



# Antimicrobial Potential of *Glycyrrhiza glabra*

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**Abstract:** This study was carried out to evaluate the antimicrobial activity of methanolic extract and different fractions (*n*-butanol, ethyl acetate, chloroform and *n*-hexane) of *Glycyrrhiza glabra* root. The antimicrobial activity was determined by the disc diffusion method and minimum inhibitory concentration (MIC) against a panel of microorganisms (four bacterial strains, *i.e.* *P. multocida*, *E. coli*, *B. subtilis* and *S. aureus* and three fungal strains, *i.e.* *A. flavus*, *A. niger* and *R. solani*). The results indicated that plant extract and fractions mildly potent as antimicrobial agent.

**Keywords:** Antimicrobial Activity, Resazurin, *A. flavus*, Minimum Inhibitory Concentration

## 1. Introduction

There has also been a rising interest in the research for natural products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades and in recent times [1]. Mulathi (*Glycyrrhiza glabra*) is of considerable importance in term of its medicinal and aromatic values. This plant belongs to Fabaceae family (Genus, *Glycyrrhiza*) and is native of south-east Europe and south-west Asia, including Iran. Liquorice (UK) or licorice (North America) is the root of *Glycyrrhiza glabra* (from the Greek "sweet root"), from which a sweet flavour can be extracted. The flavor of licorice comes mainly from a sweet-tasting compound called anethole ("trans"-1-methoxy-4-(prop-1-enyl)benzene), an aromatic, unsaturated ether compound also founds in many other herbs. Additional sweetness in licorice comes from glycyrrhizic acid (an antiviral compound significantly sweeter than sugar). The roots of *G. glabra* are rich in bioactive compounds like antiviral, anticancer, anti-ulcer, anti-diabetic, anti-inflammatory, antioxidant, antimalarial, antifungal, anti-bacterial, estrogenic, anti-allergenic and expectorant activities [2, 3] and anticonvulsant activities. *Glycyrrhiza glabra* (liquorice) root is an effective expectorant, and has been used for this purpose since ancient times, especially in Ayurvedic medicine where it is also used in tooth powders and is known as Jastimadhu.

Anti-*Helicobacter pylori* and antibacterial activities of flavonoids from the licorice extract have already been reported [4-6] Antimicrobial activities of roots and rhizomes have been studied in previous researches, but there are a few

reports about the effect of licorice roots different extracts and fractions against microorganisms [7]. Gupta and co-workers [8] reported the antimicrobial potential of *Glycyrrhiza glabra* roots. The purpose of the present study is to evaluate the antioxidant and antimicrobial effectiveness of different extracts and fractions of *G. glabra* roots.

## 2. Materials and Methods

### 2.1. Plant Material

Roots of the selected medicinal plant *G. glabra* were purchased from the local market of Faisalabad, and was further identified from the Department of Botany, University of Agriculture, Faisalabad, Pakistan, where a voucher specimen number has been deposited.

### 2.2. Preparation of Extract and Fractions

2.5 Kg roots of *G. glabra* washed with distilled water so that the adhering dust particles must be removed. They were dried in the shaded place. The dried roots were powdered and stored in the clean container. In the weighed amount of powdered roots the measured amount of 100% methanol (2×15 L) was added and kept it for 4-5 days at room temperature. The solvent was removed under reduced pressure. Extract (320g) became viscous which was dried on water bath and then stored at -4°C. The process was repeated three times with intervals of four days. The 100% methanolic extract was dissolved in distilled water and fractionation was done by using different polarity based solvents and obtained successively *n*-hexane (105g), chloroform (63g) and *n*-

butanol (44g) fractions. 80% methanolic extract (42g) was also obtained.

### 2.3. Phytochemical Analysis

Phytochemical screening of the extracts was carried out according to the methods described by Trease and Evans [9] for the detection of active components like saponins, tannins, alkaloids, steroids, flavonoids and Anthraquinones.

### 2.4. Antimicrobial Assay of Plant Extracts and Different Fractions

#### 2.4.1. Microbial Strains

The Mulathi roots extracts and its different fractions were individually tested against a panel of microorganisms, including four bacteria, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pasturella multocida* and three pathogenic fungi, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus solani*. The pure bacterial and fungal strains were obtained from the Biological Division of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The purity and identity of the strains were verified by the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37°C in nutrient agar (Oxoid, Hampshire, UK) while fungal strains were cultured overnight at 28°C using potato dextrose agar (Oxoid).

#### 2.4.2. Disc Diffusion Method

The antimicrobial activity of the Mulathi extracts and its different fractions was determined by the disc diffusion method [10]. The discs (6 mm in diameter) were impregnated with 10mg/mL extracts/fractions (100µL/disc) placed on the inoculated agar. Extracts/fractions were dissolved in 10% sterile dimethyl sulfoxide. Rifampicin (100 µL/disc) (Oxoid) and Fluconazole (100 µL/disc) (Oxoid) were used as positive reference (5mg/mL) bacteria and fungi, respectively. Disc without samples was used as a negative control. Antimicrobial activity was evaluated by measuring the inhibition zone.

### 2.5. Resazurin Microtitre-Plate Assay

The minimum inhibitory concentration (MIC) of the Mulathi extracts/fractions was evaluated by a modified resazurin microtitre-plate assay [11] with little modification. Briefly, a volume of 100 µL of extracts/fractions solutions in 10% dimethyl sulfoxide (DMSO, v/v) was transferred into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth and muller hinton broth for bacteria and fungi respectively were added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. To each well 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 mL of sterile distilled water) were added. Finally, 10 µL of bacterial/fungal suspension were added to each well. Each plate was wrapped loosely with cling film to ensure that

bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad spectrum antibiotics as positive control, a column with all solutions with the exception of the test samples, a column with all solutions with the exception of the bacterial/fungal solution adding 10µL of broths instead and a column with 10% DMSO (v/v) solution as a negative control. The plates were prepared in triplicate, and incubated at 37°C for 24 h and 28°C for 48 h for bacteria and fungi respectively. The absorbance was measured at 620 nm by micro quant for fungus and at 500nm for bacteria. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change appeared was taken as the MIC value.

### 2.6. Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference  $p \leq 0.05$  was considered to denote a statistically significance All data are presented as mean values  $\pm$  standard deviation (SD).

## 3. Results and Discussion

### 3.1. Phytochemical Analysis

The 100% methanol extract was tested to check the presence of secondary metabolites. Methanolic extract was found to be positive for the presence of alkaloids, flavonoids, tannins and saponins while steroids and anthraquinones were absent. Li *et al.* [12] reported the three flavonoids named licoagrosides D, E and F together with four known flavonoids, medicarpin 3-O-glucoside, calycosin 7-O-glucoside, formononetin 7-O-(6-malonylglucoside) and 2-hydroxyformononetin 7-O-glucoside isolated from *Glycyrrhiza pallidiflora* hairy root cultures.

### 3.2. Antimicrobial Activity of *Glycyrrhiza glabra*

The antimicrobial activity of *G. glabra* roots was assessed against some pathogenic microorganisms. The extract and fractions exhibited significant antimicrobial activity against all the tested strains. The results from the disc diffusion method (Table 1), followed by measurement of minimum inhibitory concentration (MIC) (Table 2), indicated that 100% methanolic extract showed good activity against *E. coli* and *B. subtilis*, showing the highest inhibition zones (33 and 27.5 mm) and the lowest MIC values (9.28 and 30.2 mg/mL), respectively. Least activity was exhibited against *A. niger* and *R. solani* with the smallest inhibition zones (16.5 and 16 mm) and the highest MIC values (150 and 152 mg/mL). 80% methanolic extract showed strong activity against *B. subtilis* and *E. coli* with inhibition zones (30 and 28.5 mm) and the lowest MIC values (12.2 and 20.1 mg/ml), respectively. Least activity was exhibited against *S. aureus* with inhibition zone (19 mm) and the highest MIC value (110 mg/ml),

respectively. 80% methanolic fraction showed magnificent activity against *A. niger* as compared to standard drug fluconazole. Chloroform fraction showed good activity against *A. flavus*, showing the inhibition zone (30.5 mm) and the lowest MIC value (12.2 mg/ml), respectively. Least activity was exhibited against *R. solani* with the smallest inhibition zone (12.5 mm) and the highest MIC value (210 mg/ml). *n*-butanol fraction showed good activity against *P. multocida* and *S. aureus*, showing the inhibition zones (27.4 and 23.5 mm) and the lowest MIC values (30.5 and 70.5 mg/ml), respectively. Least activity was exhibited against *E. coli* with the smallest inhibition zone (17 mm) and the highest MIC value (140 mg/ml). *n*-hexane fraction showed good activity against *P. multocida* showing the inhibition zone (20.5 mm) and the lowest MIC value (90.2 mg/ml), respectively. Least activity was exhibited against *R. solani* and *E. coli* with the smallest inhibition zones (13.5 and 13 mm) and the highest MIC values (192 and 192.2 mg/ml). In general, the antimicrobial activity of the tested extracts and fractions was comparable with the standard drugs, rifampicin and fluconazole. Nitalikar *et al.* [13] reported that the *G. glabra* root extracts has potent antibacterial activity against *E.*

*coli*, *S. aureus*, *B. subtilis* where acetone extract exhibited magnificent activity against *S. aureus* with maximum inhibited zone (32mm) as compared to standard drug Tetracyclin (25 mm) these results are in agreement with our findings where extracts and fractions of the plant roots showed potent activity against pathogens and 80% methanolic extract showed magnificent activity against *A. niger* (26 mm) as compared to standard fluconazole (17.5mm).

This antimicrobial activity may be due to Alkaloids, flavonoids, tannins, steroids, saponins, anthraquinones found in extracts. These phytochemical groups are known to possess antimicrobial compounds [14, 15]. The majority of antimicrobial effects from licorice is due to isoflavonoid components particularly hispaglabridin and B, 4'-O-methylglabridin, glabridin, glabriol and 3-hydroxyglabrol [16].

Further purification and characterization of the active principles from the extracts and fractions (for antibacterial and antifungal studies) will provide a better understanding of the antimicrobial mechanism and serves as a tool for potential lead compounds for microbial infectious diseases.

**Table 1.** Antimicrobial potential of *G. glabra* roots methanolic extracts and various organic fractions by disc diffusion method.

Microbial Strain		Inhibition Zone(mm) <sup>a</sup>				
		Methanolic extract and different organic fractions				
	Antibiotic	100% Methanol	80% Methanol	Chloroform	<i>n</i> -butanol	<i>n</i> -hexane
<b>Bacteria</b>	<b>Rifampicin</b>					
<i>B. subtilis</i>	37.0±1.73 <sup>a</sup>	27.5±1.41 <sup>b</sup>	30.0±1.65 <sup>b</sup>	19.0±1.0 <sup>c</sup>	27.4±2.9 <sup>b</sup>	18.5±1.5 <sup>c</sup>
<i>P. multocida</i>	27.5±1.65 <sup>c</sup>	24.0±1.41 <sup>a</sup>	22.0±1.41 <sup>ab</sup>	13.5±1.65 <sup>d</sup>	22.5±1.5 <sup>ab</sup>	20.5±0.86 <sup>b</sup>
<i>S. aureus</i>	29.4±0.866 <sup>a</sup>	19.0±1.0 <sup>c</sup>	19.0±1.0 <sup>c</sup>	15.5±1.65 <sup>d</sup>	23.5±1.5 <sup>b</sup>	18.5±1.5 <sup>c</sup>
<i>E. coli</i>	34.2±1.65 <sup>c</sup>	33.0±1.73 <sup>a</sup>	28.5±2.23 <sup>d</sup>	13.0±1.0 <sup>e</sup>	17.0±1.5 <sup>b</sup>	13.5±1.5 <sup>c</sup>
<b>Fungi</b>	<b>Fluconazole</b>					
<i>A. niger</i>	17.5±1.65 <sup>c</sup>	16.5±1.65 <sup>cd</sup>	26.0±1.65 <sup>b</sup>	13.0±1.0 <sup>e</sup>	21.5±2.44 <sup>a</sup>	14.5±0.866 <sup>de</sup>
<i>A. flavus</i>	31.5±1.65 <sup>a</sup>	17.5±1.65 <sup>c</sup>	28.0±1.0 <sup>c</sup>	30.5±1.65 <sup>ab</sup>	19.0±1.41 <sup>b</sup>	14.0±1.41 <sup>d</sup>
<i>R. solani</i>	23.0±2.23 <sup>c</sup>	16.0±1.41 <sup>b</sup>	20.0±1.41 <sup>a</sup>	12.5±0.86 <sup>e</sup>	18.0±1.41 <sup>b</sup>	13.0±1.0 <sup>c</sup>

Values are mean ± SD of three separate experiments

<sup>a</sup>Diameter of inhibition zone (mm) including disc diameter of 6 mm.

Letters in superscript show the significance of the results.

**Table 2.** Minimum inhibitory concentration.

Microbial Strain		MIC (mg/mL).				
		Methanolic extract and different organic fractions				
	Antibiotics	100% Methanol	80% Methanol	Chloroform	<i>n</i> -butanol	<i>n</i> -hexane
<b>Bacteria</b>	<b>Rifampicin</b>					
<i>B. subtilis</i>	3.24±0.51	30.2±1.25	12.2±1.43	110±1.65	30.5±1.2	120±2.1
<i>P. multocida</i>	300±5.1	60.1±1.43	83.2±2.3	192±1.15	82.2±1.5	90.2±1.94
<i>S. aureus</i>	15.2±1.43	110±2.1	110±3.1	172±2.24	70.5±1.15	112±1.43
<i>E. coli</i>	8.25±1.0	9.28±0.86	20.1±1.0	198±1.15	140±2.6	192±1.5
<b>Fungi</b>	<b>Fluconazole</b>					
<i>A. niger</i>	140±2.54	150±1.23	40.2±1.2	192±0.216	80.1±1.42	182±1.56
<i>A. flavus</i>	10.1±0.86	140±2.1	20.1±0.9	12.2±0.34	110±1.53	184±2.0
<i>R. solani</i>	82.3±1.4	152±2.6	82.2±1.5	210±4.5	120±2.1	192.2±1.8

Values are mean ± SD of three separate experiments.

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