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Research Article

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACT ROOTS OF GLYCYRRHIZA GLABRA AND HPLC ANALYSIS

P.K.P. GAITRY CHOPRA[a*], BINDA D. SARAF[a], FARHIN INAM[a] AND SUJATA S. DEO[a]

[a]Department of Chemistry, Institute of Science, Civil lines, R.T. Road, Nagpur, India. Email: gaitry_chopra@rediffmail.com

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ABSTRACT

This research evaluated the antimicrobial and the *in vitro* antioxidant potential of methanol extract of roots of *Glycyrrhiza glabra*. The dried root powder of *Glycyrrhiza glabra* was analysed for the presence of heavy metals using the XRF scanning method. A preliminary phytochemical screening was also performed for the extract and its HPLC analysis was also undertaken. Methanol extract showed the presence of phytoconstituent saponins, flavonoids and sugars. The extract was further tested for its antimicrobial activity against four bacteria viz. *S. aureus*, *S. sciuri*, *S. typhi* and *E. coli* as well as two fungi *A. awamorii* and *Rhizopus spp.* The results have shown that methanol extract of the roots is a moderate antimicrobial agent. The scavenging activity on the DPPH free radical was determined and the extract was found to have good antioxidant activity of 67.22% at 500µg/mL with the IC_{50} value 359.45µg/mL.

Keywords: Antimicrobial, Antioxidant, *Glycyrrhiza glabra,* HPLC, XRF scanning.

INTRODUCTION

Licorice is the name applied to the roots and stolons of some *Glycyrrhiza* species and has been used by human beings for at least 4000 years. It is a component of many traditional medicinal systems. The genus *Glycyrrhiza* consists of about 30 species, and chemical studies have so far been carried out on 15 of them. Glycyrrhizic acid [1], a triterpene glucoside, is the principal constituent of *G. glabra* which is 50 times sweeter than sugar. The saponin is used frequently as a tool for recognizing the herb and has been obtained from *G. glabra*, *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi*, and *G. eurycarpa*, and thus, these plants are generally accepted as licorice [2, 3]. *G. glabra* is considered to consist of 3 main varieties. *G. glabra* L. var. *typica* is commonly referred to as Spanish or Italian licorice. *G. glabra* L. var. *violacea* is known as Persian or Turkish licorice. *G. glabra* L. var. *glandulifera* is mainly known as Russian licorice [4, 5].

In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* have been in clinical use for centuries. Roots have demulcent, antacid, anti-ulcer [6], anti-inflammatory, expectorant, tonic, diuretic, laxative and sedative properties [7, 8]. They also possess antipyretic [9], antimicrobial, antiherpes [10], and anxiolytic [11] activities. In the traditional system of medicine *G. glabra* is recommended for the treatment of epilepsy [6]. Studies have shown that the extract has estrogenic activity and may help regulate the estrogen-progesterone ratio [12, 13]. Compounds in licorice root may also prevent heart disease in post-menopausal women [14] and have a growth-inhibitory effect on breast cancer cells [15, 16]. Many of the phenolic compounds isolated from licorice root may also help to protect low density lipoprotein (LDL) and red blood cells from oxidative damage [17-19]. Licorice root extract has also been shown to be beneficial for the liver. It has been used in Japan for more than twenty years as a treatment for chronic hepatitis, and studies with licorice root have shown a significant reduction of serum amino-transferase and a significant improvement in liver histology [20-22]. It has also been found that the licorice juice cause differences in the salivary pharmacokinetics of paracetamol if consumed with paracetamol [23].

In the present work we have attempted to compile and document evidence of essential and non-essential heavy metals in *G. glabra* and highlight the need for research and development. The preliminary phytochemical screening and HPLC fingerprinting of the methanolic extract of *G. glabra* has also been done. The biological evaluation of the extract was carried out and the extract was screened for its antimicrobial activity against four bacteria and two fungi namely, *Staphylococcus aureus*, *Staphylococcus sciuri*, *Salmonella typhi*, *Escherichia coli*, *Aspergillus awamorii* and *Rhizopus spp*. The anti-oxidant property of the extract was also studied using the DPPH assay.

MATERIALS AND METHODS

Plant material

The roots of plant *Glycyrrhiza glabra* Linn. were collected from Shri Sai Medi farms, Nagpur. The plants were botanically identified and authenticated from the Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur. The plants specimens were dried and herbarium sheets were made. The specimens of the plants are deposited in the Department for further references.

Preparation of extract

About 20 g of powder of the dried roots of *Glycyrrhiza glabra* was charged into Soxhlet apparatus and extraction was carried out continuously for 6 to 8 h using following solvents:

1. Petroleum ether (60°C– 80°C)

2. Methanol

The marc in the Soxhlet apparatus was dried every time before changing the solvent. The resulting extracts were reduced in vacuum/distilled and stored in glass bottles.

Phytochemical screening

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids, etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites, the methanolic extract of *G. glabra* was subjected to various chemical tests [24].

Evaluation of anti-microbial activity

In vitro antibacterial activity was determined by using Mueller Hinton Agar while *in vitro* antifungal activity was determined by using Sabouraud Dextrose Agar obtained from Himedia Ltd., Mumbai. Freshly prepared suspensions in sterile water of pure isolated cultures of *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus sciuri*, *Escherichia coli*, *Aspergillus awamorii* and *Rhizopus spp*. were mixed with the sterilized Mueller Hinton agar for bacteria and Sabouraud Dextrose Agar for fungi maintained at 42.0 + 2.0^0C and poured in petri dish and allowed to solidify. Test solution was prepared with known weight of extract in dimethyl sulphoxide (DMSO) and diluted suitably to give the resultant concentration of 100, 250 and 500 µg/mL. Whatman no. 1 sterile filter paper discs (6 mm) were impregnated with solution and allowed to dry at room temperature. The discs were then applied on the petri plates and the plates were incubated at 37 ◦ C for 24 h (bacteria), 28 ◦ C for 72–96

h (fungi) and the diameter of inhibition zone was measured for the estimation of the potency of the antimicrobial extract.

Determination of free radical scavenging activity by DPPH method

The antioxidant potential of the methanolic extract was determined on the basis of its scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The DPPH assay was carried out as per the procedure outlined by Blois [25]. Free radical form of DDPH shows absorbance at 517 nm but upon reduction by anti-oxidant its absorbance decreases. Briefly, 0.1 mM solution of DPPH was prepared in methanol and 4 mL of this solution was added to 1 mL of sample solution in DMSO at different concentrations (100, 200, 300, 400, 500 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm using Shimadzu-1700 spectrophotometer. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

% inhibition = $[(A_B - A_S) / A_B] \times 100$

Where, A_B is the absorbance of the control reaction (containing all reagents except the test compound) and A_S is the absorbance of the test compound. Ascorbic acid was used as positive control. The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration.

High Performance Liquid Chromatography fingerprinting

The HPLC system binary gradient Shimadzu LC-10 VP with a UV detector was used for determination of fingerprinting of the methanolic extract of *G. glabra.* Reverse- phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 x 4.6 mm internal diameter, particle size 5 µm, Luna 5 µm C-18), phenomenex, at 26^0C.

Running conditions include: injection volume, 5µl; mobile phase, methanol: water (70:30 v/v); flow rate, 1 mL/min and the chromatogram monitored at 254.0 nm. Sample was filtered through an ultra membrane filter (pore size 0.45 µm; E-Merck, Darmstadt, Germany) and sonicated for 45 min before being used.

Heavy metal analysis

The medicinal plants contains varying amount of various heavy metals. These could be both essential as well as non-essential. It has been reported that whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals. The deficiency and excess of essential micronutrients and trace toxic metals can cause serious effects on health.

The plant sample (powdered root) was analyzed for various elements by taking XRF [26] scan on PW 2403 MagiX using IQ+ software. The levels of lead, arsenic, iron, copper, rubidium and some common elements were measured in the plant root powder.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of the crude petroleum ether and methanolic extract of *G. glabra* revealed the presence of steroids in the petroleum ether extract and flavonoids, saponins and sugars in the methanolic extract. Alkaloids, proteins and tannins were not detected; the result of phytochemical test has been summarized in Table 1.

Table 1: Phytochemical results of methanolic and petroleum ether extracts of *G. glabra*

Sr. No.	Plant constituents	Test/ reagent		Petroleum ether extract	Methanolic extract
1.	Steroids	a)	Salkowski test	+	-
		b)	Liebermann- Burchard test	+	-
2.	Alkaloids	a)	Mayer's reagent test	-	-
		b)	Hager's reagent test	-	-
		c)	Wagner's reagent test	-	-
3.	Saponins	a)	Foam test	-	+
4.	Tannins	a)	Ferric chloride test	-	-
		b)	Lead acetate test	-	-
5.	Flavonoids	a)	Shinoda's test	-	+
6.	Proteins	a)	Biuret test	-	-
		b)	Xanthoproteic test	-	-
7.	Sugars (Carbohydrates)	a)	Molisch's test	-	+
		b)	Barfoed's test	-	+

Key:+ - present; - - absent

Table 2: Antimicrobial screening of methanolic extract of *G. glabra*

Sr. No.	Organism	Zone of inhibition (mm) for different concentrations (µg/mL)		
		100	250	500
1.	*S. aureus*	8	10	13
2.	*S. sciuri*	6	9	11
3.	*S. typhi*	7	9	12
4.	*E. coli*	9	11	15
5.	*A. awamorii*	4	7	9
6.	*R. spp.*	7	10	11

Evaluation of anti-microbial activity

The results of the antimicrobial study show that the methanolic extract of *G. glabra* is a moderate antimicrobial agent. The extract was most potent against *S. aureus* at 500µg/mL (inhibition zone 13 mm) amongst bacteria and showed maximum potency against *R. spp*. at 500µg/ml (inhibition zone 11 mm) amongst fungi. It was lest active against *A. awamorri*. The results obtained are reported in Table 2.

Determination of free radical scavenging activity by DPPH method

The antioxidant activity of each extract is tested with DPPH scavenging assay. The result of the DPPH scavenging assay is shown in Figure 1. It is found that antioxidant compounds in methanol extract have the free radical scavenging ability. The presence of flavonoids in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [27]. From the graph it can be concluded that the methanolic extract of *G. glabra* is a potent anti-oxidant agent with a maximum of 67.22% at a concentration of 500µg/mL. The calculated IC_{50} for the methanol extract of *G. glabra* is 359.45µg/mL and that of the standard ascorbic acid is 14.70µg/mL.

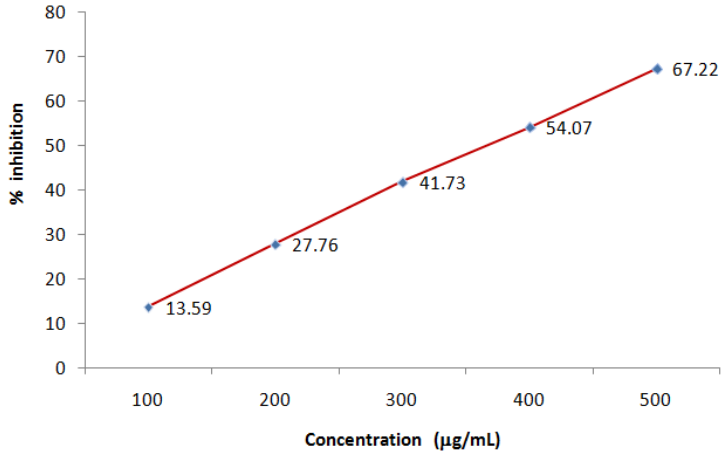

Fig. 1: Antioxidant activity of methanol extract of *G. glabra*

High Performance Liquid Chromatography fingerprinting

The fingerprint profile of the extract obtained could be used for identification purpose. The HPLC fingerprint of methanolic extract of *G. glabra* shows the presence of 13 different compounds. The major peaks (components with highest concentration) were obtained at the retention time (min.) of 2.383, 2.607, 3.683 and 4.087. The components with moderate concentration were found at retention time of 1.590, 2.003, 8.253, 8.910 and 10.127. The fingerprint of the methanolic extract of *G. glabra* is shown in Figure 2.

Heavy metal analysis

The heavy metal analysis revealed that investigated medicinal plant powder i.e. root powder of *G. glabra* is a good source of essential heavy metals. Calcium has the highest percent presence whereas Titanium, Manganese and Arsenic were found in traces. The results are tabulated below in Table 3.

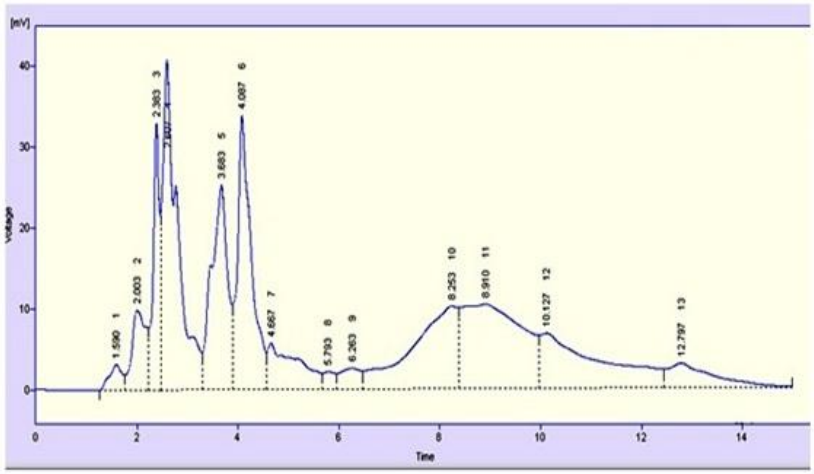

Fig. 2: HPLC fingerprint of methanolic extract of *G. glabra*

Table 3: Heavy metal analysis of root powder of *G. glabra*

Sr. No.	Metal	% Occurrence
1.	Potassium (K)	0.66
2.	Calcium (Ca)	1.87
3.	Chlorine (Cl)	Nd
4.	Sulphur (S)	0.09
5.	Iron (Fe)	0.14
6.	Aluminium (Al)	0.05
7.	Phosphorous (P)	0.06
8.	Silicon (Si)	0.12
9.	Magnesium (Mg)	0.17
10.	Rubidium (Rb)	Nd
11.	Titanium (Ti)	Trs
12.	Manganese (Mn)	Trs
13.	Sodium (Na)	0.04
14.	Strontium (Sr)	0.06
15.	Lead (Pb)	Nd
16.	Arsenic (As)	Trs
17.	Copper (Cu)	Nd

Trs = Traces; Nd = Not detected

CONCLUSION

In conclusion, the results of the present study support the usage of the studied plant and suggest that the methanolic extract of *G. glabra* possess compounds with antimicrobial potentials that can be further explored for antimicrobial activity. The extract is a potential antimicrobial agent at higher concentrations but shows moderate activity at lower concentrations. The extract is also a good antioxidant agent.

The fingerprint profile of the *G. glabra* extract showing potential antimicrobial activity, obtained by HPLC can be used for identification purposes. The fingerprint of the extract in the present study may enable the drug manufacturers to adjust the proportion and prepare a standardised product with consistent biological activity.

The powdered roots of G. glabra shows the presence of important metals like Ca, K, Fe and Mg which are beneficial for the metabolism and also traces of non essential metals like Pb and As were detected. After collection and transformation into dosage form, the heavy metals confined in plants finally enter the human body and may disturb the normal functions. It is important to have good quality control practices for herbal products and standardized extracts screening in order to protect consumers from toxicity.

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