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PHYTOCHEMICAL SCREENING AND DETERMINATION OF ANTI-BACTERIAL AND ANTI-OXIDANT POTENTIAL OF *Glycyrrhiza glabra* ROOT EXTRACTS

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ABSTRACT

The present study was undertaken to explore the phytochemical screening, anti-bacterial and anti-oxidant activities of the hydro-methanolic root extract of *Glycyrrhiza glabra* (*Leguminosea*) using standard screening methods, disc diffusion and de-oxyribose methods respectively. Different degrees of effect was noticed incase of different methods of study. In phytochemical screening, *Glycyrrhiza glabra* showed presence of secondary metabolites flavonoids, saponins, glycosides, terpenoids etc. It also showed potent antibacterial activities against almost all the test organisms. It exhibited highest sensitivity against *Shigella flexineri* with the zone of inhibition 10 mm. The extracts possessed potent hydroxyl radical scavenging activity having IC50 value 80µg/ml (52.5±0.79) against the positive control standard Ascorbic acid having IC50 value 50µg/ml (51.11±0.66). Results denote the presence of hydroxyl radical scavenging principles in the extracts.

Key Words : Antibacterial, Antioxidant, Phytochemical, *Glycorrhiza glabra,* Hydromethanolic

INTRODUCTION

Plants and their secondary metabolite constituents have a long history of use in modern western medicine and in certain systems of traditional medicine and are the sources of important drugs such as atropine, codiene, digoxine, morphine, quinine and vincristine etc. Use of herbal medicine in developed countries has expanded sharply in the latter half of twentieth century. The pharmacological treatment of disease began long ago with the use of herbs.[1]

(Glycyrrhiza glabra L., *Legumenacea* / *Fabaceae*) is a well-known medicinal herb that grows in various parts of the world. It is one of the oldest and widely used herbs from the ancient medical history of Ayurveda, both as a medicine and also as a flavoring to disguise the unpleasant flavor of other medications.[2] In the traditional system of medicine, the roots and rhizomes of *G. glabra* (family: *Legumenacea/ Fabaceae*) have been employed clinically for centuries for their anti inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities.[3]. *Glycyrrhiza inflate* (species of *Glycyrrhiza* family) has been shown to have great antioxidant, free radical scavenging[4] and anticonvulsant activities.[5] The main taproot, which is harvested for medicinal use, is soft, fibrous and has a bright yellow interior. This complex is composed of triterpene saponin, flavonoids, polysaccharides, pectin, simple sugars, amino acids, mineral salts and various other substances. The major bio-active constituent of root is a triterpenoids saponin. *Glycyrrhiza glabra* root extract have been used for more than 60 years in Japan to treat chronic hepatitis, and also have therapeutic benefit against other viruses. Expectorant, anti-tussive, mild laxative and anti aging activities has been reported.[6-7]

AIMS AND OBJECTIVES

The present study carried out on the investigation of phyto-chemicals screening, anti-oxidant and anti-bacterial activities using *in vitro* models.

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MATERIAL AND METHODS

Plant collection and identification

The roots of *Glycyrrhiza glabra* were procured from Bhopal (Madhya Pradesh) and authenticated by Botanist, Dr. Zia Ul Hasan (Voucher Specimen No: 441/BOT/Safia/13) Prof. and Head, Dept. of Botany, Safia Science College, Bhopal, Madhya Pradesh (India).

Chemicals

All the materials and reagents used for the study were from CDH, Renchem and Hi-Media Ltd., India.

Preparation of *Glycyrrhiza glabra* root extract

The collected roots WEre dried in shade and grind with mechanical grinder. About 30g powder poured in separating funnel with 50% methanol for 48hrs. The collected residues were kept at 55-60°C in water bath to concentrate it and finally transfer into the Hot Air Oven to dry it. About 5.8g crude extract was prepared (Yield= 19%) and used for the further studies.

Phytochemical screening

Phytochemicals are nonnutritive plant chemicals that contain protective, disease-preventing compounds. Standard screening test were carried out for various plant constituents. Hydro-methanolic crude extract were screened for presence or absence of secondary metabolites such as alkaloids, tannins, steroids, phenols, flavonoids, saponins and Phlobatannins etc. using standard procedures to identify the constituents as described by Sofowara, Trease and Evans, Harborne.

Test for Carbohydrates

A small quantity of extract was dissolved in 4ml of double distilled water and filtered. The filtrate was subjected to molisch's test to detect the presence of carbohydrates and further addition of Fehling's reagent. It showed the brick red color confirmed the presence of reducing sugar.

Test for Proteins

About 2ml of filtrate was treated with 2ml of 10% sodium hydroxide solution in a test tube and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet color indicates presence of proteins.

Test for Glycosides (Keller-Killani test)

A portion of the hydro-methanolic plant extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer showed presence of glycosides.

Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample.

Test for Free Flavonoids :

A portion of the powdered plant sample was heated with 10ml of ethyl acetate over a steam bath for 3min. the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonium solution. A yellow coloration was observed indicating a positive test for flavonoids.

Lead acetate test :

0.5gm plant extract was heated with double distilled water followed by addition of 1ml of 10% lead acetate solution. The yellow precipitate indicates the presence of flavonoids.

Reaction with Sodium hydroxide :

0.5gm plant sample was heated with double distilled water followed by addition of dilute sodium hydroxide . The yellow color indicates presence of flavonoid.

Test for alkaloids

Preliminary test :

100mg of plant extract was dissolve in dilute Hcl afterward the solution was filtered, filtrate was tested with Dragendroff's and Mayer's reagents.

Confirmatory test :

The 5gm of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper and then extracted twice with 10ml portion of chloroform. Chloroform extract was spotted on thin layer plates. Solvent system (n-haxane-ethyl acetate 4:1) was used to develop chromatogram and detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. An orange or dark colored spots against pale a yellow

background was confirmatory evidence for the presence of alkaloids.

Test for Steroids (Lieberman's test)

0.5gm of hydro-methanolic plant extract was dissolved in 2ml of acetic anhydride, cooled in ice bath, conc. H_2SO_4 was added. Color changed from violet to blue or green indicates presence of steroid compounds.

Test for Terpenoids (Salkowski's test)

0.5gm plant extract was dissolved in 2ml of $CHCl_3$, and conc. H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for Saponins (Froth test)

About 0.5gm extract was dissolved in 10ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allotted to stand in a vertical position and observed over 30 minutes period of time. If a "honey comb" froth above the surface of liquid persists after 30 minutes for sample is suspected to contain saponins.

Test for Tannins (Ferric chloride test)

About 0.5g of the dried powered samples were boiled in 20ml of double distilled water in a test-tube and then filtered. A few drops of 0.1% ferric chloride added and observed for brownish green or a blue-black coloration.

Test for Phlobatannins

Deposition of a red precipitate when a hydro-methanolic extract of plant sample were boiled with 1% aqueous hydrochloric acid taken as evidence for the presence of phlobatannins.

Test for Anthraquinones

About 200mg plant extract were boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 5 ml of benzene, filtered and 2 ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink and violet color indicated the presence of anthraquinones.

Test for Phenolic compounds

The dried plant extract about 100mg was dissolve in double distilled water, few crystals of ferric sulfate were added. Formation of dark violet color indicates the presence of phenolic compound.

Anti-bacterial activities

Antibacterial activities of hydro-methanolic extract from roots of *Glycyrrhiza glabra* was investigated using the Disk diffusion method given by Kerby-Bauer Disk Diffusion Susceptibility test.

Bacterial strain :

Following gram negative and gram positive bacterial strain i.e. *Pseudomonas aeroginosa, Escherichia coli, Shigella flexneri, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* used for the Antibacterial activities which were received from stock culture of our laboratory.

Media

Nutrient agar broth media were used for the antibacterial activities. Nutrient broth is prepared i.e. 1.3g in 100ml of double distilled water, poured in 6 different test-tubes and added 6 bacterial strain in each test-tube. Nutrient Agar media prepared poured in Petri-plates after solidifying swab the bacterial cultures on the plates and allowed for incubation at 37^0C for 24 hrs.

Concentration

4 different concentrations of crude extract were prepared (100%, 75%, 50%, 25%). 100%= 1g crude extract in 1ml of double distilled water freshly prepared afterward serial dilution prepared 75%= 75mg in 1ml, 50%= 50mg in 1ml, 25%= 25mg in 1ml.

Study parameter

Measurement of Zone of Inhibition (In mm).

Anti-oxidant activities

Anti-oxidant activities of *G. glabra* root extract (10-100 µg/ml) were determined according De-oxyribose method (Fenton reaction) of Halliwell and Aruoma. The Hydroxyl radical attacked to de-oxyribose and initiated a series of reaction that eventually resulted in the formation of Thiobarbeturic Acid Reaction Substances (TBARS).

Concentration

Ascorbic acid= 1mg in 1ml, *Glycyrrhiza glabra*= 5mg in 1ml

Formula:

$$\% \text{ Inhibition} = \frac{\text{Abs. 532nm control} - \text{Abs. 532nm test}}{\text{Abs. 532nm control}} \times 100$$

Study parameter

Results are concluded on the basis of plotting graph = % inhibition of TBARS vs Concentration.

RESULTS AND DISCUSSION

Phytochemical screening

The hydro-methanolic extracts of *G. glabra* was found to be positive for the presence of Saponin, flavonoids, alkaloids, steroids, terpenoids, tannins and glycosides but carbohydrates, Proteins, Phlobatannins, Phenolic compounds and anthraquinones were absent. The results are summarized in **Table 1**.

Anti-bacterial activities:

The Zone of Inhibition (In mm) of *Glycyrrhiza glabra* root extract exhibited strong anti-bacterial activities for both strain [gram (+) and gram (-) bacteria]. The diameter of zone of inhibition in different standard drugs and different concentration of *Glycyrrhiza glabra* is shown in **Table 2(a)** and **Table 2(b)** and represented with the graphs also **(Fig. 1** and **Fig. 2)** .No strain in this study showed resistance for this extract and the inhibitory zone is significantly increase in dose depending manner.

Table 1 : Preliminary phytochemical screening for hydro-methanolic root extract of *Glycyrrhiza glabra* Linn

S/N	Phytochonstituents	Test performed	Result
1.	Carbohydrates	Molisch's test	(-)
2.	Proteins	Copper sulphate test	(-)
3.	Flavonoids	Lead acetate test, NaOH solution test	(+)
4.	Alkaloids	Dragendroff's test	(+)
5.	Steroids	Lieberman's test	(+)
6.	Terpenoids	Salkowski's test	(+)
7.	Saponins	Froth test	(+)
8.	Tannins	Ferric chloride test	(+)
9.	Phlobatannins	HCL test	(-)
10.	Anthraquinones	Benzene test	(-)
11.	Glycosides	Keller-Killani test	(+)
12.	Phenolic Compounds	Ferric sulphate test	(-)

(+) Denotes Presence of Phytochemical
(-) Denotes Absence of Phytochemical

Table 2(a) : The study of anti-bacterial activities of standard antibiotics using disk diffusion method

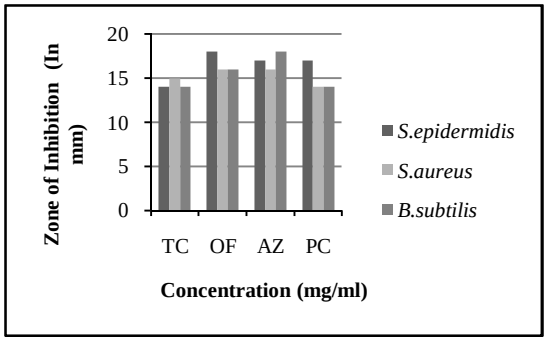
S/N	Bacteria name	Zone of inhibition (In mm)			
1.	Gram negative(-)	NF	GM	CT	NR
	Ps. Aeruginosa	14	13	18	19
	Sh. flexneri	18	18	12	20
	E. coli	12	16	8.0	16
2.	Gram positive(+)	TC	OX	AM	PC
	S. epidermidis	14	18	17	17
	S. aureus	15	16	16	14
	B. subtilis	14	16	18	14

NF = Nitrofurantoine, GM = Gentamycine, CT = Cefotaxime, NR = Norfloxaci
TC = Tetracycline, OX = Oflaxacine, AM = Azithromycine, PC = Pipracilline

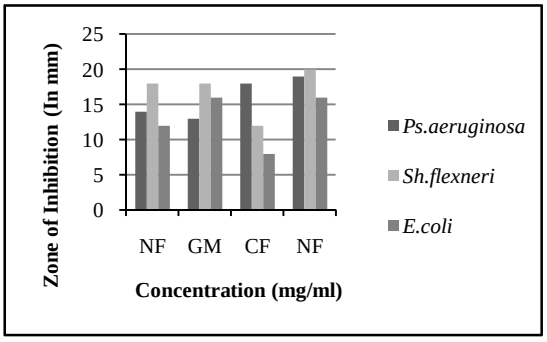
Table 2(b) : The study of anti-bacterial activities of *Glycyrrhiza glabra* root extracts using Disk Diffusion method

S/N	Bacterial strain	Bacteria name	Zone of inhibition (In mm)			
			100%	75%	50%	25%
1.	Gram Negative(-)	*Ps. aeruginosa*	9	8	6	5
		Sh. flexneri	10	7	6	6
		E. coli	8	8	7	6
2.	Gram positive (+)	*S. epidermidis*	9	8	6	5
		S. aureus	9	6	7	5
		B. subtilis	9	8	5	5

Drug conc. = 100%, 75%, 50%, 25%

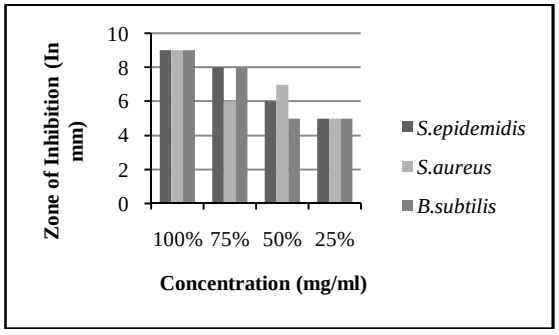


Gram (+) bacteria

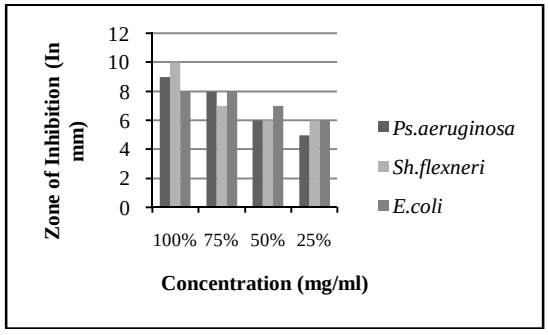


Gram (-) bacteria

Fig. 1 : Graph showing anti-bacterial activities of Standard anti-biotics



Gram (+) bacteria



Gram (-) bacteria

Fig. 2 : Graph showing anti-bacterial activities of *Glycyrrhiza glabra* hydro-methanolic root extracts

Anti-oxidant activities :

The % of Inhibition versus different concentration (10-100ug/ml) of *Glycyrrhiza glabra* root extract with standard drug ascorbic acid shows in **Table 3(a)** and represented with the graph also **(Fig. 3)** .The graph of % of Inhibition versus different concentration of *Glycyrrhiza glabra* root extract was plotted.

In which IC50 value (**Table 3(b)**) of *G. glabra* was observed = 80 µg/ml (52.5±0.79), Ascorbic acid = 50 µg/ml (51.11 ± 0.66). *In vitro* antioxidant activities of *Glycyrrhiza glabra* root extract showed significant inhibitory concentration as compared to ascorbic acid. The phytochemical screening of *Glycyrrhiza glabra* studied showed that the roots

Table 3(a) : The study of anti-oxidant activities of *Glycyrrhiza glabra* root extracts using fenton reaction method

S/N	Concentration (µg/ ml)	% of Inhibition (TBARs)	
		Ascorbic acid	*Glycyrrhiza glabra* root extract
1.	10	20.53±0.55	7.90±0.71
2.	20	25.14±0.57	9.76±0.28
3.	30	33.36±0.59	13.86±0.97
4.	40	45.85±0.33	19.22±0.43
5.	50	51.11±0.66	29.63±0.84
6.	60	59.38±0.74	37.48±0.80
7.	70	68.35±0.51	45.29±1.12
8.	80	71.51±0.40	52.5±0.79
9.	90	77.42±0.36	61.33±1.36
10.	100	81.92±0.80	73.77±0.86

Table 3(b) : IC50 Values

S/N	% of inhibition (TBARs)	Concentration (µg/ ml)	IC50 Value (µg/ ml)
1.	Ascorbic acid	50	51.11±0.66
2.	*Glycyrrhiza glabra* root extract	80	52.5±0.79

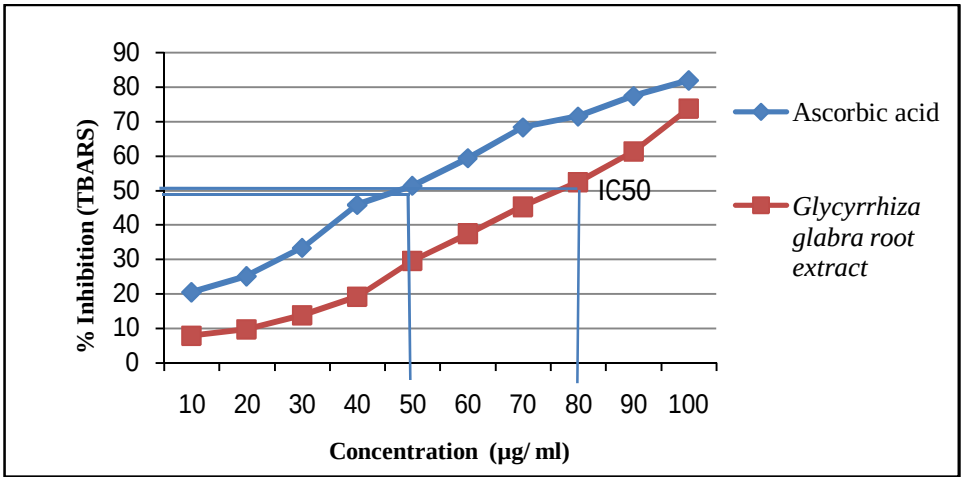


Fig. 3 : Graph showing Anti-oxidant activities of *Glycyrrhiza glabra* hydro-methanolic root extract

were rich in flavonoids, alkaloids, terpenoids, saponins, tannins, steroids and glycosides. They were known to show medicinal activities as well as exhibiting physiological activities. Tannins and flavanoides have been shown to have numerous health protective benefits, which include lowering of blood lipids.[8] The alcoholic extracts of *G. glabra* inhibit *B. cereus, K. pneumoniae, S. aureus* the acetone extracts inhibit *B. cereus, B. subtilis, K. pneumoniae, S. aureus* the chloroform extracts showed inhibition effect against *B. cereus, B.*

subtilis, E. faecalis, K. pneumoniae, S. aureus.[9] *S. aureus* is a major clinical pathogen. During the past decade, this bacterium has developed resistance to many commonly used antibiotics. In this study, the hydro-methanolic extract of *Glycyrrhiza glabra* L. showed activities against *S. aureus* and can be used as raw materials for phytotherapy. Isolation of Isoliquiritigenin (IL), a potent antioxidant agent from *Glycyrrhia glabra* have been reported in the literature of Chin *et al.*[10]

CONCLUSION

The *Glycyrrhiza glabra* root extract (Hydro-methanolic crude) have significant Anti-bacterial and hydroxyl radical scavenging activities. It is able to fight against bacterial infection and scavenging hydroxyl radical. It may be an important drug for prevention of bacterial infection and scavenging of hydroxyl radicals which are generated during carcinogenesis.

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