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DETERMINATION OF PHYSICOCHEMICAL AND PHYTOCHEMICAL PARAMETERS OF DIFFERENT EXTRACTS OF *GLYCYRRHIZA GLABRA*

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ABSTRACT

Glycyrrhiza glabra has been used for several diseases. *Glycyrrhiza glabra* commonly known as "Licirice", and is belongs to family Fabaceae. The present study comprises physico-chemical and phytochemical evaluation of different extracts of *Glycyrrhiza glabra* by using different standard methods. The investigation of physico chemical parameters was carried out by the determination of ash values, extractive values and moisture content. Whereas phytochemical analysis was carried out to estimate the presence of carbohydrates, glycosides, flavonoids, tannins, phytosterols and phenolic compounds in different extracts of *Glycyrrhiza glabra*. Results revealed the presence of carbohydrates, alkaloids, saponons, phytosterols, flavonoids, fats and oils. The present investigation will helpful in Assessing the quality and purity of a crude drug and laying down pharmacopoieal standards for *Glycyrrhiza glabra.*

INTRODUCTION:

Glycyrrhiza glabra belonging to family Fabaceae is commonly known as Licorice. In Sanskrit, it is well known as Yashti-madhu and madhuka. It is mostly cultivated in India, Iran, Iraq, China, Pakistan and Turkey. [1] The plant is a perennial herb, which grows to a height of 1 mtr. It is well grown in well drained soils and in deep valleys. It was harvested in autumn. [2] The leaves of this plant are high pinnate shape and about 7-15cm long. A single leaf is having 9 to 17 leaflets. The flowers of this plant are 0.8 to 1.2cm long and having purple to pale whitish blue color and the fruit is an oblong pod of 2-3cm long which consists of many seeds. The *Glycyrrhiza glabra* is having antibacterial activity. [3] It is also having Anti fungal activity. [4] The plant is rich in antioxidants and so it is having enormous antioxidant activity. [5,6,7,8] It also suppress cough. [9] The plant is also used to treat ulcers. [10] It is also having hepatoprotective activity. [11,12] The whole plant is also having analgesic and antiinflammatory activity. [13] It is also having estrogenic activity. [14] Previously reported chemical constituents of *Glycyrrhiza glabra* are anethole and glycyrrhizin. It also consists of asparagin and glycyrhetic acid. [15, 16]

MATERIALS AND METHODS:

I. Collection of plant material

The crude drug of *Glycyrrhiza glabra* was collected from local market of Tirupati. They were verified taxonomically and authenticated in the Department of Botany, S.V.University, Tirupati. The plant material was coarsely powdered by using a rotary

grinder and the powder is stored in airtight plastic containers. The prepared powder was used for all phytochemical analysis.

II. Preparation of extracts

The collected plant material was washed with water and dried at room temperature for 15-20 days and it was subjected for size reduction. The powder was used for extract preparation. The 100 g of plant material was extracted with Soxhlet apparatus by using 400 ml petroleum ether for about 48 h. After defatting, the marc was dried by using hot air oven at 50°C and it is packed in Soxhlet apparatus for further extraction with 400 ml of 95% ethanol until it does not shows the presence of residue on evaporation. The aqueous extract was prepared by cold maceration with 3% methanol-water for seven days with frequent shaking. Solvents were removed from the extracts by using rotary vacuum evaporator.

III. Physicochemical evaluations

1. Moisture content

Weighed quantity of the shade dried powder of *Glycyrrhiza glabra* (3 g) was taken in a tared glass bottle and initial weight was taken. The powder was heated in an oven at 105°C and is weighed. This procedure was repeated till the constant weight was obtained. The moisture content of the sample was calculated in the percentage with reference to shade dried plant powder by using formula.[17]

% Moisture content = Loss in weight of the sample/ Weight of the sample x 100

2. Ash values [18]:

a) Determination of total ash

An accurately weighed quantity of the shade dried powder of *Glycyrrhiza glabra* (2 g) was incinerated in a crucible at a temperature of 450°C in a muffle furnace untill carbon free ash was obtained. Then cooled and weighed. The percentage of total ash was calculated with reference to the shade dried powder by using the following formula.

% Total ash value = Weight of total ash/ Weight of the crude drug taken x 100

b) Determination of acid insoluble ash

The ash obtained was boiled with 25 ml of 2 M HCl for five minutes and it was filtered using an ash less filter paper. Insoluble matter retains on the filter paper and it was washed by using hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the shade dried plant powder by using the following formula.

% acid insoluble ash value= Weight of acid insoluble ash/ Weight of the crude drug taken x 100

c) Determination of water soluble ash

The ash above obtained, was boiled for 5min with 25 ml of water, cooled and the insoluble matter was collected on an ash less filter paper. Paper was washed with hot water and ignited at a temperature not exceeding 450°C, for 15min in a muffle furnace. The difference in the weight of ash and the weight of water insoluble matter gave the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the shade dried plant powder by using the following formula.

% Water soluble ash value = Weight of total ash-Weight of water insoluble ash/ Weight of the crude drug taken x 100

3. Extractive values [19]:

Extractive values of shade-dried powder of *Glycyrrhiza glabra* were determined using following methods.

a) Determination of alcohol soluble extractive

An accurately weighed quantity of the shade dried powder of *Glycyrrhiza glabra* (5 g) was macerated with alcohol (100 ml) (Ethanol) in a closed flask for 24 h, with occasional shaking during the first 6 h. It was then allowed to stand for 18 h and then filtered rapidly to prevent any loss during evaporation. Evaporate approximately 25 ml of the filtrate in a porcelain dish and dried at 105°C and weighed. The percentage of alcohol (Ethanol) soluble extractive was calculated with reference to the shade dried plant powder.

b) Determination of water soluble extractive

Weighed quantity of the shade dried powder of *Glycyrrhiza glabra* (5 g) was macerated with 100 ml of water in a closed flask with frequent shaking for the first 6 hrs and allowed to stand for 18 hrs. After that, it was filtered taking precaution against loss of water. Evaporate 25 ml of filtrate in a tared flat bottom shallow dish and it was dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the shade dried plant powder.

c) Determination of petroleum ether soluble extractive

Weighed quantity of the shade dried powder of *Glycyrrhiza glabra* (5 g) was macerated with 100 ml petroleum ether in a closed flask for 24 h, with frequent shaking for the first 6 hrs and allowed to stand for 18 hrs. After that, it was filtered rapidly taking precaution against loss of petroleum ether due to its volatility. Evaporate 25 ml of filtrate in a porcelain dish and dried at a temperature of 105°C and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to the shade dried plant powder.

IV. Phytochemical Evaluation: The freshly prepared petroleum ether, methanolic and aqueous extracts of *Glycyrrhiza glabra* were qualitatively analyzed for the presence of major phytochemical constituents using the following standard procedures.

1. Detection of Carbohydrates [20]:

100 mg of extracts were dissolved in 10 ml of water and filtered. The filtrate prepared was used to test the presence of proteins and amino acids.

(a) Molisch's Test: To the 1 ml of filtrate add Molisch's reagent (2 drops) in a test tube and add 2 ml of concentrated sulphuric acid carefully along the sides of the test tube. Formation of violet color at the interface of two liquids indicates the presence of carbohydrates.

(b) Fehling's Test: To the 1 ml of filtrate add 4 ml of Fehling's reagent (2 ml Fehling A and 2 ml Fehling B solutions) in a test tube and heated for 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

(c) Barfoed's Test: 1 ml of Barfoed's reagent is heated with 5 drops of filtrate in a test tube on boiling water bath. Formation of a brick-red precipitate within five minutes of period, indicates the presence of monosaccharides. Disaccharides generally don't give any reaction even for ten minutes.

2. Detection of Proteins and Amino acid [21]: 100 mg of extracts were dissolved in water (10 ml) and it was filtered. The filtrate was used to test the presence of proteins and amino acids.

(a) Millon's Test: 2 ml of filtrate was treated with 2 ml of Millon's reagent in a test tube and then it was heated in a water bath for about 5 min, cooled and few drops of NaNO2 solution were added. Formation of white precipitate and it turns to red upon heating indicates the presence of proteins and amino acids.

(b) Ninhydrin Test: To the 2 ml of filtrate add 2-3 drops of Ninhydrin reagent in a test tube and boiled for 2 min. Formation of deep blue colour indicates the presence of amino acids.

(c) Biuret Test: To the 2 ml of filtrate add 2 ml of 10% sodium hydroxide solution in a test tube and heated for 10 min, to the above solution, add a drop of 7% of copper sulphate. Formation of violet colour confirms the presence of proteins.

3. Detection of Glycosides [22] : 0.5 g of extract was hydrolyzed with 20 ml of dilute 0.1 N HCL and filtered. The filtrate obtained was used to test the presence of glycosides.

(a) Legal Test : To 1 ml of filtrate add 3 ml of sodium nitropruside, in pyridine and methanolic alkali (KOH) in a test tube. Appearance of blue colour in alkaline layer indicates the presence of glycosides.

(b) Keller-killiani Test: One ml of filtrate was shaken with 1 ml of glacial acetic acid which contains traces of ferric chloride. Add 1 ml of concentrated H_2SO_4 slowly along the sides of the test tubes. Appearance of blue colour in acetic acid layer and red colour at the

junction of the two liquids indicates the presence of glycosides

(c) Modified Borntrager Test: To the 1ml of filtrate add 2 ml of 1% ferric chloride solution in a test tube and heated for about 10 min in boiling water bath. The mixture was cooled and then it was shaken with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Appearance of pink colour in the ammonical layer indicates the presence of glycosides.

4. Detection of Alkaloids [23] : 0.5 g. of extract was taken and it was dissolved in 10 ml of dilute 0.1 N HCL and filtered. The filtrate was used to test the presence of alkaloids.

(a) Dragendorff's Test: To the 2 ml of filtrate, 2-3 drops of Dragendorff's reagent were added. Appearance of reddish brown colored precipitate indicates the presence of alkaloids.

(b) Hager's Test: To the 2 ml of filtrate add Hager's reagent which leads to formation of yellow colored precipitate indicates the presence of alkaloids.

(c) Mayer's Test: To the 2 ml of filtrate, 2-3 drops of Mayer's reagent were added, leads to formation of cream colored precipitate indicates the presence of alkaloids.

(d) Wagner's Test: To the 1 ml of the extract, add 2 ml of Wagner's reagent. Appearance of reddish brown precipitate indicates the presence of alkaloids.

5. Detection of Flavonoids [24]:

(a) Shinoda Test

To the 100 mg of extract in a test tube add few fragments of magnesium metal. To the test tube add 3 to 4 drops of conc. HCL. Formation of magenta colour or light pink colour indicates the presence of flavonoids.

(b) Alkaline Reagent Test: To the 100 mg of extract in a test tube add few drops of NaOH solution. Intense yellow colour is formed to which add few drops of dilute hydrochloric acid, then the yellow colour becomes colourless which indicates the presence of flavonoids.

(c) Fluroscence test: To the 100 mg of extract add 0.3 ml boric acid solution (3 %w/v) and to that add 1 ml oxalic acid solution (10 %w/ v) and evaporated to dryness. The residue obtained was dissolved in ether (10 ml). Under UV light the ethereal layer shows greenish fluorescence which indicates presence of flavanoids.

6. Detection of Phenolic Compounds and Tannins [25]: 100 mg of extract mixed with one ml of water and then it was boiled and filtered. The filtrate was used for the following test.

(a) Ferric Chloride Test: Take 2 ml of filtrate in a test tube to that add two ml of 1% ferric chloride solution. Formation of bluish to black colour indicates the presence of phenolic nucleus.

(b) Lead Acetate Test: To the two ml of filtrate in a test tube add few drops of lead acetate solution. Appearance of yellowish precipitate indicates the presence of tannins.

7. Detection of Fats and Oils [26]:

Oily Spot Test

One drop of the extract was placed on the filter paper and the solvent was allowed to evaporate. Appearance of oily stain on the filter paper indicates the presence of fixed oil.

8. Detection of Saponins [27]:

Foam Test: To 1 ml of extract add 20 ml of distilled water and then it was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of the foam in test tube indicates the presence of saponins.

9. Detection of Phytosterols [28]: To 0.5 g of extract add 10 ml of chloroform and then it was filtered. The filtrate was used to test the presence of phytosterols and triterpenoids.

(a) Libermann's Test: To the 2 ml of filtrate in hot alcohol in a test tube add few drops of acetic anhydride. Formation of brown precipitate indicates the presence of sterols.

Table. 1: Physico-chemical investigation of *Glycyrrhiza glabra*

S.No.	Quality parameters		Results
1	Moisture content		5.8
2	Ash value		
	A	Total ash value	6.2
	B	Acid insoluble ash value	0.8
	C	Water soluble ash value	2.4
3	Extractive values		
	A	Petroleum ether soluble extract	5.7
	B	Methanol soluble extract	6.3
	C	Aqueous soluble extract	7.6

Table. 2: Phyto-Chemical investigation of *Glycyrrhiza glabra*

S.No	Tests	Petroleum ether extract	Methanolic extract	Aqueous extract
1	Carbohydrates	-	+	+
2	Proteins and aminoacids	-	-	-
3	Glycosides	-	-	-
4	Alkaloids	-	+	-
5	Flavonoids	-	+	+
6	Phenolic compounds	-	-	-
7	Tannins	-	-	-
8	Saponins	-	+	+
9	Phytosterols	+	+	+
10	Fats and oils	+	-	-

(b) Salkowski Test: To the 2 ml of extract add few drops of concentrate sulfuric acid d then it was shaken and then allowed to stand. Appearance of red colour in lower layer indicates the presence of sterols.

RESULTS AND DISCUSSION:

Glycyrrhiza glabra was subjected to systematic physicochemical and phytochemical screening by extracting with different organic solvents in the order of increasing polarity to determine the soluble constituents in a given amount of plant material. The present work is helpful in determining the quality and purity of a the crude drug. In this study the parameters used for the evaluation of *Glycyrrhiza glabra* were moisture content, extractive values by different solvents (includes petroleum ether, methanol and water), ash values (total ash, water soluble and acid insoluble ash) (Table 1). On incineration, drugs leave an ash and it consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash value is useful for detecting the exhausted drugs, low-grade products and excess of sandy matter which is applicable to powdered drugs. Phytochemical analysis was performed on the petroleum ether, methanol and aqueous extracts of *Glycyrrhiza glabra*. Petroleum ether extract was found to contain phytosterols, fats and oils. Methanolic extract contains carbohydrates, alkaloids, flavonoids, saponins and phytosterols. Aqueous extract contains carbohydrates, flavonoids saponons and phytosterols (Table 2).

CONCLUSION:

Herbal remedies serve as the important means of therapeutic medical treatment. The people are turning to use medicinal plants and phyto-chemicals in health care. India has one of the oldest cultural traditions of use of its herbal plants since from vedic period. Ayurveda, Unani, Siddha and other traditional systems of medicine are the oldest systems of medicine and utilize vast number of medicinal plants. Phytochemical screening, biological screening of randomly collected plants and phytochemical examination of plants have proved to be

helpful in discovering the new drugs. *Glycyrrhiza glabra,* generally known as Licorice, is very important medicinal plant belonging to family Fabaceae. The present study concluded that the plant *Glycyrrhiza glabra* contains variety of phytoconstituents. The physicochemical evaluation of *Glycyrrhiza glabra* revealed that the standard quality and purity of drug. Phytochemical studies on the extracts of *Glycyrrhiza glabra* showed presence of phytosterols, carbohydrates, flavonoids, fats and oils. This information may be further useful for isolation of various compounds from *Glycyrrhiza glabra* for treatment of diseases in human beings.

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