Invitro Anti-Bacterial and Anti-Oxidative Activity of *GlycyrrhizaGlabra* L. from North of Golestan Province

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ABSTRACT

Background and Objective: *Glycyrrhizaglabra* L. is one of the most widely used medicinal herbs in Golestan province that is known for its anti-inflammatory, carminative, antiviral, anti-infection and anti-ulcer properties in Iranian traditional medicine. This study aimed to assess the anti-bacterial and anti-oxidative activity of *G. glabra*from the Golestan province.

Methods: The rip root of the plant was collected in autumn 2013. The ethanolic extract of the plant was prepared by maceration method. The anti-oxidative property of the plant was assessed by total antioxidant capacity (TAC), reducing power (RP) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assays. The anti-bacterial activity was assessed using agar-well diffusion method and the minimum inhibitory concentration (MIC) assay.

Results: The ethanolic extract of *G. glabra*had relatively high anti-oxidative activity with IC50 value of 130 µg/ml, especially in the DPPH method. The extract also exhibited high anti-bacterial activity against the following Gram-positive bacteria: *Staphylococcus aureus* (21.1±0.7 mm), *Staphylococcus epidermidis* (19.6±0.2 mm), *Bacillus subtilus* (19.3±0.6 mm), followed by *Escherichia coli* (12.1±0.8 mm), *Enterococcus faecalis* (13.2±0.1 mm) and *Kelebsiellapneumoniae* (11.5±0.4 mm) with MIC values in the range of 31 - 132 mg. mL^{-1}.

Conclusion: According to results, the root extract of *G.glabra*is a good source of antioxidant compounds with suitable anti-bacterial activity, which can be used as natural anti-infection and anti-inflammatory agent for treatment of many diseases.

KEYWORDS: Anti-Bacterial, Anti-Oxidant, *Glycyrrhiza*, Golestan Province.

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INTRODUCTION

In recent decades, the increased level of oxidative stress, cell injury and cell death generated during chemotherapy, and antibiotics resistance have been considered the major health problems. Screening of antioxidant and antibacterial activities of natural compounds (polyphenols, terpenoids and flavonoids) present in many wild plants, especially endemic medicinal plants (1- 4) has attracted global interest (5-7). Licorice (*Glycyrrhizaglabra* L.) is a perennial herb with sweet taste in its root that grows wild in subtropical areas of Europe, Middle East and Western Asia. The root extract of the plant and its principal component (glycyrrhizin) have extensive use in food and tea industries, and herbal medicine as strong anti-inflammatory, anti-infection, anti-coagulative, anti-allergic, expectorant and especially anti-viral agent for treatment of gastric inflammations, gastric ulcer, jaundice and hepatitis (8, 9). Several studies have reported that the root extract of *G. glabra* L. is rich in triterpenes (glycyrrhizin, glycyrrhetinic acid, liquirtic acid) and flavonoids (liquirtin and formononetin) (10). Various chemical constituents including glycyrrhizin, glycyrrhizinic acid, glabridin, glabrene, glabrol, licoflavonol, glycyrol, licoricone, formononetin, glabridin, glabranin isomer andnarigenin have been previously isolated (11). The anti-microbial and anti-viral activity of the *G. glabra* root extract has been previously reported (12, 13); therefore, this study aimed to determine the anti-oxidative and anti-bacterial activity of *G. glabra* from Gorgan, Golestan Province, Iran.

MATERIAL AND METHODS

The root of 3-year-old plant was collected in September 2013 from meadows in Northern areas of Gorgan (35m), located in Northwest of the Golestan province (latitude of 36° 37' 24" to 36° 34' 28" and longitude of 54° 35' 26" to 54° 24' 32"). The collected roots were kept in silty clay loam soil. A voucher specimen of the plant was identified and deposited at the Herbarium of Research Center of Medicinal plants of Islamic Azad University of Gorgan. The roots were dried in shade, powdered and stored at 4°C until invitro testing. One gram of the plant with 100 ml of solvent (methanol 80%) was extracted by maceration method. The extracts were filtered with Whatman No. 1 filter paper. The filtrates were evaporated in dry rotary evaporator at 40°C and were later stored at 4°C (14). 2, 2'-diphenyl-1picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St., Louis, USA). Other chemical substances and culture plates were purchased from Merck Co. (Germany).

This assay was performed according to Arabshahi-Delouee method. First, the dried extract (12.5–1000 µg) in 1 ml of the solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 gl^{-1}). The mixture was incubated at 50°C for 30 min. Then, 2.5 ml of trichloroacetic acid (100 g l^{-1}) were added and the mixture was centrifuged at 1650 g for 10 min. Next, 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (1g l^{-1}). Finally, the samples' absorbance was measured at 700 nm (15).

The free radical scavenging activity of the extract was assessed by the method described by a previous study (15). Briefly, 1 ml of 1 mMmethanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 12.5–1000 µg dried extract). The mixture was then vortexed vigorously and left for 30 min in the dark at room temperature. The absorbance was measured at 517 nm and DPPH scavenging activity was expressed as percentages relative to controls using the following equation:

DPPH scavenging activity (%) = [(Absorbance of control – Absorbance of sample) / Absorbance of control] ×100

This experimental procedure was adapted from Arabshahi-Delouee method, which is based on the reduction of Mo (VI) to Mo (V) by the sample and formation of a green phosphate/Mo (V) complex at acidic pH. An aliquot of 0.1 ml of the sample solution (containing 12.5- 1000 µg of dried extract in the corresponding solvent) was combined in a tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tube was incubated in a thermal block at 95 °C for 90 mins. The samples were cooled and their absorbance was measured at 695 nm. A typical blank solution containing 1 ml of the reagent solution and appropriate volume of the solvent was prepared and incubated under the same conditions as the rest of the samples (15).

The bacterial strains were obtained from the Microbiology Laboratory of Golestan University of Medical Sciences. The ethanolic extract of the roots were individually tested against nine strains of Gram positive and Gram negative bacteria including *Shigelladysenteria (*PTCC1188*), Pseudomonas aeroginosa* (PTCC1430),*Escherichia coli* (PTCC1399), *Staphylococcus aureous* (PTCC1431), *Bacillus cereus* (PTCC1015), *Salmonella typhimurium*(ATCC1596),*Staphylocuccusepid ermidis* (PTCC1114), *Enterococcus faecalis* (PTCC1393) and *Kelebsiellapnumonie*(PTCC1291). In the first screening, the extracts were tested against the mentioned bacteria. Minimal inhibitory concentrations (MICs) were determined by the agar serial dilution method at concentrations ranging from 0.93 to 60 µg/mL. Two-fold serial dilutions were prepared from the plant extract in molten Mueller Hinton agar (Pronadisa- Madrid) andcooled to 45-50 ^{o}C in a water bath. The plant extract was dispersed in the mixture using dimethyl sulfoxide (DMSO). Then, 0.01 mL of every bacterial suspension equivalent to a 0.5 McFarland standard (108 CFU/mL) was inoculated on the agar of each well. The culture plates were then incubated at 37 ^{o}C for 24 hours. The MIC was defined as the lowest concentration at which no visible growth was observed (16). The Mueller Hinton agar containing DMSO without the essential oil was used as negative control, while gentamycin was used as positive control. ANOVA was used to compare the anti-*Candida* activities of the essential oil and drug. P-value of less than 0.05 was considered statistically significant.

RESULTS

As shown in Table 1, the results showed that the root extract of *G. glabra* had suitable antioxidant activity with IC50 value of 130±1.4 µg/ml in free radical scavenging, especially in the DPPH method. The ethanolic extract of the root had a good potential antimicrobial activity against some Gram-positivebacteria (Table 2). The maximum antibacterial activity of the extract was observed against Gram positive bacteria including *S. aureus* (21.1±0.7 mm), *S. epidermidis* (19.6±0.2 mm), *B. subtilus* (19.3±0.6 mm), followed by *E. coli* (12.1±0.8 mm), *E. faecalis* (13.2±0.1 mm) and *K. pneumoniae* (11.5±0.4 mm) with MIC values in the range of 31 - 132 mg. mL^{-1}.

Table 1- Evaluation oftheantioxidant activity of *G. glabra*L. (collected from Gorgan)using different methods

	IC50 (µg/ml)		
Antioxidant activity	Root extract	BHA	BHT
TAC	510±1		
RP	799 ±1.3	492.6±0.3	423.6±0.5
DPPH	130 ±1.4		

Table 2- the anti-bacterial activity of ethanolic extract of *G.glabra* from Gorgan

Bacteria	Inhibition zone (mm) ±SD	MIC (mg/mL)	Gentamycin
. aureus	21.1±0.7	31.4	20.7
S. epidermidis	19.6±0.2	42.1	22.3
B. subtilis	19.3±0.6	49.2	16.5
E. faecalis	13.2±0.1	93.2	9.6
E. coli	12.1±0.8	91.6	17
P. aeroginosa	11.1±0.9	110.5	9
K. pneumonia	11.5±0.4	124.5	na
S. typhymorium	10.2±0.7	132	na
S. disentria	11.1±0.5	132	na

na: non active

DISCUSSION

The results of this study showed that the ethanolic extract of *G. glabra* root had good anti-bacterial and antioxidant activity in free radical scavenging, especially compared to other species (7). Thus, it can be suggested that terpenoids and polyphenols were the secondary compounds in the *G. glabra* extract, which might be responsible for the anti-oxidative, anti-inflammatory, anti-infection and sedative properties of this plant. Several studies reported that the root extract of *G. glabra* L. is rich in triterpenes (glycyrrhizin, glycyrrhetinic acid, liquirtic acid) and flavonoids (liquirtin and formononetin) (10). Many studies have also reported the isolation of various chemical constituents with anti-oxidative and anti-bacterial properties including glycyrrhizin, glycyrrhizinic acid glabridin, glabrene, glabrol, licoflavonol, glycyrol, licoricone, formononetin, glabridin, glabranin isomer andnarigenin (11, 17). In a similar study, it was found that licorice prevents replication of Herpes simplex virus in vitro (12). Some studies indicated that licorice root can be used as a strong anti-inflammatory agent and immune system strengthener for treatment of asthma, immunodeficiency, allergy and liver inflammation, due to its ability to synthesize many antioxidant compounds such as phenolic, terpenoids and flavonoids. These metabolites can also act as antiviral and antiseptic agents that nourishand stimulate the immune system (18, 19). The natural antioxidants in *G. glabra* such as glycyrrhizicacid can reduce the risk of developing infectious and inflammatory diseases and cancer. They can also reduce stomach acid and be used to treat stomach ulcer and disorders of the gastric mucosa (20). It was reported that *S. aureus*, *E. coli* and *S. epidermidis* are the main causes of infectious furuncles, sores, wounds, nosocomial infections and gasteric ulcer (21). Furthermore, it was shown that the methanolic extract of licorice root have a good anti-bacterial effect on 12 bacteria, especially against *Helicobacter* and *E. coli*, which is in agreement with our findings. Thus, these results prove the medicinal applications of this plant's extract for the treatment of many diseases such as stomach ulcer, disorders of the gastric mucosa and jaundice (22).However, it was reported that Gram-negative bacteria are often more resistant to *G. glabra* (22), which is consistent with the results of the present study. Recently, finding naturally occurring anti-oxidative, anti-inflammatory and anti-bacterial agents to replace synthetic drugs for use in food or pharmaceutical industrieshave attracted a lot of attention (23,24).

CONCLUSION

The results of this study indicate that the root of *G. glabra* has suitable anti-oxidative and anti-bacterial activity and confirm the traditional uses of this plant in the Golestan province.

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CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

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