Research Article

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PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF YASHTIMADHU (*GLYCYRRHIZA GLABRA)* IN AQUEOUS AND ETHANOLIC EXTRACT

Revathi S [1]*, Madhavan K [2], Naresh R [2], Priyanka S [2], Ramyadevi S [2], Selvam B [2], Manivannan R [3]

[1] HOD, Department of Pharmaceutics, Excel College of Pharmacy, Namakkal, Tamil Nadu, India

[2] B.Pharm Student, Excel College of Pharmacy, Namakkal, Tamil Nadu, India

[3] Principal, Excel College of Pharmacy, Namakkal, Tamil Nadu, India

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***Corresponding author**
E-mail: revathis79@gmail.com

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ABSTRACT

The study aims to analyse the physicochemical and phytochemical characteristics of Yashtimadhu (*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 Yashtimadhu drugs for 20 hours using water and ethanol as solvents. The analysis of physicochemical properties of Yashtimadhu extracts includes ash value and extractive value etc. The study of phytochemical screening of Yashtimadhu 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 found within limits. The ethanolic extract of Yashtimadhu showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, glycosides and phytosterol. The amounts of total flavonoids, phenol and ascorbic acid were 185.14 mg, 481.47 mg per 1 gm, and 33.81 µg/ml of aqueous extract 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 Yashtimadhu (*Glycyrrhiza glabra*) extract and can be used for the development of new formulations.

Keywords: Yashtimadhu, *Glycyrrhiza glabra*, physicochemical, phytochemical, estimation

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 necessary for man's survival. Nature has offered many 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 for assuring health care.[1]

Since the dawn of human gardening activities, plants have played a significant part in medicine. *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. It is still commonly used in folk medicine to treat gastritis, peptic ulcers, respiratory infections, and tremors.[2]

Plant Profile
***Glycyrrhiza glabra* Linn.**

In north India, *Glycyrrhiza glabra* is known as mulaithi. *Glycyrrhiza glabra*, popularly known as Yashtimadhu or sweet wood, is a Mediterranean and Asian plant endemic to the Mediterranean and certain parts of Asia. *Glycyrrhiza glabra* is a plant in the genus *Glycyrrhiza*, generally known as Yashtimadhu in India. Traditional healers have claimed the efficacy of *Glycyrrhiza* species as a diuretic, choleretic, and insecticide for various pathological disorders. It is used in traditional medicine for coughs, colds, and uncomfortable swellings.[3]

Plant Description

Classification
Kingdom: Plantae
Division: Angiospermae
Class: Dicotyledoneae
Order: Rosales
Family: Fabaceae
Genus: *Glycyrrhiza*
Species: *glabra*
Binomial Name: *Glycyrrhiza glabra* L. [3]

MATERIALS AND METHODS

The crude powdered drug of analytical grade was purchased from Yucca Enterprises in Mumbai. Yucca Enterprises is a certified herbal drugs manufacturer, retailer and service provider – GST No - 27ACEPL2903R1Z9. All the other chemicals used were of pharmaceutical or analytical grade.

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 around 20 hours using a heated continuous extraction method. After extraction, the extract was filtered using Whatman 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 in a water bath, collected and dried. The dried extract was sealed in an airtight container and used in subsequent research such as phytochemical screeningand estimation of phytoconstituents. [4]

Physicochemical Evaluation of Crude Drug

The following procedures determined the different ash and extractive values of the Yashtimadhu (*Glycyrrhiza glabra*) crude drug.

Determination of ash values
Determining ash values aims to discover exhausted low-grade products and sandy or earthy particles. It can also detect chemical components using water-soluble and acid-insoluble ash. [5]

Total ash
The total ash was calculated by incinerating the fine powder of crude medicine (2 g) in a tarred silica crucible at 450ºC until the carbon was removed entirely. 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. [5]

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 preceding process was put into 25 ml of dil. HCl is maintained on the heating mantle to estimate the acid-insoluble value. The mixture was filtered through ash-free filter paper, washed, burned and weighed. [5]

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. [5]

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 weight. The proportions of alcohol-soluble extractive values were calculated using the air-dried medication as a reference. [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, frequently shaking 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. [4]

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 105ºC until two consecutive weighs 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 the 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
The extract was dissolved in 10 mL of pure water to determine the pH. A digital pH meter was used to determine the pH. The pH is measured three times. [5]

Determination of foreign matter
About 100g of the drug sample to be analysed is weighed and spread into a thin layer, inspected with the unaided eye and a lens to find the foreign matter (6x). The foreign matters are then separated, weighed and noted. [7]

Phytochemical Screening of Yashtimadhu (*Glycyrrhiza glabra*)

Test for alkaloids
A small quantity of the solvent-free extract was filtered after agitation 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 Dragendorff's reagent (Reddish-brown ppt) were used to test the filtrate for the presence of alkaloids (Orange brown ppt). [4]

Tests for carbohydrates
A small amount of the extract was diluted in 4 mL distilled water and filtered separately. Molisch's and Fehling's tests were used to determine the carbohydrate present in the filtrate. [4]

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. [4]

Fehling's test
The extract was stored in the water bath. A and B Fehling solutions were mixed. The presence of reducing sugars was visible in the red brick precipitate. [5]

Tests for glycosides
Another portion of the extract was hydrolysed with hydrochloric acid for a few hours in a water bath. The hydrolysate was tested for various glycosides using Legal's and Borntrager's tests. [4]

Legal's test
1 mL pyridine and a few drops of sodium nitroprusside solutions were added to the hydrolysate, then alkaline with sodium hydroxide solution. The presence of glycosides was indicated by the appearance of a pink to red tint. [4]

Borntrager's tests
The chloroform layer was removed from the hydrolysate after being treated with chloroform. An equal amount of weak ammonia solution was added to this. The ammonia layer turns pink, indicating that glycosides are present. [4]

Test for saponins (Foam test)
With 20 mL of distilled water, the extract was dissolved and agitated for 15 minutes. The presence of saponins was demonstrated by creating a 1cm layer of foam over time. [5]

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. [5]

With concentrated sulphuric acid
Concentrated sulphuric acid was added to the extract. The presence of anthocyanins is indicated by a yellow-orange colour, while orange indicates the presence of flavones with a red tint. [5]

Shinoda test
The extract was dissolved in ethanol, and magnesium turnings were added to perform Shinoda's test. Conc. Hydrochloric acid was added to this combination. A change in hue indicates the presence of flavonoids 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 and its swelling qualities. [4]

Test for phytosterol
The extract was heated in a solution of alcoholic potassium hydroxide until it was utterly saponified. Ethyl ether was used to dilute the mixture and extract it. The ether layer was evaporated, and the residue was examined for phytosterol. [4]

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. A bluish-green tint showed the presence of phytosterol. [4]

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.

Dil. Ferric chloride solution (5%) – Violet colour.
1% solution of gelatin containing 10% sodium chloride-White ppt
10% lead acetate solution-White ppt. [6]

Estimation of Phytochemical Constituents

Estimation of total phenol
The total phenolic content 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 the prepared gallic acid solution. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight. [6]

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). A blank solution was prepared with the standard working solution. The amount of ascorbic acid per gm is calculated as follows. [9]

$$\text{Amount of ascorbic acid mg per 100 g sample} = \frac{0.5 \text{ mg x V2 x } 100\text{ml}}{\text{V1x 5 ml x weight of the sample}} \text{X } 100$$

Estimation of Total Flavonoids

A colourimetric test was used to determine total flavonoids. An aliquot of diluted (+)-catechin sample or standard solution was added to 75 mL NaNO2 solution (5%) and stirred for 6 minutes before adding 0.15 mL AlCl3 (10 percent). 0.5 mL of NaOH was added after 5 minutes. The final volume was adjusted to 2.5 mL with distilled water and carefully mixed. At 510 nm, absorbance was measured against a blank. The total flavonoid concentration is measured in milligrams of catechin per gram of dry weight (mgCE/g DW) 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. [10]

RESULTS AND DISCUSSION

Table 1: Quantitative Standards[8]

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%

Figure 1: Yashtimadhu – crude drug

Figure 2: Yashtimadhu – extract

Table 2: Organoleptic characters of Yashtimadhu (*Glycyrrhiza glabra*)

Parameters	Yashtimadhu
Odour	Sweet smell
Colour	Yellowish or pale brown
Taste	Sweet
Consistency	Solid – powder

Table 3: Percentage yield of extract preparations

Content	Solvent	Colour	Type of extract	Consistency	% Yield
Yashtimadhu	Aqueous	Brown	Crude	Solid	7.86 %
Yashtimadhu	Ethanol	Dark brown	Crude	Solid	9.73 %

Table 4: Physicochemical evaluation of Yashtimadhu (*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
Alcohol soluble extractives	34.5% w/w
Loss on drying	4.85 w/w
Foreign matter	Nil
pH (aqueous solution)	5.7

Table 5: Phytochemical screening of Yashtimadhu (*Glycyrrhiza glabra*)

S. No	Name of tests	Results	
		Aqueous Extract	Ethanolic extract
Test for alkaloids			
1	Dragendorff's	+	+
2	Mayer's	+	+
Test for flavonoids			
3	With sodium hydroxide	+	+
4	With conc. sulphuric acid	-	+
5	Shinoda	+	+
Test for tannins			
6	FeCl3	+	+
Test for phenols			
7	FeCl3	+	+
Test for saponins			
8	Froth test	+	+
Test for carbohydrates			
9	Molisch's test	+	+
10	Fehling's test	+	+
Tests for glycosides			
11	Legal's test	-	+
12	Borntrager's tests	+	+
Test for phytosterol			
13	Liebermann-Burchard test	-	+

Table 6: Estimation of phytochemical constituents in Yashtimadhu (*Glycyrrhiza glabra*) extract in distilled water and Ethanol

Herbal drug name	Solvent used	Total flavonoid * (mg)	Total phenol* (mg)	Ascorbic acid (µg/ml)
Yashtimadhu	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

* – mg equivalent to 1 gm of the Ethanolic extract

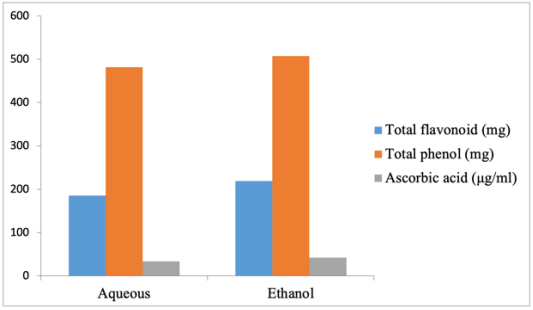

Figure 3: Estimation of phytochemical constituents in Yashtimadhu (*Glycyrrhiza glabra*) extract in distilled water and ethanol

Extraction of Yashtimadhu powder

The crude drug of Yashtimadhu was extracted 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 solubilises more phytochemical constituents when compared to that 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, and foreign and pH of the aqueous solutions were analysed and are found to be within limits. (Table 4)

Phytochemical screening

The phytochemical constituents such as alkaloids, tannins, phenols, saponins and carbohydrates are identified in both aqueous and ethanol extracts of Yashtimadhu. Also, flavonoids, glycosides and phytosterol are present in ethanol extract but not found in aqueous extract. So, the ethanolic extract is used for further research findings. (Table 5)

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 and Folin-Ciocalteu methods, respectively. A colourimetric assay estimated the flavonoid. The ascorbic acid was estimated by the volumetric method. The amounts of total flavonoids, phenol, and ascorbic acid were 185.14 mg, 481.47 mg per 1 gm, and 33.81 µg/ml of aqueous 218.92 mg, 507.62 mg per 1 gm, and 42.38 µg/ml of the ethanol extract. When ethanol extract is compared to aqueous extract, the amount of phytochemical constituents is higher in ethanol extracts. (Table 6, Figure 3)

CONCLUSION

Yashtimadhu (*Glycyrrhiza glabra*) showed an excellent extractive value in ethanol than in water, suggesting that the phytoconstituents would be more concentrated in ethanolic extract. The ethanolic extract of Yashtimadhu 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 Yashtimadhu extract and can be used for the development of new formulations.

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