RESEARCH ARTICLE

Qualitative Phytochemical Analysis and Antibacterial Activity Evaluation of *Glycyrrhiza glabra* against Some Human Pathogenic Bacteria

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ABSTRACT

In the present investigation, the methanolic root extracts of *Glycyrrhiza glabra* were evaluated for antimicrobial activity against some human pathogenic bacterial strains and subsequently phytochemical analysis of the crude extracts was carried out to determine the active phytochemical constituents responsible for antimicrobial activity. It was observed that the methanolic root extract of *G. glabra* shows a significant effect on the tested pathogenic bacterial strains with a zone of inhibition ranging between 21 to 31 mm. Preliminary phytochemical screening of the extract revealed that they were positive for glycosides, flavonoids, steroids, phenols, saponins, alkaloids, tannins, and anthraquinones. Infrared spectroscopy (IR) and proton nuclear magnetic resonance spectroscopy (^{1}HNMR) studies confirmed the presence of three metabolites, such as, glycyrrhizin (Gg I), glabridin (Gg II), and 18-β-glycyrrhetinic acid (Gg III). The results of the antimicrobial study revealed efficient activity of the crude extract, which might be due to the presence of soluble bioactive components, and were confirmed by phytochemical analysis, IR, and ^{1}HNMR. Thus, it was observed that the roots of *G. glabra* could be used for the extraction of medicinally important bioactive compounds.

Keywords: ^{1}HNMR, Antimicrobial activity, *Glycyrrhiza glabra*, IR, Phytochemical analysis, Zone of inhibition.

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INTRODUCTION

Traditional medicine has been practiced for many centuries in many parts of the world, including India, especially in rural areas due to availability and low cost. Nature has provided a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine.[1] There has been an increasing incidence of multiple resistances in human pathogenic microorganisms, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases.[2] The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity, as well as, for the discovery of new antimicrobial compounds.[3-6] The efforts of scientists are establishing plants with promising antimicrobial property is yielding fruitful results as a number of plants with the high antimicrobial property have been elucidated.[7-13]

G. glabra belongs to the family Fabaceae. *G. glabra,* commonly known as licorice, is an herbaceous perennial, and has been used as a flavoring agent in foods and medicinal remedies for thousands of years. Licorice root has been widely used around the world to treat cough since ancient times. It has been widely used in folk medicine for the treatment of different diseases.[14-17] Moreover, licorice has been reported for having antibacterial and antiviral activities.[18]

G. glabra contains several chemical constituents[19] with inhibitory effects. Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to investigate *in vitro* antimicrobial activity and phytochemical analysis of *G. glabra* methanolic root extract against some human pathogenic bacterial strains (standard microbial cultures).

MATERIAL AND METHODS

Preparation of Plant Extracts

Twenty plant species were collected and predicting to posses bioactive compounds, and plant species were collected based on the information available from literature,[20-22] folklore,[23] and through field observations. The plant materials were collected in and around Visakhapatnam district, Andhra Pradesh, India. The collected plant materials were washed thoroughly with running tap water, and finally, with distilled water; the material

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was chopped into small pieces and then, air-dried on a sterile blotter under shade for 20 to 30 days.

The completely shade dried plant materials were coarsely powdered and allowed to Soxhlet extraction with methanol for 5 to 6 hours at a temperature not exceeding the boiling point of the solvent and then filtered through Whatman no. 1 filter paper. The extracted liquid obtained was subjected to a rotary evaporator and subsequently concentrated under reduced pressure (in vacuum at 40°C). The residues obtained were designated as crude extracts, were labeled and stored in the refrigerator for further study.[24] The dried plant extract residues obtained were redissolved in 0.1% dimethyl sulfoxide (DMSO) to get the necessary concentration of crude extracts and filtration through a 0.45 µm membrane filter and stored in sterile brown bottles in a freezer at 20°C, until bioassayed.

Microorganisms

Based on the disease index, eight bacterial strains were selected, listed in Table 1. All the eight microorganisms tested were purchased from microbial type culture collection and gene bank (MTCC) Chandigarh, India. All the pure cultures were obtained in the lyophilized or freeze-dried form are reconstituted in sterile water and produced a suspension of the microbial cells, inoculation was done with a sterile inoculating loop to liquid broth medium. Liquid cultures are then incubated to allow cell replication and adequate growth of the culture, for use in bioassays. Following incubation, liquid cultures are refrigerated to store for further use. Typically, 24 hours will provide sufficient growth to allow the visibly thick spread of the microbes for bioassay. The bacterial strains are maintained and tested on nutrient agar medium.

In vitro Antimicrobial Assays

The development of simple *in vitro* pre-screens could offer an initial idea of the biological activity of plant extracts and its compounds. The antimicrobial activity of bacterial strains listed in Table 1 was performed by the agar ditch/ well/ cup diffusion method[25-27] at desired concentration with DMSO solvent, which did not affect the growth of microorganisms. The antimicrobial activity was evaluated by measuring the zone of inhibition of the organisms against extracts.

Preliminary Phytochemical Screening

To detect the presence of various biologically active constituents like glycosides, flavonoids, steroids, phenols, saponins, alkaloids, tannins, and anthraquinones in the root methanolic extracts of *G. glabra* the preliminary phytochemical screening was done by standard methods.[28-31]

Isolation and Characterization of Compounds

In view of its medicinal and antimicrobial importance, as evidenced in Table 2, the root extract of *G. glabra* was subjected to column chromatography for separation of pure compounds by gradient elution method. The crude extract was chromatographed over a column of silica gel (G 100–200 mesh acme), using eluents of the increasing polarity of solvent mixtures hexane, hexane-ethyl acetate, pure ethyl acetate, and methanol. Fractions of 250 mL were collected and monitored

Table 1: Pathogen index

S. No.	*Pathogen*	*MTCC code*	*Disease*
1	*Bacillus subtilis*	121	Septicemia, wound, and burn infections
2	*Klebsiella pneumoniae*	39	Pneumonia, bloodstream infections
3	*Streptococcus*	889	Scarlet fever, rheumatic fever
4	*Psuedomonas mirabilis*	425	Urinary tract infections
5	*Escherichia coli*	476	Urinary tract infections, pneumonia
6	*Micrococcus*	7527	Bacteremia, meningitis
7	*Enterococcus faecalis*	439	Urinary tract and nosocomical infections
8	*Enterobacter cloacae*	509	Skin infections, meningitis, bacteremia

Table 2: Antimicrobial activity of plant extracts and isolated phytochemicals on pathogens

		DIZ (mm)			
S. No.	*Pathogen*	**Gg extract*	*Gg I*	*Gg II*	*Gg III*
1	*Bacillus subtilis*	23	24	20	22
2	*Klebsiella pneumoniae*	31	20	20	27
3	*Streptococcus*	26	19	21	22
4	*Psuedomonas mirabilis*	25	18	20	21
5	*Escherichia coli*	27	26	22	23
6	*Micrococcus*	28	23	24	20
7	*Enterococcus faecalis*	25	18	20	19
8	*Enterobacter cloacae*	30	21	17	19

DIZ diameter of zone of inhibition in mm, including well diameter 6 mm; *Gg extract concentration of *G. glabra* methanolic root extract (100 mg/mL); Gg I, II, III - phytochemicals isolated from *G. glabra* (1 mg/mL)

Table 3: Preliminary phytochemical analysis of root extracts of *G. glabra*

Phytochemical test	*Test result*
1. Alkaloids	
Dragendorff's test	+
Mayer's test	+
Hager's test	+
Wagner's test	+
Tannic acid test	+
2. Flavonoids	
Alkaline reagent test	+
Lead acetate test	+
Zn-HCl reduction test	+
Shinoda's test	+
3. Quinones	
NaOH test	+
Alcoholic KOH test	+
4. Anthraquinones	
Borntrager's test	+
Modified Borntrager's test	+
5. Glycosides	
Raymond's test	+
Legal's test	+
Kellar Kiliani test	+
Conc. H_2SO_4 test	+
Bromine water test	+
Molisch test	+
6. Tannins	
Gelatin test	+
Mitchell's test	+
7. Steroids	
Salkowiski test	+
Libermann-Buchard test	+
8. Phenols	
Ellagic acid test	+
9. Saponnins	
Froth test	+

+: positive (presence of the constituent)

through silica gel thin layer Chromatography (TLC), the visualization of spots under UV light or iodine vapor, or by spraying 5% methanolic sulphuric acid and heating at 110°C. Fractions with similar spots were combined, the purification of each fraction was affected by extensive re-chromatography over small silica gel columns, and recrystallization from suitable solvents and the purity of the compounds was ascertained by homogeneity over silica gel (G 60–120) TLC.

Identification and Characterization of the Compounds

For identification, characterization, and structural elucidation of the compounds, spectroscopic methods like HPCL, GC/MS, UV, IR, and NMR spectra were used, the IR spectral data has been recorded on KBr disc at Indian Institute of Chemical Technology (IICT), Hyderabad; GC/MS (GP 5050A) at Andhra University; ^{1}H and ^{13}C NMR recorded on MHz 400 at Central Drug Research Institute (CDRI), SAIF, Lucknow, and Indian Institute of Science (IISc), Bangalore. The compounds isolated were named with the prefix "Gg" signifying that these compounds were isolated from *G. glabra*.

RESULTS AND DISCUSSION

In the present investigation, the antimicrobial activity of methanolic extracts of twenty plant species was evaluated against eight bacterial strains listed in Table 1, in which *G. glabra* root methanolic extract shows significant activity against all eight (gram +ve and gram –ve) pathogenic bacterial strains. Data presented in Table 2 revealed that the methanolic root extract of *G. glabra* shows a significant effect on all pathogenic strains with a zone of inhibition ranging from 21 to 31 mm (including well diameter 6 mm). Chloramphenicol and penicillin were used as positive control antibiotics, which produced the zones ranging from 25 to 35 mm.

Preliminary phytochemical screening revealed the presence of glycosides, flavonoids, steroids, phenols, saponins, alkaloids, and tannins (Table 3) in the methanolic root extract of *G. glabra*. The inhibitory effects of this extract on the microorganisms may, therefore, be due to the presence of the above mentioned phytochemical components.[32]

Three biologically active compounds (Gg I-glycyrrhizin, Gg II-Glabridin, Gg III-18-β-glycyrrhetinic acid) from the methanolic root extract of *G. glabra* were isolated by chromatographic and recrystallization techniques, and were tested for antimicrobial activity against the bacterial strains

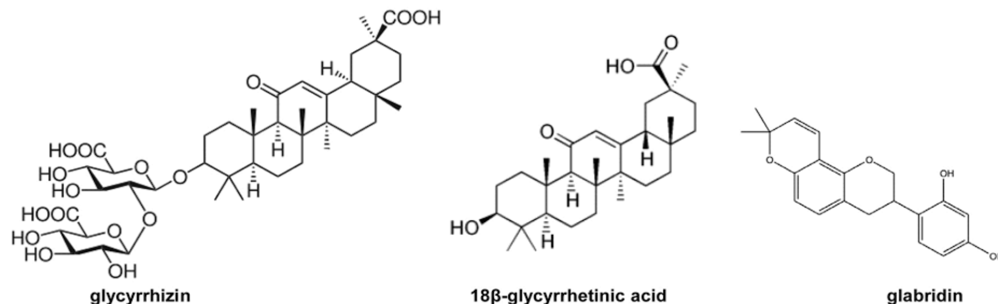

Figure 1: Phytochemical compounds isolated from *G. glabra*

(Table 2) which exhibited effective inhibition on all the pathogens.

Physical and Spectral Data of the Compounds

Gg I: Glycyrrhizin or glycyrrhizinic acid (Figure 1). The white crystalline powder obtained from the fraction of hexane:ethyl acetate (98:2) mp 295–302°C. ^{1}HNMR (CDCl3, 400 MHz) spectrum showed multiplet between 1.6, 2.15, and 7.2.

Gg II: Glabridin (Figure 1).[25,26] Red brown powder obtained from the fraction of hexane:ethyl acetate (95:5) mp 154 to 156°C. ^{1}HNMR (DMSO), 400 MHz spectrum showed multiplet between 6.82 (d, 1H, J = 8.3 Hz), 6.38 (dd, 1H, J = 8.4 Hz), 6.3 (d, 1H, J = 9.9 Hz), 6.31 (d, 1H, J = 2.6 Hz), 2.85 (m, 1H), 1.43 (s, 3H), MS at m/z is 325.3 (M^+ + H), 189.21, 149.15, 130.60, 123.03, 110.01, and 82.97.

Gg III: 18-β-glycyrrhetinic acid (Figure 1). White crystalline powders obtained from the fraction of hexane:ethyl acetate (95:5) mp 292 to 295°C. ^{1}HNMR (CDCl3, 400 MHz) spectrum showed multiplet between 7.2, 5.7, 3.2, 2.8, 2.7, 2.3, 2.1, 1.9, 1.8, 1.6, 1.5, 1.4, and 1.1. MS m/z is 471.53 (M^+, 100), 469.34, 457.46, 409.37, 337.33, 317.24, 277.26, 212.22, 191.06, 189.21, and 122.22.

CONCLUSION

In conclusion, it is observed that to standardize the botanical identity of the drug in crude form and the bioactive compounds in pure form, revealed that the antimicrobial activity of *G. glabra* root methanolic extract is largely due to the presence of biologically active constituents and their synergistic effect. The encouraging results indicate that the methanolic root extracts of *G. glabra* might be exploited as a natural drug for the treatment of several infectious diseases caused by the organisms and could be useful in understanding the relations between traditional cures and current medicine. The present phytochemical studies should further focus on exploring the uninvestigated phytomolecules that may possess more promising medical benefits.

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