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

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Received: 07 Oct 2015  
Revised: 01 Dec 2015  
Accepted: 19 Dec 2015

**ABSTRACT**

**Background and Objective:** *Glycyrrhiza glabra* 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 subtilis* (19.3±0.6 mm), followed by *Escherichia coli* (12.1±0.8 mm), *Enterococcus faecalis* (13.2±0.1 mm) and *Klebsiella pneumoniae* (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.

This paper should be cited as: Varasteh Moradi A, Zhand S [Invitro Anti-Bacterial and Anti-Oxidative Activity of *Glycyrrhiza* *Glabra* L. from North of Golestan Province]. mljgoums. 2016; 10(3): 48-52
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 (Glycyrrhiza glabra 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, liquiritic acid) and flavonoids (liquiritin and formononetin) (10). Various chemical constituents including glycyrrhizin, glycyrrhizinic acid, glabridin, glabrene, glabrol, licoflavonol, glycyrol, licoricone, formononetin, glabridin, glabranin isomer andnarinogenin 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-1-picrylhydrazyl (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 (K₃Fe(CN)₆; 10 g l⁻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 FeCl₃ (1 g 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:

\[
\text{DPPH scavenging activity (\%) = } \left[ \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100
\]

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 culture plates were then incubated at 37 °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-

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-positive bacteria (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. subtilis (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 of the antioxidant activity of G. glabra (collected from Gorgan) using different methods

<table>
<thead>
<tr>
<th>Antioxidant activity</th>
<th>IC50 (μg/ml)</th>
<th>BHA</th>
<th>BHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root extract</td>
<td>TAC 510±1</td>
<td>492.6±0.3</td>
<td>423.6±0.5</td>
</tr>
<tr>
<td></td>
<td>RP 799±1.3</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>DPPH 130±1.4</td>
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</tbody>
</table>

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

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

na: non active
epidermidis are the main causes of infectious furuncles, sores, wounds, nosocomial infections and gastric 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 industries have 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.

AKNOWLEDGEMENTS

The authors would like to appreciate honest efforts of respectful personnel of Research Center of Medicinal Plants at Islamic Azad University of Gorgan, as well as Golestan University of Medical Sciences for their cooperation.

CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.
REFERENCES


