Glycyrrhiza (root) . The plant is used fre q uently 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. [2]

Plant profile

Glycyrrhiza glabra L inn: In north India, Glycyrrhiza glabra is kno w n as mulaithi.Glycyrrhiza glabra popularly kno w n as li q uorice or s w eet w ood is a Mediterranean and Asian plant endemic to the mediterranean and certain parts of Asia.Glycyrrhiza glabra is a plant in the genus Glycyrrhiza that is generally kno w n as li q uorice in India. Traditional healers have claimed the efficacy of Glycyrrhiza species as a diuretic, choleretic, and insecticide for a variety of pathological disorders and it is used in traditional medicine for coughs, colds and uncomfortable s w ellings.

Plant description

Classification:

K ingdom: Plantae.

Division : Angiospermae.

Class : Dicotyledoneae.

O rder: Rosales.

F amily: F abaceae.

Genus : Glycyrrhiza.

Species: Glabra L inn.

Binomial name : Glycyrrhiza glabra L .

MATERIA L S AND MET H O DS

The crude po w dered drug of analytical grade w as purchased from yucca enterprises in Mumbai. All the other chemicals used w ere of pharmaceutical or analytical grade.

Extraction method

In the soxhlet apparatus crude drug material w as e q ually packed. It w as extracted w ith ethanol and distilled w ater as solvents. The extraction w as carried out over a period of around 2 0 hours using a heated continuous extraction method. After extraction, the extract w as filtered using w hat man filter paper w hile still hot to eliminate any contaminants. The concentrated extract w as transferred to a 1 00 ml beaker and the remaining solvent w as evaporated on a w ater bath collected and dried. The dried extract w as sealed in an airtight container and used in subse q uent research such as phytochemical screening and estimation of phytoconstituents [3] .

Physicochemical evaluation of crude drug

The follo w ing procedures w ere used to determine the different ash values and extractive values of Glycyrrhiza glabra stolon po w der.

Determination of ash values

The purpose of determining ash values is to discover lo w -grade products that are exhausted and sandy or earthy particles. It can also be used to detect chemical components using w ater-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 [4].

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 [5, 6].

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 [3].

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 [4].

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 [4].

Determination of foreign matter

About 100 g of the drug sample to be analysed is weighed and spread into a thin layer and inspected with the unaided eye and with the use of a lens to find the foreign matter. The foreign matters are then separated, weighed and noted [6] (Table 1).

Quantitative standards

Total ash	Not more than 4.5%
Acid insoluble ash	Not more than 1.15%
Water soluble ash	Not more than 1%
Aqueous extractive value	Not less than 20%
Ethanolic extractive value	Not less than 15%
Chloroform extractive value	Not less than 5%
Water soluble extractive	Not less than 20%
Moisture content	Not more than 7.45%

Table 1: Quantitative standards.

PHYTOCHEMICAL SCREENING OF GLYCRRHIZA GLABRA

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) [3].

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 [3].

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 [3].

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 [4].

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 [3].

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 [3].

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 [3].

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 [4].

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 [4].

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 [4].

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. [4]

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 [3].

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 [3].

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 [3].

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 [3]. 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% Na_2CO_3 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 [7].

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 (V_2 ml). Blank solution was prepared with the working standard solution.

Amount of ascorbic acid mg per 100 g sample = (0.5 mg × V_2 × 100 ml)/ (V_1 × 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 (Tables 2 and 3) (Figures 1 and 2) [8-10].

RESULTS AND DISCUSSION

Organoleptic characters of glycyrrhiza glabra

S. No	Parameters	Glycyrrhiza glabra
1	Odour	Sweet smell
2	Colour	Yellowish or pale brown
3	Taste	Sweet
4	Consistency	Solid – powder

Table 2: Organoleptic characters of *Glycyrrhiza glabra.*

Figure 1: *Glycyrrhiza glabra* crude drug.

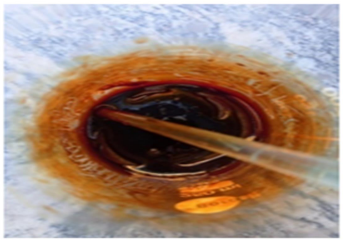


Figure 2: *Glycyrrhiza glabra* extract.

Extraction of glycyrrhiza glabra powder

S. No	Content	Solvent	Colour	Type of extract	Consistency	% Yield
1	*Glycyrrhiza glabra*	Aqueous	Brown	Crude	Solid	7.86%
2		Ethanol	Dark brown	Crude	Solid	9.73%

Table 3: Percentage yield of extract preparations.

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.

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, foreign and pH of the aqueous solutions were analysed and are found to be in limits (Table 4).

S. No	Parameters	Results
1	Total ash	4.37 % w/w
2	Acid insoluble ash	0.73 % w/w
3	Water soluble ash	0.91 % w/w
4	Water soluble extractives	23.7 % w/w
5	Alcohol soluble extractives	34.5% w/w
6	Loss on drying	4.85 w/w
7	Foreign matter	-
8	pH (aqueous solution)	5.7

Table 4: Physicochemical evaluation of *Glycyrrhiza glabra.*

Phytochemical screening

The phytochemical constituents such as alkaloids, tannins, phenols, saponins and carbohydrates are identified in both aqueous 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.

Estimation 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. 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

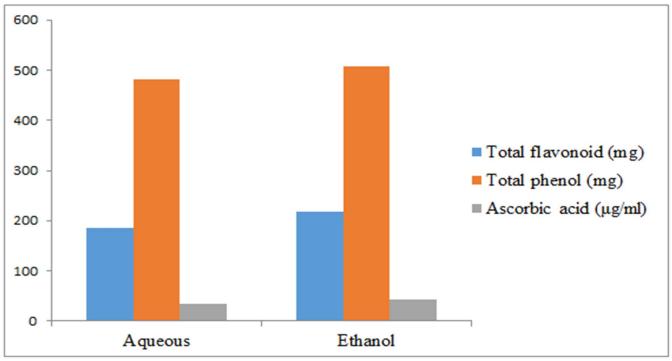
µ g/ml of the ethanol extract. When ethanol extract is compared to aqueous extract, the amount of phytochemical constituents was found to be higher in ethanol extracts (Tables 5, 6) (Figure 3).

S. No	Name of tests	Results	
		Aqueous extract	Ethanolic extract
Test for alkaloids			
1	Dragendroff's	+ ve	+ ve
2	Mayer's	+ ve	+ ve
Test for flavonoids			
3	With sodium hydroxide	+ ve	+ ve
4	With conc. sulphuric acid	- ve	+ ve
5	Shinoda	+ ve	+ ve
Test for tannins			
6	$FeCl_3$	+ ve	+ ve
Test for phenols			
7	$FeCl_3$	+ ve	+ ve
Test for saponins			
8	Froth test	+ ve	+ ve
Test for carbohydrates			
9	Molisch's test	+ ve	+ ve
10	Fehling's test	+ ve	+ ve
Tests for glycosides			
11	Legal's test	- ve	+ ve
12	Borntrager's tests	+ ve	+ ve
Test for phytosterol			
13	Liebermann-Burchard test	- ve	+ ve

Table 5: Phytochemical screening of *Glycyrrhiza glabra.*

S. No	Herbal drug name	Solvent used	Total flavonoid (mg)	Total phenol (mg)	Ascorbic acid (µ g/ml)
1	*Glycyrrhiza glabra*	Aqueous	185.14 ± 0.25	481.47 ± 0.61	33.81 ± 0.52
2		Ethanol	218.92 ± 0.37	507.62 ± 0.04	42.38 ± 0.13

Table 6: Estimation of phytochemical constituents in *Glycyrrhiza glabra* extract in ethanol.



. **Figure 3**: Estimation of phytochemical constituents in *Glycyrrhiza glabra* extract in distilled water and ethanol.
Note: Total flavonoid(mg) Total phenol (mg) Ascorbic acid (µg/ml)
:

CONCLUSION

Glycyrrhiza glabra showed a good extractive value in ethanol than water suggesting that the phytoconstituents would be more concentrated in ethanolic extract. The 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.

ACKNOWLEDGEMENT

The author is thankful to the management of Excel Institutions and Principal Dr. R.Manivannan, Excel College of Pharmacy, Komarapalayam, Namakkal, Tamilnadu for providing laboratory facilities to carry out the research work.

REFERNCES

[1] Kokate C K., Purohit.A P., Gokhale SB., et al., *Pharmacogn*, **2000**.
[2] Pastorino G., Cornara L., Soares S., et al., *Phytotherapy Res*, **2018**, 32(12):2323-2339.
[3] Vashist H., Sharma D. *Asian J Pharm Clin Res*, **2013**, 6(4):55-59.
[4] Chauhan S., Gulati N., Nagaich U. *Ars Pharm*. **2018**, 59(2): 61-67.
[5] Sam S. *J pharmacogn phytochem*, **2019**, 8(2):354-357.
[6] Bysani S., Babu P S., Karthikeyan R. *J Med Plants Studies*, **2017**, 5(3):373-383.
[7] Ainsworth E A., Gillespie K M. *Nature Protocol*, **2007**, 2(4):875-877.
[8] Katoch R. *Spring Sci Business Media*, **2011**.
[9] Medini F., Fellah H., Ksouri R., et al., J Taibah Uni Sci, **2014**,8(3):216-24.
[10] Sharma V A., Agrawal R C. *Mintage J Pharm Med Sci*, **2013**, 2(3):15-20.