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Physicochemical and phytochemical analysis of *Glycyrrhiza glabra* root extract

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ABSTRACT

Glycyrrhiza glabra L., also known as licorice, belongs to the Fabaceae family and is one of the most commercially valuable plants worldwide, being used in the pharmaceutical, cosmetic, and food industries, both for its therapeutic benefits as well as for the sweetening properties of the extract. The aim of the study is to analyse the physicochemical and phytochemical characteristics of *Glycyrrhiza glabra* in aqueous and ethanolic extracts and determine the ash value and extractive value of the drug. Using soxhlet equipment the extract was prepared from powdered *Glycyrrhiza glabra* drugs for 20 hours using water and ethanol as solvents. The analysis of phytochemical screening of *Glycyrrhiza glabra* includes tests for carbohydrates, saponins, flavonoids, alkaloids, etc. The percentage yield of ethanol extract is found to be 9.73%. The physicochemical parameters of the aqueous solution and ethanol were analysed and are found to be in limits. The ethanolic extract of *Glycyrrhiza glabra* showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, glycosides and phytosterol. The amounts of total flavonoids, total phenol and ascorbic acid were found to be 185.14 mg, 481.47 mg per 1 gm, and 33.81 µg/ml of aqueous extract and 218.92 mg, 507.62 mg per 1 gm, and 42.38 µg/ml of the ethanol extract, respectively. It was found that the phytochemical constituents are very much enriched in the *Glycyrrhiza glabra* extract and can be used for development of new formulations.

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INTRODUCTION

Nature is always a shining example of the long-standing phenomena of symbiosis. All of the biotic and abiotic factors are interconnected. Plants are absolutely necessary for man's survival.

Nature has offered a vast array of cures to treat all of humanity's diseases. As a result of man's inquisitive inclination, drug knowledge has gathered over thousands of years, and we now have numerous effective techniques of assuring health care (Kokate *et al.*, 2000).

Licorice (*G. glabra* L.) is a perennial shrub belonging to the Fabaceae (Leguminosae) family. It was first described and listed by Pedanius Dioscorides in the first century AD (ca. 40–90), placing it among the 650 medicinal plants listed in De Materia Medica (Fiore *et al.*, 2005).

Licorice is grown commercially in Italy, Spain, Greece, France, Iran, Iraq, Turkey, Turkmenistan, Uzbekistan, Syria, Afghanistan, Azerbaijan, India, China, the United States, and England. It is one of the most commercially valuable plants globally, being used in the pharmaceutical, cosmetic, and food industries, both for its therapeutic benefits as well as for the sweetening properties of the extract (Wahab *et al.*, 2021).

Plants have played a significant part in medicine since the dawn of human gardening activities. *Glycyrrhiza glabra* is one of the most well-known medicinal plants in the Fabaceae (formerly known as Leguminosae) family, and its relatives are now widely used as feed and food. The Greek words glykos (sweet) and rhiza (root) are combined to form the genus *Glycyrrhiza* (root). The plant is used frequently in traditional chinese medicine for gastrointestinal issues, coughs, bronchitis, and arthritis, for example. It is still commonly used in folk medicine to treat gastritis, peptic ulcers, respiratory infections, and tremors (Pastorino *et al.*, 2018).

Glycyrrhiza glabra L. (Family: Fabaceae) is commonly known as Liquorice, sweet wood, and mulethi (Pastorino *et al.*, 2018; Lim, 2005). Liquorice roots are used for the treatment of many digestive system disorders, and respiratory tract disorders like asthma, cough, tonsillitis, sore throat, paralysis, etc. It was traditionally used as an anti-inflammatory, anti-diabetic, anti-ulcer, antibiotic, anti-arthritic, antiviral, memory stimulant, anti-mycotic, oestrogenic, antioxidant, anticancer, and anti-diuretic agent (Tan *et al.*, 2022; Mamedov and Egamberdieva, 2019). The root components are also marketed as artificial sweetener. In the recent COVID-19 pandemic situation, people have used the liquorice roots to treat sore throat problem (Diomede *et al.*, 2021). *Glycyrrhiza glabra* is known to be 50 times sweeter than sugar and hence, in this study, we pursued for its anti-diabetic potential (Quirós-Sauceda *et al.*, 2015; Thakur and Raj, 2017).

Roots are the most important medicinal parts of licorice that have been reported to be used alone or in combination with other herbs to treat many digestive disorders like obesity, stomach ulcers, excessive thirst, intestinal gas and abdominal pain; respiratory tract disorders like coughs, asthma, tonsillitis, and sore throat; liver disorders like jaundice. *G. glabra* is well documented to exhibit antibacterial, anti-inflammatory, antiviral, antioxidant, and antidiabetic activities. The present study therefore aimed at evaluating the physicochemical and phytochemical analysis of *Glycyrrhiza glabra* root extract and potential mechanisms driving its putative protective and therapeutic effects.

MATERIALS AND METHODS

Collection and identification of plant material

For the study, the *Glycyrrhiza glabra* belongs to family Fabaceae was collected from Super market, Thanjavur, Tamilnadu, South India. The Plant was authenticated by The Director, Plant Anatomy and Research centre, Chennai.

Preparation of powder

The *Glycyrrhiza glabra* was collected and washed and dried at room temperature (37°C) for a week and made into powder by using mixture for further analysis.

Extraction method

In the soxhlet apparatus crude drug material was equally packed. It was extracted with ethanol and distilled water as solvents. The extraction was carried out over a period of around 20 hours using a heated continuous extraction method. After extraction, the extract was filtered using what man filter paper while still hot to eliminate any contaminants. The concentrated extract was transferred to a 100 ml beaker and the remaining solvent was evaporated on a water bath collected and dried. The dried extract was sealed in an airtight container and used in subsequent research such as phytochemical screening and estimation of phytoconstituents (Vashist and Sharma, 2013).

Physicochemical evaluation of crude drug

The following procedures were used to determine the different ash values and extractive values of *Glycyrrhiza glabra* root powder.

Determination of ash values

The purpose of determining ash values is to discover low-grade products that are exhausted and sandy or earthy particles. It can also be used to detect chemical components using water-soluble and acid-insoluble ash.

Total ash

The total ash was calculated by incinerating the fine powder of crude medicine (2 g) in a tarred silica crucible at 450°C until the carbon was completely removed. After that the ash was allowed to cool before being weighed. The weighed value of ash and powdered crude medication were used to compute the percentage of total ash.

Acid insoluble ash

The ash value was calculated to identify any unwanted, toxic or earthy substances that may have been present in the crude medication. The ash obtained from the foregoing process was put into 25 ml of dilute HCl is maintained on the heating mantle to estimate the acid insoluble value. The mixture was filtered through ash-free filter paper then washed, burned and weighed.

Water soluble ash

The ash obtained from the total ash process was combined with 25 ml of water to determine the water-soluble ash value. The mixture was filtered, collected and weighed on the filter paper. The water-soluble ash value was calculated by subtracting the weighed amount of insoluble matter from the weighed amount of ash. The percentage of water soluble ash value was calculated using this weighted quantity (Chauhan *et al.*, 2018).

Determination of extractive values

Alcohol soluble extractive value

In a closed flask 5 g of coarsely powdered air-dried powder was macerated with 100 ml of ethanol of the appropriate strength for twenty-four hours shaking regularly during the first six hours and allowing it to stand for eighteen hours. To avoid solvent loss it was quickly filtered and 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish and dried at 105 °C to a consistent weight and weigh. The proportions of alcohol soluble extractive vales were calculated using the air-dried medication as a reference (Bysani *et al.*, 2017; Sam, 2019*)*.

Water soluble extractive value

In a closed flask 5 g of coarsely powdered air-dried medication was macerated with 100 ml of chloroform water for 24 hours, shaking frequently during the first six hours and then left to stand for eighteen hours. It was then quickly filtered to prevent the loss of chloroform water. In a tared flat-bottomed plate dried at 105 °C, 25 ml of the filtrate was evaporated to dryness and weighed (Vashist and Sharma, 2013).

Methanol soluble extractive value

In a closed flask 5 g of coarsely powdered air-dried powder was macerated with 100 ml of methanol of the appropriate strength for twenty-four hours shaking regularly during the first six hours and allowing it to stand for eighteen hours. To avoid solvent loss it was quickly filtered and 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish and dried at 105°C to a consistent weight and weigh. The proportions of alcohol soluble extractive vales were calculated using the air-dried medication as a reference (Bysani *et al.*, 2017; Sam, 2019*)*.

Loss on drying

The approach provided was used to calculate the loss on drying. A measured amount of extract was poured into a weighed petri dish. The petri dish was placed in the oven and weighed at various intervals at 105°C until two consecutive weighing did not deviate by more than 0.25 mg, indicating the drug's final loss of moisture. The percentage loss on drying was estimated using the formula below (Chauhan *et al.*, 2018).

LOD (%) = (Weight of porcelain dish with drug at time 0-Weight of porcelain dish after 6 h)/(Weight of porcelain dish at time 0-Weight of empty porcelain dish)

PH determination

To determine the pH, the extract was dissolved in 10 ml of pure water. A digital pH meter was used to determine the pH. The pH is measured 3 times (Chauhan *et al.*, 2018).

Phytochemical screening of *Glycrrhiza gabra:*

Test for alkaloids

A little quantity of the solvent-free extract was filtered after being agitated with a few drops of weak hydrochloric acid. Mayer's reagent (cream ppt), Hager's reagent (yellow ppt), Wagner's reagent (reddish brown ppt), and Dragendroff's reagent (reddish brown ppt) were used to test the filtrate for the presence of alkaloids (orange brown ppt) (Vashist and Sharma, 2013).

Tests for carbohydrates

A little amount of the extract was diluted in 4 ml distilled water and filtered separately.

Molisch's and Fehling's tests were used to determine carbohydrate presence in the filtrate (Vashist and Sharma, 2013).

Molisch's test

2-3 drops of 1 percent alcoholic alpha-naphthol solution were added to the filtrate and 2 ml of Conc. Sulphuric acid was poured along the edges of the test tube. The presence of carbohydrates was shown by the appearance of a brown ring at the intersection of two liquids (Vashist and Sharma, 2013).

Fehling's test

Extract was stored in the water bath A and B Fehling solutions were mixed together. The presence of reducing sugars was visible in the brick red precipitate (Chauhan *et al.*, 2018).

Tests for glycosides

Another portion of the extract was hydrolysed with hydrochloric acid for a few hours on a water bath and the hydrolysate was tested for the presence of various glycosides using Legal's and Borntrager's tests (Vashist and Sharma, 2013).

Legal's test

1 ml pyridine and a few drops of sodium nitroprusside solutions were added to the hydrolysate, which was then made alkaline with sodium hydroxide solution. The presence of glycosides was indicated by the appearance of a pink to red tint (Vashist and Sharma, 2013).

Borntrager's tests

The chloroform layer was removed from the hydrolysate after it was treated with chloroform. An equal amount of weak ammonia solution was added to this. The ammonia layer turns pink, indicating that glycosides are present (Vashist and Sharma, 2013).

Test for saponins

With 20 ml of distilled water, the extract was dissolved and agitated for 15 minutes. The presence of saponins was demonstrated by the creation of a 1 cm layer of foam over time (Chauhan *et al.*, 2018).

Tests for flavonoids

With sodium hydroxide: 1 ml sodium hydroxide solution was added to the extract. Anthocyanins are found in blue to violet colours, Flavanones are found in yellow to orange colours and flavones are found in yellow (Chauhan *et al.*, 2018).

With concentrated sulphuric acid

Concentrated sulphuric acid was added to the extract. The presence of anthocyanin is indicated by a yellow orange colour, while the presence of flavones is indicated by an orange to red tint (Chauhan *et al.*, 2018).

Shinoda test

Extract was dissolved in ethanol and magnesium turnings were added to perform Shinoda's test concentration hydrochloric acid was added to this combination. The presence of flavonoids is indicated by a change in hue from magenta to purple (Chauhan *et al.*, 2018).

Test for mucilage

Small amounts of the extract were added individually to 25 ml of pure alcohol and filtered while constantly stirring. The precipitate was dried in the air and analysed for the presence of mucilage as well as its swelling qualities (Vashist and Sharma, 2013).

Test for phytosterol

The extract was heated in a solution of alcoholic potassium hydroxide until it was completely saponified. Ethyl ether was used to dilute the mixture and extract it. The ether layer was evaporated, and the residue was examined for phytosterol (Vashist and Sharma, 2013)..

Liebermann-burchard test

The residue was dissolved in a few drops of diluted acetic acid, followed by 3 ml of acetic anhydride and a few drops of concentrated sulphuric acid. The presence of phytosterol was shown by the presence of a bluish green tint (Vashist and Sharma, 2013).

Test for phenolic compounds and tannins

Small amounts of the extract were separated in water and tested for the presence of phenolic compounds and tannins using the reagents listed below (Vashist and Sharma, 2013). Dilute Ferric chloride solution (5%)-Violet colour, 1% solution of gelatine containing 10% sodium chloride - White ppt, 10% lead acetate solution - White ppt.

Estimation of phytochemical constituents

Estimation of total phenol

The TPC is determined according to the Folin-Ciocalteu spectrophotometric method. Briefly 0.5 ml of sample extract was mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 2 ml of 7.5% Na2CO3 solution was added and the final volume was made up to 10 ml with distilled water. After 1 h of reaction at room temperature the absorbance at 760 nm was measured. The measurements were compared to a standard curve of prepared gallic acid solution and the total phenolic content was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of dry weight (Ainsworth and Gillespie, 2007).

Estimation of ascorbic acid

1 gm of the sample extract is dissolved in 4% oxalic acid and made up to a known volume of 100 ml and centrifuged. 5 ml of the supernatant is pipette out and 10 ml of 4% oxalic acid is added and titrated against the dye (V2 ml). Blank solution was prepared with the working standard solution.

Amount of ascorbic acid mg per 100 g sample = (0.5 mg × V2 × 100 ml)/ (V1 × 5 ml × weight of the sample) × 100

Estimation of total flavonoids

A colorimetric test was used to determine total flavonoids. An aliquot of diluted (+)-catechin sample or standard solution was added to 75 ml $NaNO_2$ solution (5%) and stirred for 6 minutes before adding 0.15 ml $AlCl_3$ (10 percent). 0.5 mL of sodium hydroxide was added after 5 minutes. With distilled water the final volume was adjusted to 2.5 ml and carefully mixed. At 510 nm, absorbance was measured against a blank. The total flavonoid concentration is measured in milligrams of catechins per gram of dry weight and compared to the (+)-catechin calibration curve, which ranges from 0 to 400 mg/ml. All of the samples were examined in three different ways.

RESULTS AND DISCUSSION

Organoleptic characters of *Glycyrrhiza glabra*

The extraction of crude drug of *Glycyrrhiza glabra* was carried out as per the methodology in aqueous and ethanol solvents. The percentage yield of crude extract was found to be more in ethanol when compared to that of the aqueous solvent. The percentage yield of ethanol extract is found to be 9.73%. This shows that ethanol diffuses and solubilizes more phytochemical constituents when compared to that of distilled water (Table 1).

Table 1. Organoleptic characters of *Glycyrrhiza glabra*

Parameters	*Glycyrrhiza glabra*
Odour	Sweet smell
Colour	Yellowish or pale brown
Taste	Sweet
Consistency	Solid – powder

Physicochemical parameters

The physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, loss on drying and pH of the aqueous solutions were analysed and are found to be in limits (Table 2).

Table 2. Physicochemical evaluation of *Glycyrrhiza glabra*

Parameters	Results
Total ash	4.37 % w/w
Acid insoluble ash	0.73 % w/w
Water soluble ash	0.91 % w/w
Water soluble extractives	23.7 % w/w
Ethanol soluble extractives	34.5% w/w
Methanol soluble extractives	39.2% w/w
Loss on drying	4.85 w/w
pH (aqueous solution)	5.7

Phytochemical screening

The phytochemical constituents such as alkaloids, tannins, phenols, saponins and carbohydrates are identified in both aqueous, methanol and ethanol extracts of *Glycyrrhiza glabra*. Also, the flavonoids, glycosides and phytosterol are present in ethanol extract but not found in aqueous extract. So, the ethanolic extract is used for the further research findings (Table 3).

Table 3. Phytochemical screening of *Glycyrrhiza glabra*.

Name of tests	Results		
	Aqueous extract	Ethanolic extract	Methanolic extract
Test for alkaloids			
Dragendroff's	+ ve	+ ve	+ ve
Mayer's	+ ve	+ ve	+ ve
Test for flavonoids			
With sodium hydroxide	+ ve	+ ve	+ ve
With conc. sulphuric acid	- ve	+ ve	+ ve
Shinoda	+ ve	+ ve	+ ve
Test for tannins			
FeCl3	+ ve	+ ve	+ ve
Test for phenols			
FeCl3	+ ve	+ ve	+ ve
Test for saponins			
Froth test	+ ve	+ ve	+ ve
Test for carbohydrates			
Molisch's test	+ ve	+ ve	+ ve
Fehling's test	+ ve	+ ve	+ ve
Tests for glycosides			
Legal's test	- ve	+ ve	+ ve
Borntrager's tests	+ ve	+ ve	+ ve
Test for phytosterol			
Liebermann-Burchard test	- ve	+ ve	+ ve

Quantitative analysis of phytochemical constituents

The major phytochemical constituents present in this herbal powder are believed to be total flavonoid, total phenol and ascorbic acid. The presence of total flavonoid, gallic acid equivalent for total phenol and ascorbic acid can be used to identify these phytochemical constituents. The total phenol was estimated by the Folin-denis method and the Folin-ciocalteu method, respectively. The flavonoid was estimated by a colorimetric assay. The ascorbic acid was estimated by the volumetric method (Table 4). The amounts of total flavonoids, total phenol, and ascorbic acid were found to be 185.14 mg, 481.47 mg per 1 gm, and 33.81 µ g/ml of aqueous extract, and 218.92 mg, 507.62 mg per 1 gm, and 42.38 µ g/ml of the ethanol extract. When ethanol extract is compared to aqueous extract, the amount of phytochemical constituents was found to be higher in ethanol extracts (Katoch, 2011; Medini *et al.*, 2014; Sharma and Agrawal, 2013).

Table 4. Estimation of phytochemical constituents in *Glycyrrhiza glabra* extract

Solvent used	Total flavonoid (mg)	Total phenol (mg)	Ascorbic acid (µ g/ml)
Aqueous	185.14 ± 0.25	481.47 ± 0.61	33.81 ± 0.52
Ethanol	218.92 ± 0.37	507.62 ± 0.04	42.38 ± 0.13
Methanol	221.29 ± 0.45	517.16 ± 0.05	43.32 ± 0.15

CONCLUSION

Glycyrrhiza glabra showed a good extractive value in ethanol than water suggesting that the phytoconstituents would be more concentrated in methanol and ethanolic extract. The methanolic and ethanolic extract of *Glycyrrhiza glabra* showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, glycosides and phytosterol. It was found that the phytochemical constituents are very much enriched

in the *Glycyrrhiza glabra* extract and can be used for development of new formulations.

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