 0.29	8.5	–	–
S pneumoniae	13.2 ± 0.29	11.7 ± 0.58	8	–	–
S pyogenes	13	11.7 ± 0.29	9	–	–
L monocytogenes	14 ± 0.5	11.5 ± 0.5	7	–	–
B cereus	13.2 ± 0.29	11	7.33 ± 0.58	–	–

Table 2: Comparison of average of ZDI (mm) of pathogenic bacterial strains for *Glycyrrhiza glabra* root aqueous extract (mg/mL).

Bacteria	600	400	200	100	50
S aureus	21	19.5 ± 0.5	18.2 ± 0.29	13.7 ± 0.29	8.67 ± 1.15
S enterica	18.2 ± 0.29	15.3 ± 0.58	14.7 ± 0.29	11.5	7.33 ± 0.58
E *coli*	19.7 ± 0.29	17.5 ± 0.5	16.3 ± 0.29	11.3 ± 0.58	8.33 ± 0.29
P aeruginosa	14.2 ± 0.29	11.3 ± 0.29	8.33 ± 0.58	–	–
Y enterocolitica	18.7 ± 0.58	16.3 ± 0.68	17.2 ± 0.29	12.2 ± 0.29	–
S dysenteriae	19.2 ± 0.29	17.3 ± 0.58	14.5	12.3 ± 0.58	–
S *pneumoniae*	16.7 ± 0.29	13.3 ± 0.58	9.83 ± 0.29	9	–
S pyogenes	16.2 ± 0.29	14	12.5 ± 0.29	7.17 ± 0.29	–
L monocytogenes	17.8	13.2 ± 0.29	11.5 ± 0.5	9	–
B cereus	16.3 ± 0.58	14	12.3 ± 0.58	9.83 ± 0.29	–

Table 3: Comparison of average of ZDI (mm) of pathogenic bacterial strains for *Glycyrrhiza glabra* root methanolic extract (mg/mL).

Bacteria	600	400	200	100	50
S aureus	18.3 ± 0.58	16.8 ± 0.76	15	13.5	7.33 ± 0.6
S enterica	17	15.3 ± 0.29	13.7 ± 0.3	9.33 ± 0.6	7
E *coli*	18.2 ± 0.29	16.3 ± 0.29	14.3 ± 0.6	9 ± 0.9	7.5 ± 0.5
P aeruginosa	13.3 ± 0.58	10.5 ± 0.87	8.17 ± 0.3	_	_
Y enterocolitica	16.7 ± 0.58	15 ± 0.5	14.3	9.5	_
S *dysenteriae*	16.5 ± 0.5	16	14.3 ± 0.3	9.67 ± 0.6	_
S *pneumoniae*	15	13	9.17 ± 0.3	_	_
S pyogenes	14.7 ± 0.29	13.3 ± 0.58	10.7 ± 0.6	_	_
L monocyto-genes	16.2 ± 0.29	13.3 ± 0.58	9	_	_
B cereus	15.3 ± 0.58	12.3 ± 0.58	9 ± 0.9	_	_

The results showed there was significant correlation between an increase of extract concentration and increase of ZDI in the bacterial pathogens tested. In other words, The ZDI increased significantly in a dose dependent manner ($p < 0.05$). Furthermore, the results of ZDI showed that the maximum ZDI was seen with *Staphylococcus aureus* and the minimum was *Pseudomonas aeruginosa* (Figs. 1 and 2).

Table 4 shows the values of MICs and MBCs of the aqueous, ethanolic and methanolic extracts against the bacterial pathogens tested.

DISCUSSION

According to the results *P aeruginosa* was the most resistant of the pathogenic bacterial strains tested. Fukai *et al* surveyed the antimicrobial effect of flavonoids from *liquorice* extract ag ainst *H pylori* and methicillin-resistant *Staphylococcus aureus* through MIC more than 100 µg/mL (6, 18). Ateş *et al* reported *liquorice* root extracts showed various antibacterial activities (7–11 mm/20 µl inhibition zone) against the *Bacillus brevis*, *Bacillus cereus*, *Bacillus mega-terium*, *Bacillus subtilis*, *Bacillus subtilis var. niger*, *Enterococcus fecalis*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Micrococcusluteus*, *Micrococcus smegmatis, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Yersinia enterocolitica* (20). Another study obtained MIC for *liquorice* root extracts at concentrations from 2 to > 50 µg/mL against food contaminant microorganisms (8). *Liquorice* roots is reported to possess antimicrobial activity against pathogenic bacteria that cause stomach ulcers, indicating that it may cyto-protective and anti-infectious (19).

Glycyrrhetinic acid is a promising biological alternative for the topical treatment of recurrent vulvovaginal candidiasis [RVVC] (21). Moreover, *Glycyrrhiza glabra* aqueous extract (1 mg/mL) is reported to inhibit the adhesion of *Helicobacter pylori* to human stomach tissue (19).

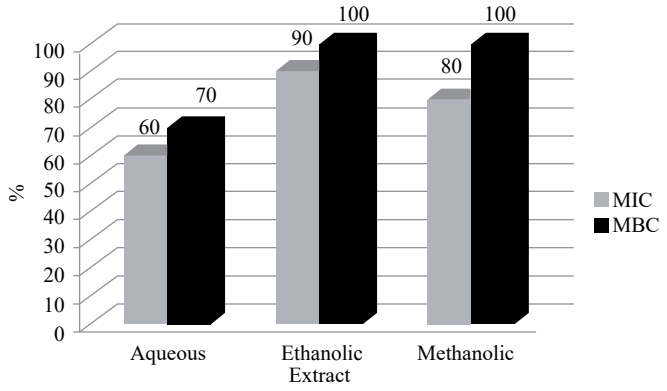


Fig. 1: Minimum inhibitory concentration and minimum bactericidal concentration values of *P aeruginosa* as the most resistant bacteria (mg/mL).

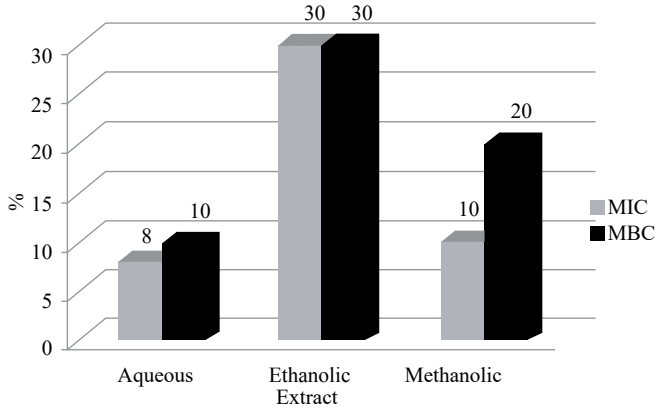


Fig. 2: Minimum inhibitory concentration and minimum bactericidal concentration values of *S aureus* as the most sensitive bacteria (mg/mL).

Table 4: Determination of MIC and MBC value (mg/mL) for *Glycyrrhiza glabra* root aqueous, ethanolic and methanolic extracts against pathogenic bacterial strains.

Extracts	*S aureus*		*S enterica*		*E coli*		*P aeruginosa*		*Y entero-colitica*		*S dysenteriae*		*S pneumoniae*		*S pyogenes*		*L monocy-togenes*		*B cereus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Water	8	10	9	10	10	10	60	70	40	40	10	20	30	30	40	50	20	30	10	10
Ethanolic	30	30	30	40	20	30	90	100	70	70	20	20	50	50	60	70	30	30	30	30
Methanolic	10	20	20	20	10	20	80	100	60	60	20	30	40	50	50	50	30	40	20	30

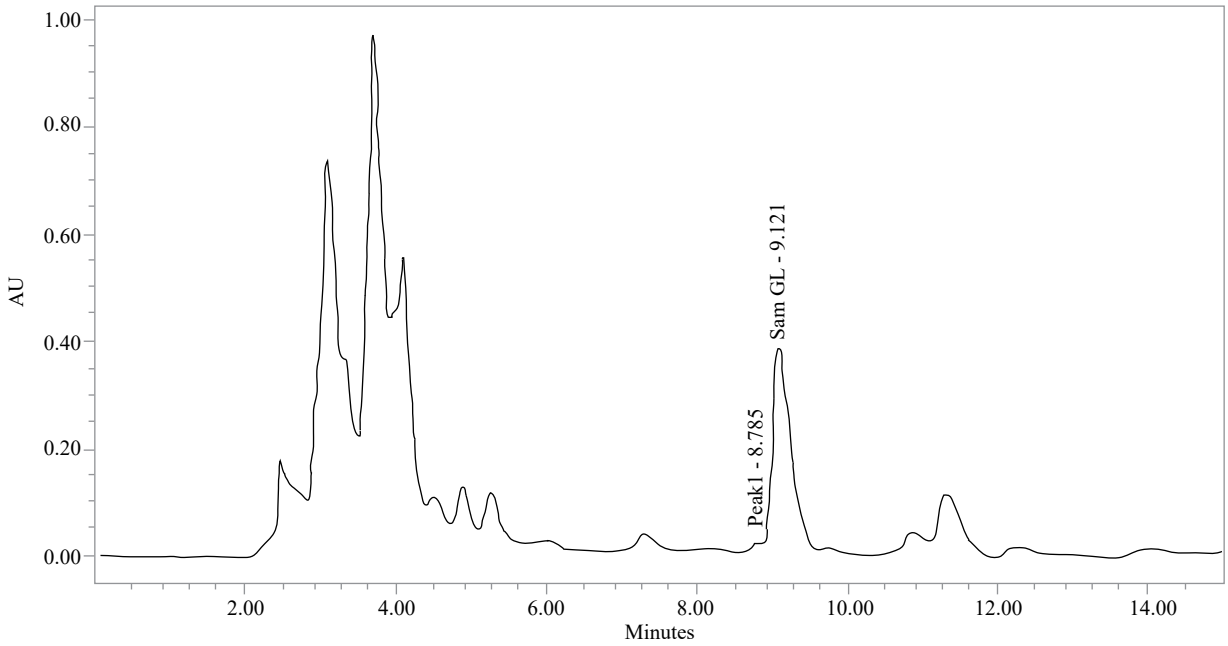
MIC: Minimum inhibitory concentration; MBC: minimum bactericidal concentration.

Xiao *et al*, showed that *licochalcone* isolated from the root of *Glycyrrhiza glabra* has the potential to be used in the treatment of gastric cancer (22). Messier *et al* (2012) reported that *liquorice* and its constituents can be used for preventing/treating oro-dental diseases (23). Glycyrrhetinic acid, has a protective effect against staphylococcal pneumonia in a mice model system (24). Irani *et al* reported the most active extract against gram-positive bacteria was related to the ethanolic extract of the leaves of *Glycyrrhiza glabra*. Other studies in Iran have reported the antimicrobial effect of Liquorice ethanolic extract against *Helicobacter pylori* through MIC ranging from 125 to above 500 µg/mL (25) and 500 µg/mL effective against *Malassezia Furfur* (26), MIC, and MBC for *Lactobacillus delbrueckii*, 0.8 and 0.7 mg/mL, respectively (27). Our results demonstrated that the aqueous extract had the greatest anti-bacterial activity. This outcome would be influenced by genotype differences of *Glycyrrhiza glabra* used in the study along with the growth ecosystem (habitat, temperature, height), experimental conditions (pH and temperature), and differences in the tested strains. Growth and performance of the plants in the ecosystems is under the effect of many factors such as type, habitat, soil, height and geographical position. Each one of the factors may have considerable influence on the quality and quantity of the result.

A study, performed on food-borne pathogens, showed that the allicin and lysozyme conjugated nanocellulose had good antifungal and antibacterial effects against standard strains of *Candida albicans*, *Aspergillus niger*, *Staphylococcus aureus* and *Escherichia coli* (28).

The result of agar disk diffusion method, is affected by physical and chemical factors such as the type of culture media, diffusing characteristic of the culture media and the size and stability of the molecule. Standardizing the experimental conditions is a necessity in order to achieve more reliable results. Results of the diffusion must be interpreted based on diluting and diffusing methods and the inhibition rates of different concentrations of extracts against different bacteria.

Results of phytochemical analysis based on HPLC revealed that presence of glycyrrhizic acid in the aqueous extract was more effective compared to the others. Moreover, among the tested bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most sensitive and the most resistant, respectively. A HPLC chromatogram of *Glycyrrhiza glabra* is shown in Fig. 3.



	Peak Name	RT	Area	% Area	Height
1	Peak1	8.785	151975	2.37	14811
2	Sam GL	9.121	6258782	97.63	378855

Fig. 3: A chromatogram obtained for the *Glycyrrhiza glabra.*

The difference in sensitivity between gram-positive and gram-negative bacteria may be due to the differences in the composition and structure of the cell wall.

CONCLUSION

Antimicrobial activity of *Glycyrrhiza glabra* root extract against several food-borne bacterial pathogens was demonstrated, because of its antibacterial properties, *Glycyrrhiza glabra* root extracts may be a suitable replacement for synthetic antibiotics. However, further investigations are required to determine the suitability of using the extract components to increase the shelf-life of food and as anti-bacterial agents.

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