

**PHARMACOGNOSTICAL ASPECTS OF GLYCYRRHIZA GLABRA****HEMRAJ VASHIST\*, DIKSHA SHARMA**

Department of Pharmacy, L.R.Institute of Pharmacy, Solan (H.P), India, Email: shimla\_pharmacy@rediff.com

Received: 23 July 2013, Revised and Accepted: 13 August 2013

**ABSTRACT**

Objective-Pharmacognostical evaluations like thin layer chromatography, ash value and extractive values, Hydrodistillation, powder microscopy, extraction of ammonium and calcium glycyrrhizinate was performed for licorice powder.

Method- Dried powder of licorice root was extracted with ethanol by soxhlation. Ash values, extractive values and ammonium glycyrrhizinate were obtained by standard procedures.

Results-Total ash value was obtained 9.33% while acid insoluble and water soluble ash was recorded as 2.1 whereas water soluble extractive and alcohol soluble extractive value was calculated as 19.6% w/v and 9% w/v. 3.5% ammonium glycyrrhizinate from 20g powder of licorice and 4% of calcium glycyrrhizinate was obtained from the same amount. The preliminary phytochemical screening as performed in the results saponins, flavonoids, carbohydrates and phytosterols were found to be present.

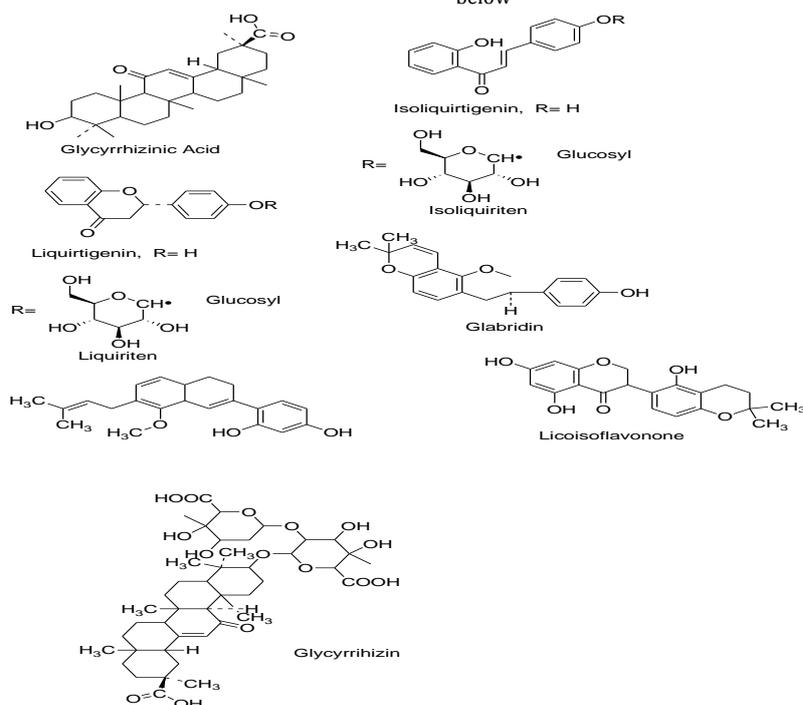
Conclusion-The Preliminary phytochemical, Pharmacognostical study, ammonium glycyrrhizinate and calcium glycyrrhizinate obtained can be used for evaluation of these aspects on licorice.

**Keywords:** Pharmacognostical evaluation, ammonium and calcium glycyrrhizinate, phytochemical screening.

**INTRODUCTION**

The licorice shrub is a member of the pea family and grows in subtropical climates in rich soil to a height of four or five feet. Glycyrrhiza plays important parts in Hindu medicine and is one of the principle drugs of the 'susruta'. In ancient Egypt, Greece and Rome Glycyrrhiza was also frequently used. Liquorice is referred to by Theophrastus. It is interesting to find that even to this day liquorice is maintaining its place in medicine and pharmacy. Licorice continues to be used as a pharmacological agent as well as an ingredient in tobacco and confectionery throughout India in the East and West. Studies over the past 50 years have yielded

information which has prompted new interest in the pharmacological and physiological effects of this plant. This research has revealed that the chemical structure of one of the principle agents in the root of the licorice plant is a glycoside of a triterpene called glycyrrhetic acid. Originally its structure and activity were thought to be similar to adrenal steroid hormones such as aldosterone and cortisol, since ingestion of licorice mimicked hyperaldosteronism and was suggested as a treatment for Addison's disease [1-2]. The drug is rich with various chemical constituents. The list of such constituents is as below-

**Fig. 1: Chemical constituents of licorice**

The layout of pharmacological action in structural form has been depicted below [3]

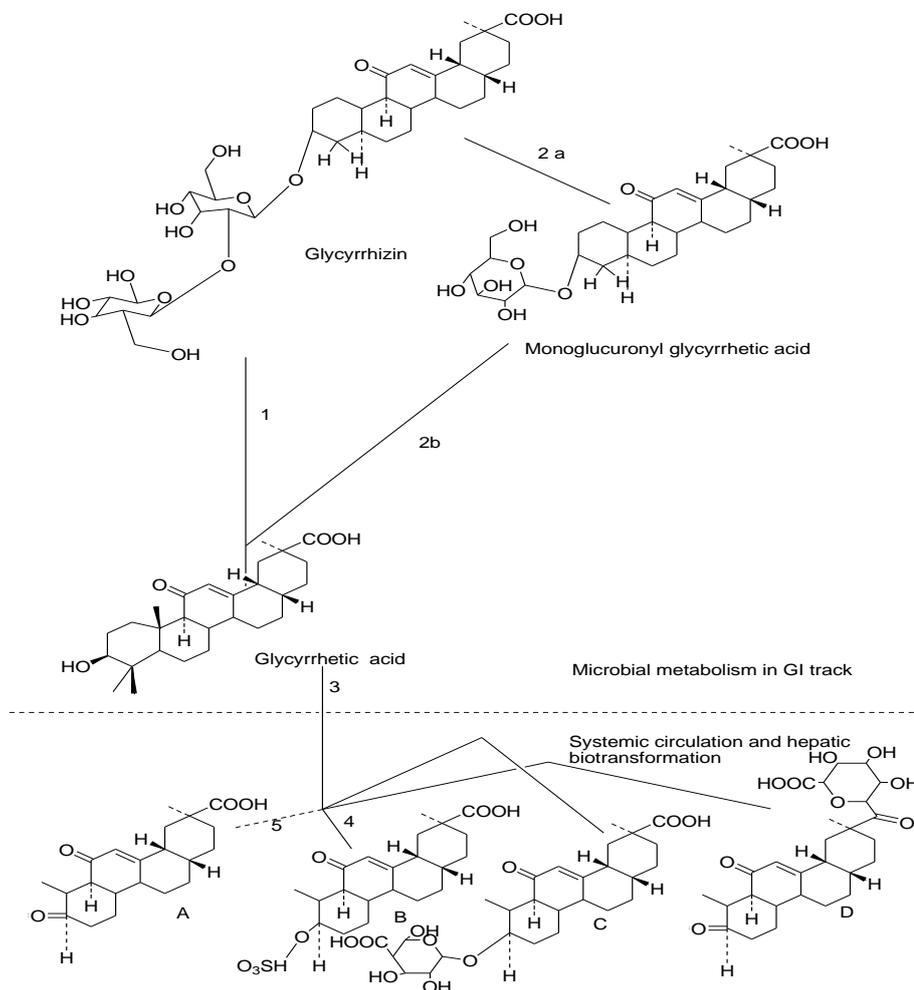


Fig. 2: Pharmacological action of glycyrrhizin [4]

## MATERIAL AND METHODS

### Physicochemical Evaluation of Crude Drug

To determine the different ash values and extractive values of the stolon powder of *Glycyrrhiza glabra* following procedures were used [5-6].

**Determination of ash values.** Determination of ash values are meant for detecting low-grade products, exhausted and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

**Total ash** Accurately about 3 g of air dried powder of stem of *Glycyrrhiza glabra* was weighed in a tarred silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated.

**Acid insoluble ash** Half of the ash obtained from the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

### Water soluble ash

Second half of the ash obtained from total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated. The results are presented in Table no. 1

### Determination of Extractive Values.

#### Alcohol soluble extractive value

5 g of coarsely powdered air-dried drug was macerated with 100 ml of alcohol in a closed flask for 24 hour, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

#### Water soluble extractive value

5 g of coarsely powdered air-dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precautions against loss of chloroform water. 25 ml of the filtrate was evaporated to dryness in tared flat-bottomed dish dried at 105°C and weighed. The results are presented in Table no. 2

### Methods for Extraction

#### Extraction of Plant Material

The extraction of the plant materials was done by hot percolation in soxhlet apparatus.

Preparation of extract -Chemical- i) Ethanol (95% v/v),

ii) Distilled water

**Extraction procedure** -The collected, cleaned and powdered leaves of *G. glabra* was taken for the extraction purpose. 160 g of powder of leaves material was evenly packed in the soxhlet apparatus. It was extracted with solvent ethanol and distilled water. The solvent used is purified before use. The extraction was carried out by hot continuous extraction for about 20 hrs. After extraction, the extract was filtered while hot through whatman filter paper to remove any impurities if present. The extract was concentrated by vacuum distillation to reduce the volume to 1/10. The concentrated extract was transferred to 100 ml beaker and the remaining solvent was evaporated on the water bath, collected and placed in desiccator to remove the excessive moisture. The dried extract was packed in air tight container and used for further studies such as phytochemical screening.

**Preliminary Phytochemical Evaluation** Test for identification of phytoconstituents [7-8]

**Test for alkaloids**-A small portion of the solvent free extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various reagents for the presence of alkaloids like Mayer's reagent (Cream ppt), Hager's reagent (Yellow ppt), Wagner's reagent (Reddish brown ppt) Dragendroff's reagent (Orange brown ppt) (Table.3)

#### Tests for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of Carbohydrates.

#### Molisch's test

Filtrate was treated with 2-3 drops of 1% alcoholic  $\alpha$  - naphthol solution and 2 ml of Conc. sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids showed the presence of carbohydrates. Another portion of the extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's test and Borntrager's test to detect the presence of different glycosides.

#### Legal's test

To the hydrolysate, 1 ml of pyridine and few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color showed the presence of glycosides.

**Borntrager's test** Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

#### Test for phytosterol

The extract was refluxed with solution of Alcoholic potassium hydroxide till complete saponification has taken place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

#### Libermann burchard test

The residue was dissolved in few drops of dil. Acetic acid; 3 ml of acetic anhydride was added followed by few drops of Conc. sulphuric acid. Appearance of bluish green color showed the presence of phytosterol.

#### Test for gums and mucilages

Small quantities of the extract were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of gums and mucilage.

#### Tests for fixed oils and fats

##### Spot test

Small quantities of extract were separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N Alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of Phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap on partial neutralization of alkali indicates the presence of fixed oils and fats.

#### Test for saponins

The ethanolic extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

#### Test for proteins and free amino acids

Small quantities of the extract was dissolved in few ml of water and treated with following reagents.

**Million's reagent**- No appearance of red color showed the absence of protein and free amino acid.

**Ninhydrin reagent**- Shows absence of proteins and free amino acids

**Biuret test** - Shows absence of proteins and free amino acid

#### Test for phenolic compounds and tannins

Small quantities of the extract were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

Dil. Ferric chloride solution (5%) - Violet color.

1% solution of gelatin containing 10% sodium chloride-White ppt

10% lead acetate solution-White ppt.

**Test for flavonoids** With sodium hydroxide solution-Blue to violet color (Anthocyanins) yellow color (flavones), yellow to orange (flavonones)

**With con. Sulphuric acid** Yellow orange color (Anthocyanins) yellow to orange color (flavones) orange to crimson (flavonones)

**Shinoda's test** Small quantities of the extract was dissolved in alcohol, to them piece of magnesium followed by Conc. hydrochloric acid drop wise added and heated. Appearance of magenta color showed the presence of flavonoids. The results are presented in Table no.3

**Isolation of ammonium and calcium Glycyrrhizinate**- Extraction of ammonium Glycyrrhizinate from licorice powder and extraction of calcium glycyrrhizinate was performed.

**Procedure [9]** - Add 50ml acetone to 20g of powdered drug. To this add 2ml of dilute  $\text{HNO}_3$ . Mix thoroughly, cork the flask and macerate for 2 hrs. Shake occasionally and filter the content. Add 20ml of acetone to the marc and warm on water bath and filter again. Combine the filtrate and concentrate preferably under vacuum. To combined acetone extract add quantity of dilute ammonia solution for precipitation of Ammonium glycyrrhizinate.

The precipitates were separated filtration, washed with 5ml of acetone twice. The product is dried and weighed. Percentage yield is calculated.

Same procedure was followed for calcium glycyrrhizinate. After addition of ammonium solution 10% calcium chloride solution is added to get precipitation of calcium glycyrrhizinate. **Microscopy procedure**- A pinch of the powder is taken on the neat and clean glass

slide. Few drops of chloral hydrates were put and warmed on the burner. The Chloral hydrate is added to remove all starch, proteins and other impurities from the powder. Add one drop of glycerin on it to prevent from drying. Observe under compound microscope.

## RESULTS

**Physicochemical analysis** The physicochemical analysis of stem powder was carried out. In this study ash values (total ash, acid insoluble ash and water soluble ash) were determined. The total ash value was found to be 3.5 % w/w indicating the considerable presence of inorganic radicals. The acid insoluble ash value was found to be 4 % w/w. The difference between the total ash value and water soluble ash value indicates that the ash of powder contains considerable amount of inorganic radicals like calcium oxalate which are acid soluble.

**Table 1: The ash values of dried stem powder of *G. glabra***

Plant name	Part used	Type of Ash	% of as % of Ash*
<i>Glycyrrhiza glabra</i>	Stem Powder	Total ash	9.33 % w/w
		Acid insoluble ash	2.1% w/w
Not more than 10% w/w			Obtained
Not more than 10% w/w			

\*Percentage ash value from herbal pharmacopoeia.

### Extractive values

Water soluble and alcohol soluble extractive values were determined. The water soluble and alcohol soluble extractive values were found to be 19.6 w/v and 9 % w/v respectively. The water soluble and alcohol soluble extractive values indicate the presence of more water soluble and more alcohol soluble constituents in the plant.

**Table 2: The extractive values of dried stem powder of *G. glabra***

Sr. No.	Parameters	Extractive values in % w/v	Percentage Extractive value
1.	Alcohol soluble	9%	10
2.	Water soluble	19.6%	20

\*Percentage ash value from herbal pharmacopoeia.

### Phytochemical screening

Preliminary Phytochemical studies of alcoholic extract of stolon of *Glycyrrhiza glabra*.

The alcoholic and distilled water extract of the stolon of *Glycyrrhiza glabra* were subjected to phytochemical screening which reveals that the presence of various pharmacologically active compound

### Isolation of ammonium glycyrrhizinate and calcium glycyrrhizinate

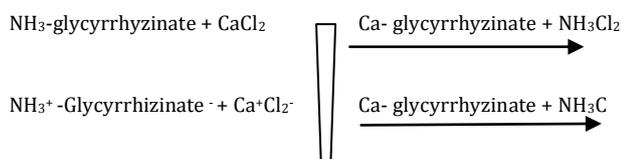
#### Ammonium glycyrrhizinate-

Amount of powder drug taken= 20g

Amount of ammonium glycyrrhizinate obtained= 70mg

Percentage yield=3.5% w/w

**Calcium glycyrrhizinate** - Amount of Ammonium glycyrrhizinate taken = 20gm Amount obtained= 80 mg Percentage yield=4 %



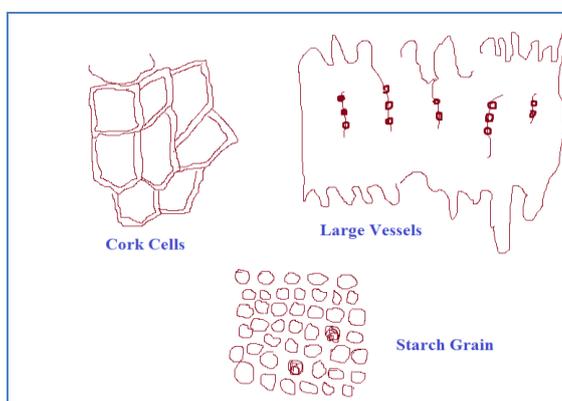
**Table 3: Preliminary phytochemical screening of *G. glabra***

Name of Test	Distilled water Extract	Ethanolic extract
Amino Acid	-	-
Anthraquinon	-	-
Alkaloids	-	-
Tannins	-	-
Flavonoids	+	+
CHB*	+	+
Phenols	-	-
Saponin	+	+
Fats & oil	-	-
Gum and mucilage	-	-
Phytosterols	+	+

+ =Present, - = Absent,\*Carbohydrates

### Microscopic Evaluation-

Microscopy showed clear  
Cork cells  
Large vessels  
Starch grains. (fig-4)



**Fig.4: Powder microscopy of licorice**

## DISCUSSION

The pharmacognostical evaluation shows ash values and Extractive value .Total ash value was reported to be 9.33, acid insoluble ash value 2.1. The standard value for the same has been reported as 10%, 2.5 % respectively.

The alcohol soluble extractive was found to be 9% whereas its value which has been mentioned in herbal pharmacopoeia should be less than 10% by same way the water soluble extractive value was reported as 19.6% and in standard book its value should not be more than 20%.

**Ammonium glycyrrhizinate** was isolated from 20g of the licorice powder. 3.5% of the ammonium glycyrrhizinate was obtained. The experiment was performed to form calcium glycyrrhizinate by following similar procedure as for ammonium glycyrrhizinate the single step changed after addition of ammonia 10% solution of calcium chloride is added. 4% of calcium glycyrrhizinate was obtained. The chemical test was performed which showed the positive result. The end colour after addition of 80% H<sub>2</sub>SO<sub>4</sub> was not intense yellow but a dull orange yellow colour was obtained. When few drops of H<sub>2</sub>SO<sub>4</sub> was added to the calcium glycyrrhizinate solution few fumes evolved but in simple licorice powder fumes were not observed.

Preliminary phytochemical evaluation was performed for ethanolic extract and distilled water extract. The result showed the presence of saponin, carbohydrates and phytosterols in both extracts.

Microscopic examination on 45x showed the presence of Cork cells, large vessels and starch granules.

**CONCLUSION-** The pharmacognostical evaluation of *Glycyrrhiza glabra* was performed and along with this the ammonium and calcium glycyrrhizinate was extracted. These studies could help to researchers further to evaluate such aspects about the plant.

**ACKNOWLEDGEMENT-** We are thankful to LR college of pharmacy, Solan to provide us all lab facilities.

#### REFERENCE-

1. Borst. J.G.G., Ten Holt. S.P., De Vries. L.A. and Moluy-son, J.A. (1953) Lancet ii, 657.
2. Conn. J.W. Rower, D.R. and Cohen, E.L. J. Am. Med. Assoc.1968; 205: 80-84.
3. Isbrucker,RA. Burdock, GA. Risk and safety assessment on the consumption of Licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin.
4. Kataria R. and Hemraj, Singh G, Gupta A, Jalhan S, Jindal A. Pharmacological Activities On *Glycyrrhiza Glabra* –A Review. Asian J Pharm Clin Res, Vol 6, Suppl 1, 2013, 5-7.
5. Ayurvedic pharmacopoeia of India Part-I vol.I, 168-169.
6. Indian Herbal Pharmacopoeia revised new edition 2002. Page 243.
7. Khandelwal, Pawar, Kokate CK, Gokhle. Practical pharmacognosy' Technique an experiments, Nirali Prakashan, pune 3<sup>rd</sup> edition.1996; 1-45.
8. Farnsworth NR. Biological and phytochemical screening of plants. *J. Pharm Sci.*1966; 55: 225-286.
9. Kokate C. K. Practical pharmacognosy. Fourth edition. Vallabh Prakashan, Pitampura, delhi.1994.