Glycyrrhizin from Glycyrrhiza glabra root: Phytochemical analysis and Characterization

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Abstract

Glycyrrhiza glabra is the scientific name for licorice. It is a well-known medicinal plant that can be found growing in many different regions of the world. It is one of the oldest and most commonly used plants that has been utilised in both western and eastern countries for a long time. Glycyrrhiza glabra (Fabaceae) roots were the main source of the triterpenoid saponin, glycyrrhizic acid (glycyrrhizin), which is a sweeter component and around 50 times sweeter than sugar, and is widely used as a sweetening addition in the food industry. Glycyrrhizin's chemical makeup has been identified as glycyrrhetinic acid. The goal of the current study was to determine the root aqueous extract of G. glabra's preliminary phytochemical analysis. Glycyrrhizin's isolation and characterisation were expanded upon.

Key words: *Glycyrrhiza glabra*, glycyrrhizin, TLC, HPLC, UV & IR.

Introduction

Triterpenoids represent a diverse, physiologically appealing type of terpenoids and have been found in both terrestrial and marine living creatures. They also have a wide structural range of secondary metabolites with more than 100 carbon skeletons. Triterpenes, steroids, limonoids, quassinoids, and triterpenoidal and steroidal saponins are among the more than 30,000 isolated and recognised molecules that make up this class of natural products. Three monoterpenes are referred to as triterpenes, and these compounds have 30 carbons arranged in six isoprenyl units[1]. Glykos, which means sweet in ancient Greek, and rhiza, which means root, are the roots of the word glycyrrhiza. In India, Glycyrrhiza glabra is generally known as mulaithi. Licorice is another name for it in the Mediterranean and some parts of Asia[2]. The plant's dried, peeled or unpeeled root and stolon are what make up this substance (Leguminosae). Both the food and pharmaceutical industries make extensive use of this plant. Glycyrrhizin (2-20%), a triterpenoid

saponin glycoside that is composed of glycyrrhizic acid salts in both potassium and calcium, is the primary component of licorice. It is 50 times sweeter than sucrose and is safe for use in treating diabetes[3]. Predictably, the plant has not been required as a prophylaxis for stomach and duodenal ulcers, or for dyspepsia as an anti-inflammatory during allergic reactions[4]. It is employed as a herbal remedy as a laxative, contraceptive, galactogogue, anti-asthmatic, and antiviral agent[5]. Previous research has shown that it is effective for treating a variety of conditions, including anaemia, gout, sore throats, tonsillitis, flatulence, sexual dysfunction, fever, coughs, skin conditions, acidity, leucorrhoea, bleeding, jaundice, and bronchitis[6].

Fig1: Root of *G.glabra*

The triterpenoid component glycyrrhizin is mostly accountable for the licorice root's sweet flavour. This substance is a component of a mixture of glycyrrhizic acid potassium, calcium, and magnesium salts. [7].

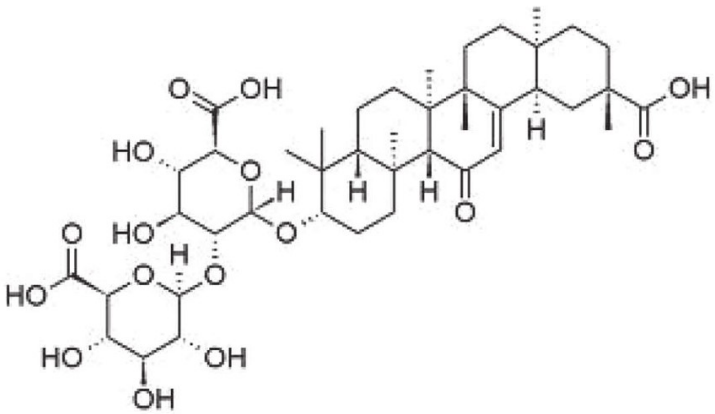


Fig:2 Structure of Glycyrrhizin

Description of Glycyrrhizin [8-9]

S.No.	Glycyrrhizin Description	
1.	**Mol. Formula**	**$C_{42}H_{62}O_{16}$**
2.	**Average Molecular Weight**	822.9 g/mol
3.	**IUPAC Name**	S,3S,4S,5R,6R)-6-[(2R,3R,4S,5S,6S)-2-[[(3S,4aR,6aR,6bS,8aS,11S,12aR,14aR,14bS)-11-carboxy-4,4,6a,6b,8a,11,14b-heptamethyl-14-oxo-2,3,4a,5,6,7,8,9,10,12,12a,14a-dodecahydro-1H-picen-3-yl]oxy]-6-carboxy-4,5-dihydroxyoxan-3-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid.
4.	**Class**	Triterpene glycoside
5.	**M.P.**	220 °C
6.	**Partition coefficient**	2.80
7.	**Pharmacology**	It is effective in the treatment of peptic ulcer and also has expectorant (antitussive) properties.
8.	**Mechanism of Action**	Inhibition of Hepatic Apoptosis and Necrosis Anti-Inflammation and Immunity Regulation Antiviral Effects Antitumor Effects Inductive Effect of Liver Enzyme Activity

MATERIAL AND METHOD

Plant material collection

The root was purchased from local market at Shivpuri (M.P.) and authenticated. They were dried in shade for several days at room temperature and then grinded as powder.

Extraction

50 grams of the powdered root was extracted by hot percolation (Soxhlet apparatus) with 250 ml of water till exhaustion. The extract was filtered and concentrated by evaporation under vacuum.

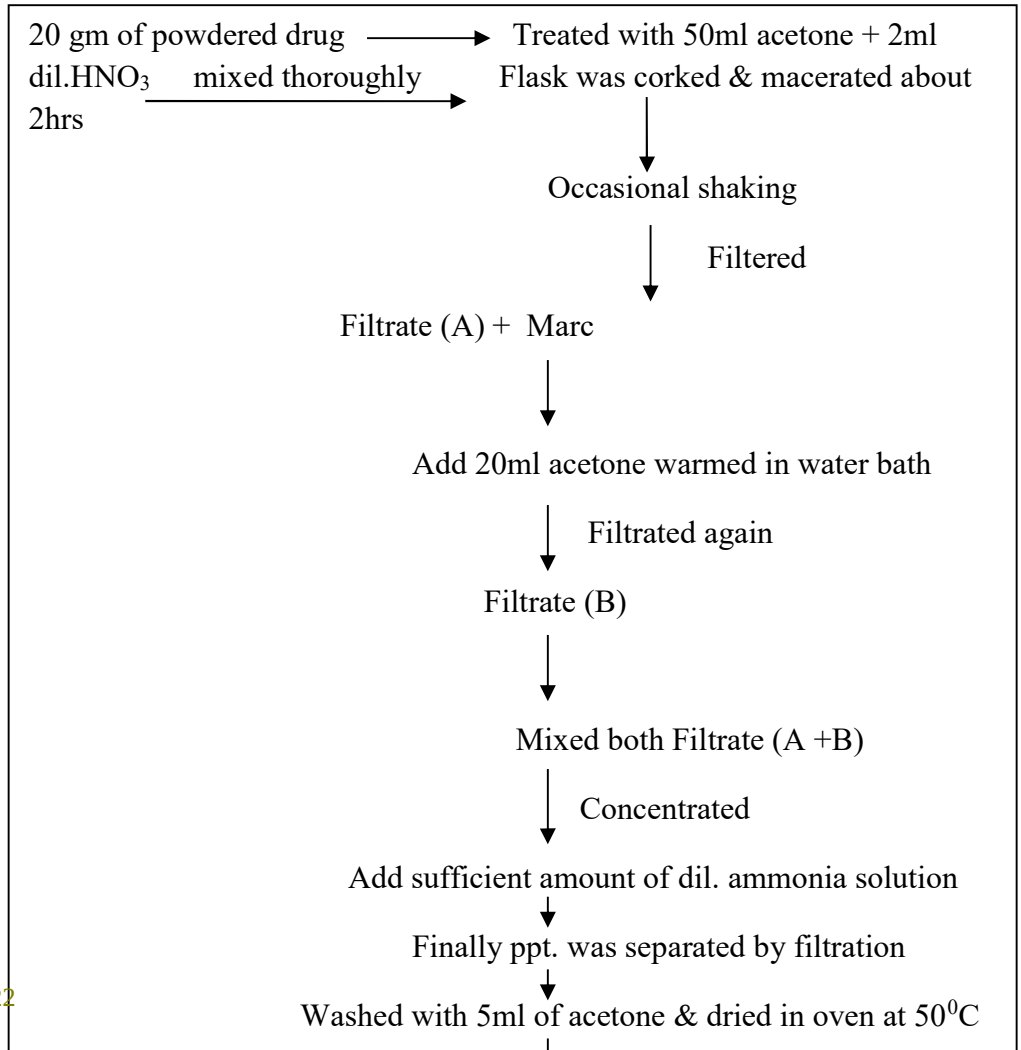
PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

Physico-chemical parameters were determined as per standard procedures[10].

Preliminary qualitative test

The extract was subjected to preliminary qualitative phytochemical investigation[11-16].

Isolation of Glycyrrhizin



General and Physical Properties: Appearance, color, solubility and melting point of the isolated constituents will be determined.

TLC chromatography

A pre-coated aluminium sheet with silica gel GF254 and the following mobile phases were used to compare isolated glycyrrhizin to standard glycyrrhizin using TLC technique. Toluene, ethyl acetate, and glacial acetic acid (12.5: 7.5: 0.5), with anisaldehyde-sulphuric acid used as a spray reagent[17–18].

Spectrophotometric analysis

Methanol was used to dissolve the isolated Glycyrrhizin, and its UV absorption peaks were measured. The Shimadzu 1700 UV spectrophotometer was used for the spectrophotometer-based analysis[19].

HPLC analysis

A LC-100 CyberlabTM, Salo Torrace, Millburry, MAO 1527, USA, with an LC-UV-100 UV detector was used for the HPLC analysis. For the chromatographic separations, a C-18 (CAPCELL) HPLC-packed column (4.6 mm I.D. x 250 mm), type MG 5 m, and number AKAD/05245 were utilized. Phosphoric acid accounted for 1% of the mobile phase: 60:40 acetone The column temperature was 25°C and the flow rate was 0.2 mL/min. The UV detection wavelength was 251 nm, and the injection volume was 25 µl.

IR analysis

IR spectral data was acquired using a Bruker (AT-IR).

Result and Discussion

Liquorice has traditionally been prescribed as a preventative treatment for gastric and duodenal ulcers. It is utilized in dyspepsia during allergenic reactions as an anti-inflammatory agent.

Glycyrrhizin can be found in stereoisomer forms of 18 and 18. Glycyrrhizin is thought to be the most widely used folk medicine for its anti-inflammatory effects on neutrophil functions, including the generation of ROS (reactive oxygen species)[20]. Thus, the current examination investigated the different phytochemicals and physiochemical boundaries found in fluid root remove. The normalization boundaries were misfortune on drying at 100-105^{0}C , complete debris esteem, corrosive insoluble debris esteem, water dissolvable debris esteem was classified in table1. Table 2 shows that the aqueous root extract contained a number of phytoceuticals. Glycyrrhizin was extracted from the aqueous root of G.glabra with a yield of 7.91 percent w/w. The melting point of isolated glycyrrhizin was 221°C, which is the same as the one that was previously reported for glycyrrhizin. Table 3 summarizes the general properties of isolated glycyrrhizin. Qualitative TLC chromatography analysis of isolated glycyrrhizin revealed Rf values that were roughly comparable to those of standard glycyrrhizin (table 4 and Fig. 4). The UV range of glycyrrhizin in methanolic arrangement shows two significant assimilation groups at 204 and 251nm, which demonstrates the presence of triterpenoid structure (Fig:5). For the purpose of quality control of herbal medicines, it is recommended to obtain chemical fingerprints through chromatographic methods. As a result, the chromatographic fingerprint should be taken into consideration when evaluating the quality of herbal medicines around the world due to the numerous components they contain[21]. The HPLC analysis was carried out with a LC-100, CyberlabTM, Salo Torrace, Millburry, MAO 1527, and USA with LC-UV-100 UV detector. The chromatograms of the standard and isolated glycyrrhizin showed, respectively, RT 8 and 7.58min (Fig. 6 and 7). Figure 8 and table 6 show the results of the IR analysis. When compared to the standard, the isolated compound's infrared spectrum revealed the same number of functional group presences.[22-47]

Conclusion

The purpose of this study was to evaluate the physiochemical and phytochemical analyses of G.glabra's aqueous root extract. The solvent precipitation method was used to further isolate glycyrrhizin from the root of G. glabra. Various chromatographic and analytical methods were used to further characterize glycyrrhizin. These measures have huge appositeness for the food and drug industry. For the quantification of glycyrrhizin in the G. glabra root, this newly

developed method proved to be straightforward, effective, dependable, and cost-effective, as this study demonstrated. The procedure is quick, easy, and very specific for glycyrrhizin.

Table: 1 Physiochemical properties of aqueous root extract of *G.glabra*

S.No	**Ash values**	**Observation in (w/w)**
1	Total ash	4.7%
2	Acid insoluble ash	0.55%
3	Water soluble ash	6.55%
4	LOD	5.5%

Table No 2: Phytochemical analysis of aqueous root extract of *G.glabra*

S.No.	**Test**	**Aqueous root extract**
1	**Tests for sterols**	
	1. Salkowski's Test	+
	2. Libermann Burchard's Test	+
2	**Test for glycosides**	
	1. Baljet's Test	+
	2. Brontrager Test	-
3	**Tests for saponins**	

	1. Foam Test	+
4	**Test for carbohydrates** 1. Molish's Test 2. Barfoed's Test 3. Benedict's Test	 - + -
5	**Tests for alkaloids** 1. Mayer's Test. 2. Wagner's Test. 3. Dragendorff's Test	**-** - - -
6	**Tests for flavonoids** 1. Ferric chloride Test. 2. Shinoda Test. 3. Alkaline Reagent Test. 4. Lead Acetate Test.	 - + - -
7	**Tests for tannins** 1. Ferric chloride Test. 2. Gelatin Test	 - -
8	**Test for amino acid and protein** 1. Biurete test	 +

Table 3: General Physical Properties of isolated Glycyrrhizin

S.No.	Physical properties	Inference
1.	Appearance	powder
2.	Color	Yellowish brown
3.	Solubility	Chloroform, methanol & slightly soluble in Pet.ether
4.	Melting point	221^0C

Fig 3: Isolated Glycyrrhizin

Table 4: TLC chromatography Analysis of Glycyrrhizin

Solvent System	R_f(standard)	R_f(isolated sample)
Toluene: ethyl acetate: glacial acetic acid (12.5:7.5:0.5)	0.43 (Dark violet purple spot)	0.41 (Dark violet purple spot)

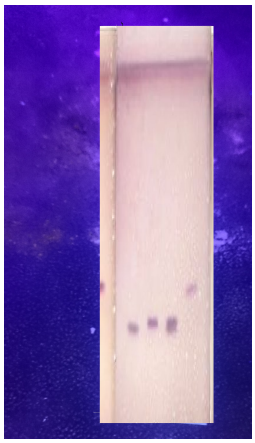


Fig 4: TLC chromatogram of isolated compound (Glycyrrhizin)

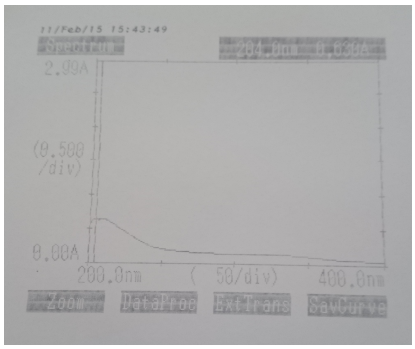


Fig 5: UV Scan of isolated compound (Glycyrrhizin)

Table: 5 HPLC Analysis of Glycyrrhizin

S.No.	Sample	Height	Area	Conc.	RT	Inference
1.	Standard Glycyrrhizin	49442	1251681.2	96.7	8.00	Glycyrrhizin
2.	Isolated Glycyrrhizin	32582	417227.2	29.0598	7.58	Glycyrrhizin

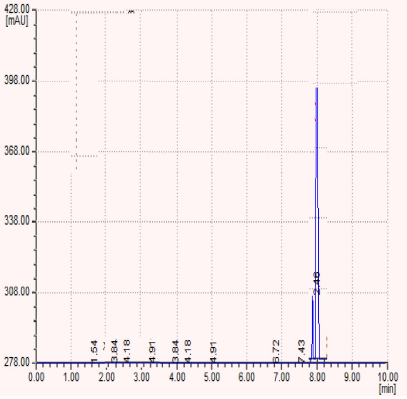


Fig 6:HPLC chromatogram of Std Glycyrrhizin

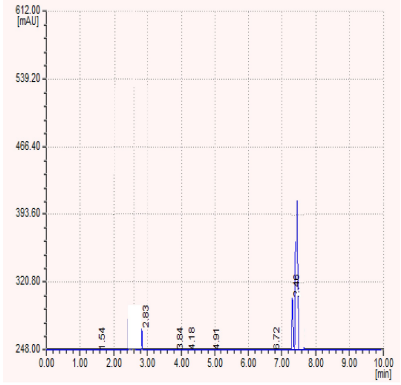


Fig 7:HPLC chromatogram of Glycyrrhizin (isolated)

Table: 6 IR Analysis of Glycyrrhizin (isolated)

cm^{-1}	**Functional Group**
3343	O-H (stretch)
3355	O-H (stretch)
3755	Aromatic
2874	CH stretch
1639	C=O stretch
1620	C=C
1364	C-O-C

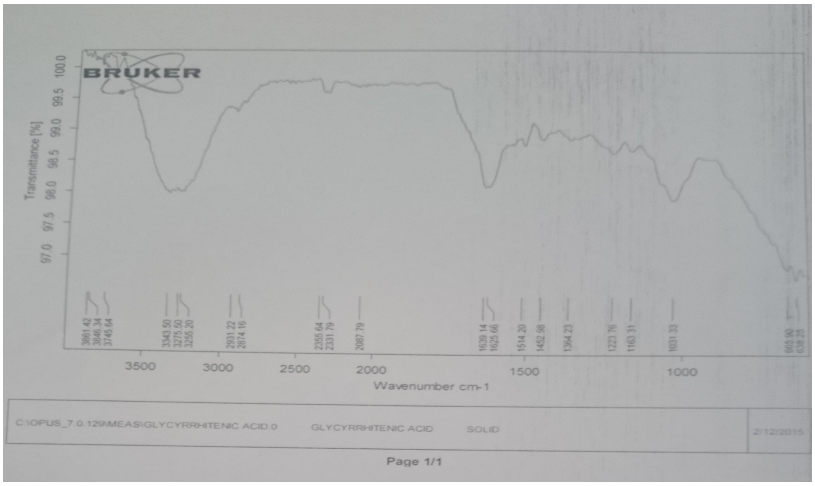


Fig 8: IR spectra of Glycyrrhizin (isolated)

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