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ISOLATION AND CHARACTERIZATION OF GLYCYRRHETINIC ACID FROM ROOT OF *GLYCYRRHIZA GLABRA*

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ABSTRACT
Glycyrrhiza glabra also well-known as Licorice. It is a famous medicinal herb that grows in various parts of the world and is one of the oldest and broadly used herbs known both in western and eastern countries since several thousand years ago. The triterpenoid saponin, glycyrrhizic acid (glycyrrhizin) was the major bio-active constituent isolated from the roots of Glycyrrhiza glabra (Fabaceae), which is a sweet-tasting material and is about 50 times sweeter than sugar, making it a widely used as a sweetening additive in the food industry. The structure of glycyrrhizin has been characterized as glycyrrhetinic acid. The present study was carried out to isolation and characterization of glycyrrhetinic acid from root of *G.glabra* from aqueous extract. The chemical compound isolated was analyzed by TLC, HPLC, UV & IR. Glycyrrhetinic acid showed the absorbance maxima at 204nm. The HPLC analysis of isolated sample revealed RT = 2.58 min which is resemblance with standard. Further quantification of glycyrrhetinic acid in the aqueous root extract was also determined by UV & HPLC method.

KEYWORDS: *Glycyrrhiza glabra*, Glycyrrhetinic acid (GA), TLC, HPLC, UV & IR.

INTRODUCTION
Triterpenoids represent a wide, biologically attractive group of terpenoids and include a large structural multiplicity of secondary metabolites with more than 100 carbon skeletons identified from terrestrial and marine living organisms. This class of natural products, including triterpenes, steroids, limonoids, quassinoids, and triterpenoidal and steroidal saponins, consists of over 30,000 compounds isolated and identified. Most of triterpenic skeletons are tetracycles, containing three six-membered and one five-membered rings, and pentacycles, either with four sixmembered and one five-membered rings or five six-membered rings. However, acyclic, mono-, di-, tri-, and hexacyclic scaffolds have also been isolated and identified from natural sources. The term triterpene refers to three monoterpenes and consequently to 30 carbons grouped in six isoprenyl units.[1] *Glycyrrhiza* is derived from the ancient Greek term *glykos*, meaning sweet, and *rhiza*, meaning root. *Glycyrrhiza glabra* is known as mulaithi in north India. *G.glabra*, also known as licorice and sweet wood, is native to the Mediterranean and certain areas of Asia. *G.glabra* belongs to genus Glycyrrhiza and is commonly called as licorice which is available in India.[2] Liquorice consists of the dried peeled or unpeeled root and stolon of plant *Glycyrrhiza glabra* L., (Family: Leguminosae). This plant is widely used in both food and pharmaceutical industries. Major constituent of licorice is a triterpenoid saponins glycoside; glycyrrhizin (2-20%), which is a mixture of potassium and calcium salts of glycyrrhizic acid and is 50 times sweeter than sucrose and safe to be used in diabetes. Glycyrrhizin loses its sweet taste and yield one molecule of glycyrrhetinic acid (aglycon) and two molecules of glucuronic acid (glycon) on hydrolysis in acidic medium due to breakage of ether bond between glycone and aglycone.[3] Conventionally the plant has been recommended as a prophylaxis for gastric and duodenal ulcers and dyspepsia as an anti-inflammatory agent during allergenic reactions.[4] It is used as a laxative, emmenagogue, contraceptive, galactagogue, anti-asthmatic drug and antiviral agent as a folk medicine.[5] Therapeutically is useful in anemia, gout, sore throat, tonsillitis, flatulence, sexual debility, fever, coughs, skin diseases, swellings, acidity, leucorrhoea, bleeding, jaundice, bronchitis etc.[6]

Fig 1: Root of *G.glabra.*

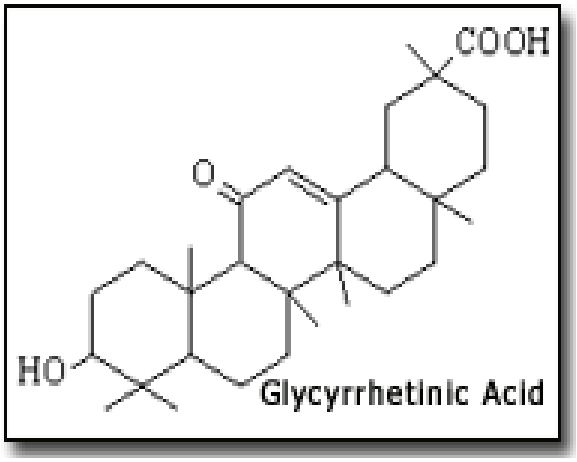

Fig 2: Structure of Glycyrrhetinic acid.

Pentacyclic triterpenoid derivative of β-amyrin type is a Glycyrrhetinic acid. It is freely soluble in chloroform and acetic acid. Clinically, it has proven various activities like antiulcer, antiasthmatic, anti-diuretic, hepatoprotective, antibacterial, antioxidant, anti-spasmodic, anti-inflammatory, estrogenic.[7]

Pharmacological action of Glycyrrhetinic acid[8-9]

S.No.	General Glycyrrhetinic acid Description	
1.	Mol. Formula	$C_{30}H_{46}O_4$
2.	Average Molecular Weight	470.6838
3.	IUPAC Name	(2S,4aS,6aS,6bR,10S,12aS)-10-hydroxy2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo 1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid
4.	Class	Triterpene glycoside (Pentacyclic triterpenoid derivative of the beta-amyrin)
5.	Pharmacology	It is effective in the treatment of peptic ulcer and also has expectorant (antitussive) properties.
6.	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 collected from botanical garden L.N.C.P.Bhopal (M.P.) and authenticated (Voucher No. 004/bot/LNCP/14. They were dried in shade for several days at room temperature and then grinded as powder.

Extraction and isolation

Fifty grams of the powdered root was extracted by Soxhlet apparatus with 250 ml of water till exhaustion. The extract was filtered and concentrated by evaporation under vacuum. Dissolve the residue in water and precipitate glycyrrhetinic acid by addition of HCl(pH= 3-3.5).The precipitate was filtered, washed with water and dried to yield crude glycyrrhetinic acid (Crude GA). Crude GA (50 mg) was treated with 50 mg oxalic acid in 20 ml of 1:1 MeOH:water and the mixture was heated at 60^{o}C for 24 hours. The reaction mixture was extracted with ethyl acetate (EtOAc) (2 x 250 ml) to give an aqueous fraction containing sugars and an EtOAc fraction containing the partial hydrolyzed product. Concentration of the EtOAC fraction followed by purification using normal phase PTLC using the solvent system n-hexane/EtOAC (70:30) yielded glycyrrhetinic acid.[10-11]

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

TLC and paper chromatography

Isolated glycyrrhetinic acid was compared with standard glycyrrhetinic acid using TLC method; a pre-coated aluminum sheet with silica gel GF_{254} with the following mobile phases: Chloroform: methanol (9:1) using vanillin sulphuric acid as spray reagent.[12]

Spectrophotometric analysis

The isolated glycyrrhetinic acid was dissolved in methanol and its UV absorption peaks were determined and compared with standard GA. Spectrophotometric analysis was carried out in Shimadzu 1700 UV spectrophotometer.

Determination of glycyrrhetinic acid in aqueous root extract of *G.glbra*

Preparation of calibration curve

3mg of isolated glycyrrhetinic acid was dissolved in 30 ml of methanol to get 100 µg/ml stock solutions separately. Lower concentrations (10, 20, 30, 40, 50µg/ml) were prepared by serially diluting stock solution. Now 100mg of crude extract was dissolved in 100ml of methanol. 1ml of above solution was taken in 10ml volumetric flask and volume made up by methanol. The absorbance was taken at 204nm taking methanol as a blank.

HPLC analysis

The HPLC analysis was performed using a LC-100, Cyberlab™, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of methanol: water (80:20). The flow rate was 1.0 mL/min, and a column temperature of 25°C. The injection volume was 25µl, and UV detection was effected at 204 nm.

$$\text{Vitamin C content \%} = \frac{A_1 \times W_2 \times P}{A_2 \times W_1}$$

Where, A_1 = Peak area of sample solution
A_2 = Peak area of standard solution
W_1 = Weight in g of sample
W_2 = weight in g of standard
P = Purity of standard GA

IR analysis

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

RESULT AND DISCUSSION

Glycyrrhetinic acid is triterpene glycoside (pentacyclic triterpenoid derivative of the beta-amyrin). Glycyrrhetinic acid was isolated from aqeous root of *G.glabra*. Isolated glycyrrhetinic acid showed a melting point at 296°C which is identical with that reported for glycyrrhetinic acid. The general physical properties observed in isolated glycyrrhetinic acid were tabulated in table 1.Qualitative analysis of isolated glycyrrhetinic acid were carried out by TLC chromatography and the results revealed that R_f value were more or less similar to standard glycyrrhetinic acid (table 2 & Fig.4). The UV spectrum of glycyrrhetinic acid in methanolic solution shows two major absorption bands at 204 & 254nm, which indicates the presence of triterpenoid structure(Fig:5). A simple, rapid, accurate, precise, and economic spectrophotometric method for estimation of glycyrrhetinic acid in aqueous root extract of *G.glabra* was also developed. Calibration equation for glycyrrhetinic acid was constructed by plotting the UV absorbance against the glycyrrhetinic acid concentration at five concentration levels (analyzed in triplicate). UV absorbance (y) of glycyrrhetinic acid over a concentration (x) range of 10-50 ppm was linear y = 0.009 x with a regression coefficient (R^2) of 0.996 (Fig: 9 & Table:3).The concentration of the glycyrrhetinic acid found was to be 70 µg/ml and % glycyrrhetinic acid in aqueous extract was found to be 3.5. IR analysis was tabulated in table 5. The infrared spectrum is shown in figure 10. The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. HPLC separations of isolated sample with reference to standard were performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ). Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally considering multiple constituents present in the herbal medicines.[13] The HPLC chromatogram of standard and isolated glycyrrhetinic acid showed RT 2.46 & 2.58 respectively (Fig.6 & 7). The HPLC analysis of aqueous root extract revealed that % GA content was found to be 3.2(Fig.8 & table 4).

Table 1: General Physical Properties of isolated GA.

S.No.	Physical properties	Inference
1.	Appearance	Crystalline powder
2.	Color	White
3.	Solubility	Chloroform, methanol & slightly soluble in Pet.ether
4.	Melting point	296^0C

Fig 3: Isolated moiety.

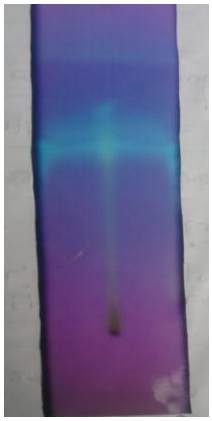

Fig 4: TLC chromatogram of GA(sample).

Table 2: TLC chromatography Analysis of GA.

Solvent System	R_f (standard)	R_f(isolated sample)
Chloroform: methanol (9:1)	0.60	0.56

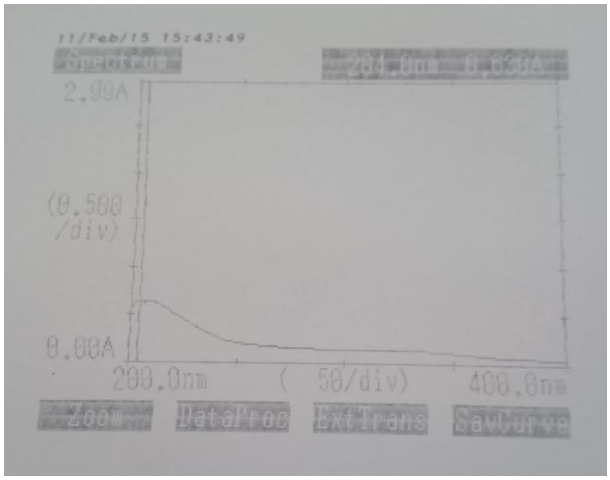

Fig 5: UV Scan of glycyrrhetinic acid.

Table 3: Quantitative estimation of glycyrrhetinic acid from UV spectrometry.

S.No.	Sample	Absorbance at 204nm	Statistical Analysis	Concentration (µg/ml)
1.	Aqueous extract of glycyrrhetinic acid	0.637	Correlation coefficient R^2= 0.996 Straight Line equation y= 0.009x	70
2.	Concentration glycyrrhetinic acid(mg)	Conc. in mg = $\frac{C \times \text{dilution factor}}{100}$		3.5

Table 4: HPLC Analysis of crude GA from aqueous root extract.

S.No	Sample	Height	Area	Conc.	RT	Inference
1.	Standard GA	49422	1251681.2	96.7	2.46	GA
2.	GA (sample)	2582	417227.2	29.0598	2.58	GA

Table: 5 IR Analysis of GA.

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

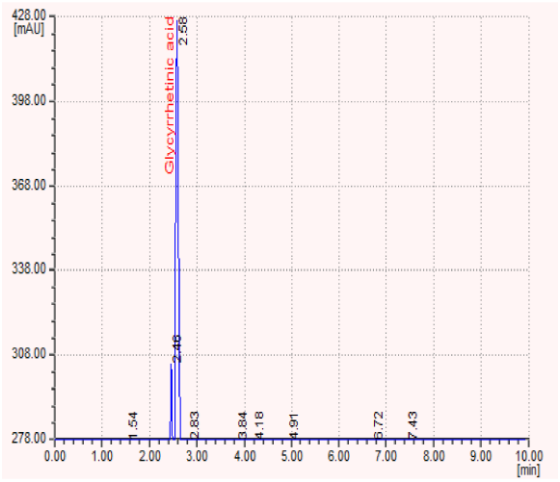

Fig 6: HPLC chromatogram of GA(Sample)

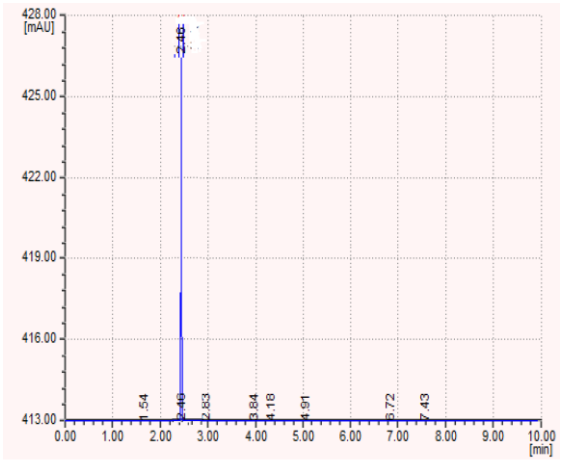

Fig 7: HPLC chromatogram of GA(Standard).

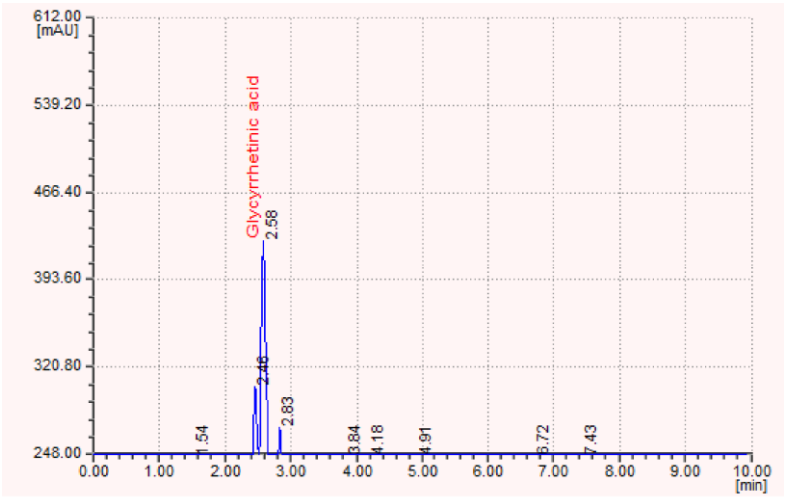

Fig 8: HPLC chromatogram of crude GA in Aqueous root extract of *G.glabra.*

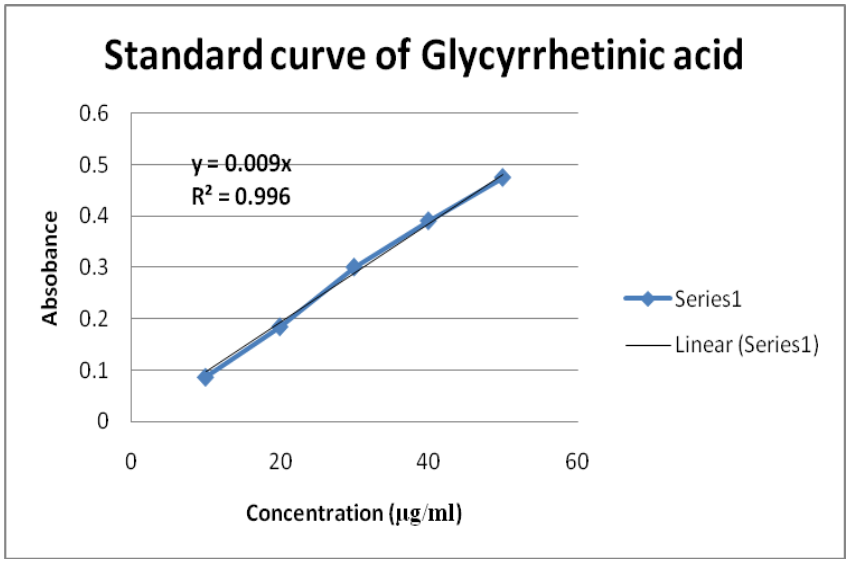

Fig 9: Standard curve of Glycyrrhetinic acid.

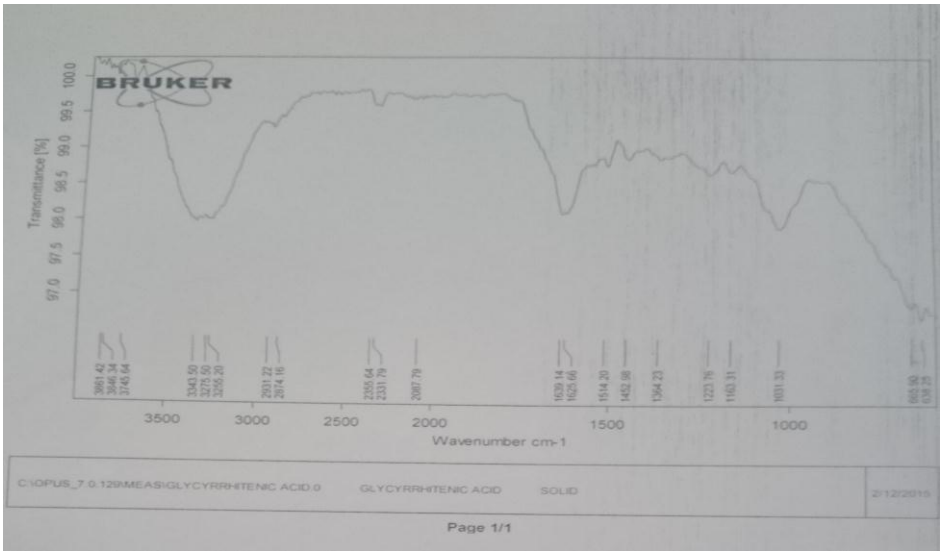

Fig 10: IR spectra of isolated GA.

CONCLUSION

The present study was carried out for the isolated glycyrrhetinic acid from root of *G.glabra.* Further characterizations of glycyrrhetinic acid were carried out by various chromatographic and analytical techniques. These assays have important appositeness for the food and pharmaceutical industry. The quantification of glycyrrhetinic acid from aqueous extract was carried out by UV spectrometry & HPLC methods. The % glycyrrhetinic acid was estimated which is more or less similar in both the methods. The present work revealed that this developed method was simple, efficient, reliable and cost-effective for the quantization of glycyrrhetinic acid in root of the *G.glabra*. The method is simple, rapid and has high specificity to glycyrrhetinic acid.

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