



PHARMACOGNOSTICAL, PHYSICOCHEMICAL AND PHYTOCHEMICAL STUDY OF *Glycyrrhiza Glabra* Linn.

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ABSTRACT

Introduction - *Glycyrrhiza glabra* Linn. is a perennial herb of family Leguminosae, used very extensively in Ayurveda classics. It is having property of aphrodisiac, trichogenous, expectorant, haemostatic, intellect promoting etc. **Objective** – Objective of this study to examine the Pharmacognostical and Phytochemical properties of *Glycyrrhiza glabra* Linn. **Material and Method** – Dry sample of *Glycyrrhiza* was collected from Market. The qualitative microscopy, phytochemical screening, physicochemical evolution and TLC studies were carried out according to the standard procedure recommended by API and other standards. **Result** –The sample is having sweetish taste, faint and characteristic odour, externally dark brown in colour and internally golden yellow. Cork, Phellogen and Phelloderm, Secondary phloem, Cambium, Secondary xylem, Medullary rays and Pith are present in microscopic examinations, from outer to inner side. Cork (in surface view), Tangentially cut medullary ray cells, Fragment of vessel, Calcium Oxalate Crystals, Starch, Mucilage were seen in powder microscopy. pH value of test sample was 5.73. **Water Soluble Extractive value** (25.0064%) was more than **Alcohol Soluble Extractive value** (12.7398 %). Total ash value of test sample was **6.131 %**. Acid insoluble ash value was **0.79 %**, Water soluble ash value was **1.819 %**. In TLC, R_f values were found 0.23, 0.58, 0.71 after derivatization with vanillin – Sulphuric acid reagent. Carbohydrate, alkaloid, amino acid, tannin, protein, saponin, glycosides, flavonoids and tannin were present in the sample. **Conclusion** - test sample of *Glycyrrhiza* complies all standard when matches

with the reference standard book. It suggests that test sample is authentic and without any impurities and adulteration.

Keywords - *Glycyrrhiza glabra* Linn., Pharmacognostical study, Microscopy, Macroscopy, Physico-chemical analysis, Phytochemical analysis, Thin Layer Chromatography.

INTRODUCTION

Medicinal plants are being used for centuries for treating a variety of ailments in different traditional systems of medicine. Now a day, global interest has been developed in different traditional health care system as a safer option. Herbal industry is the growing industry as a billion-dollar industry these days due to increasing demand of medicinal plants. As demand is increasing, quality, safety and efficacy are major concerns which come into account. There is a lot of variation in raw plant material due to various reasons, like closely related species, climate variation, seasonal variations, different drying procedures and storage practices. That's why standardization is considered necessary.

Standardization is the process of evaluation of the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and biological observations¹. Standardization starts right from the collection of raw materials upto their clinical application. In case of *Āyurveda* medicines, the therapeutic efficacy is also related to its chemical constituents. The quality and purity refer to the total profile of the drug rather than any of its character. Therefore, a multidimensional approach is essential for standardizing a *Āyurveda* drug.

Glycyrrhiza glabra is well known plant of *Ayurveda* classics and well described as name of *Madhuka*, *Yashtimadhu*, *Madhuyashti* etc. in different *Ayurveda* text. It belongs to the Leguminosae family. *G. glabra* is a typical herbaceous perennial, growing to 1 m in height, presenting pinnate leaves with a length of 7 to 15 cm. The flowers are purple to pale whitish blue, being arranged in a hermaphrodite inflorescence, whereas the fruit is an oblong legume with 2 to 3 cm of length and containing several seeds²

It is distributed in the Sub-tropical and warm temperature regions of the world chiefly in the Mediterranean countries, south Europe, Asia Minor, Egypt, Turkistan, Iran, Siberia, Persia, Arab countries and Afghanistan. In India, it is reported to be cultivated in Baramulla, Srinagar, Jammu, Dehradun and Delhi³.

It is very extensively used in *Ayurveda* for different ailments. *Acharya Charak* added *Yashtimadhu* in 11 *Mahakashayas* among 50 *mahakashayas*⁴. It is the drug, which has been used most frequently in *Mahakashaya* than any other drug. Among 36 groups of drugs (*gana*) of *Sushrut Samhita*, *Madhuka* is included in 8 groups (*Gana*)⁵. In *Ashtanga Hridaya*, *Madhuka* has been included in ten out of thirty-three *Gana*⁶. It expresses that it is a broad-spectrum drug which is used very extensively in *Ayurveda* pharmacology.

The roots are sweet, cold in potency, emetic in large doses, tonic, diuretic, demulcent, mild laxative, aphrodisiac, trichogenous, expectorant, emmenagogue, alexipharmic, haemostatic, alterant and intellect promoting⁷. Other pharmacological actions are Smooth muscle depressant, anti-microbial, hypolipidaemic, antiantherosclerotic, antiviral, hypotensive, hepatoprotective, anti-exudative, spasmolytic, antidiuretic, anti-ulcer, antimutagenic, antipyretic, antioxidant, anti-inflammatory, anti-nociceptive, expectorant⁸.

The aim of this paper is to present an overview of pharmacognostical, physico-chemical and phytochemical investigations carried out on the market sample of plant *Glycyrrhiza glabra* Linn. for proper authentication & identification of the drug and for analyze quality and safety of the drug.

Material and Methods

Collection of Sample

Dry sample of *Madhuka* was purchased from Jaipur crude drug market after proper identification. The

plant material was authenticated from Raw Material Herbarium and Museum, Delhi (RHMD) of National Institute of Science Communication and Information Resources (NISCAIR) with Ref. no. NISCAIR/RHMD/Consult/2016/2977-04.

Macroscopic Study: The collected sample of *Madhuka* studied organoleptically, with naked eye & magnifying lens, with the help of pharmacognostical procedure i.e. appearance, size, shape, colour, and odour and findings were recorded.

Microscopic Study:

Collected Sample of *Glycyrrhiza glabra* Linn was dried that's why it was be soaked in water for overnight, still it was hard, so it was boiled in water for 15-20 minutes for softening. After softening sample was cut into thin sections with the help of blade and were put in water for some time. Then the sections were soaked in safranin stain for 5 min. & then transferred to a watch glass with plain water, so that excess stain gets washed away. The sections were transferred on a glass slide & few drops of glycerin water added on it. With the help of a needle, a cover slip was placed on the section gently and then the slides were observed in microscope & photos were taken.

Powder Microscopy

For examining the characters of the powder, sufficient amount of powder was taken in different chemical reagents then after place on different slide and warmed over a low flame for a short time. A Drop of glycerin was put on the powder on the slide, covered it with the cover slip and observed under the microscope. Chemical reagents used for staining are Safranin, Dilute Ferric chloride, Methylene blue, Eosin aqueous solution and Iodine solution.

Physicochemical Analysis

Assessment of the parameters such as moisture content, foreign matter, ash value, acid insoluble ash, water-soluble extractive, alcohol soluble extractive, and pH was carried out by following standard procedures recommended by Ayurvedic Pharmacopoeia of India and other standard texts^{9, 10, 11, 12}

Phytochemical Analysis

Freshly prepared extracts were tested for the presence of various active phyto compounds. Presence of car-

bohydrates (Molisch's Test, Benedict's test, Barfoed's test, Fehling solution test), alkaloids (Mayer's test, Dragendorff's test, Wagner's Test, Hager's Test), amino acids (Ninhydrin test), proteins (Biuret test, Xanthoprotic test, Millons test), saponins (Foam test), glycosides (Keller – Killiani Test, Borntrager's test), Phenolic Compound, flavonoids (Shinoda test), steroids (Salkowski test) and tannins (Ferric chloride solution, Lead acetate test, Pot. Dichromate test) were tested¹³.

Chromatography

Procedure was done as mentioned in the Quality Standards of Indian Medicinal Plants, Vol. 9¹⁴. which is as follows -

TLC plate and Activation of Plate - T.L.C. plate coated with 0.25 mm layer of silica gel 60 F₂₅₄ with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width). Then, **Activation of pre-coated Silica gel 60 F₂₅₄ was done.** Plates were dried in hot oven at 105⁰ C for one and half hour for activation.

Preparation of mobile solution – Mobile solution was prepared with n-Butanol: Water: Glacial acetic acid in the ratio 7: 2: 1

Preparation of test solution -4 gm powdered drug was extracted with 100 ml of ethanol (90 percent) in a Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

Sample application - Sample was applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate.

Visualization - The plate was sprayed with vanillin-Sulphuric acid reagent and heated at 105° for 5 to 10 min. R_f value and colour of the resolved bands are recorded.

R_f Value- Measured and recorded the distance of each spot from the point of its application and calculated R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

Results

Macroscopic evaluation

In macroscopic examination it is observed that pieces of stolon were cylindrical, straight, variable in length, 1-2 cm in diameter, externally dark brown in colour and internally golden yellow, externally longitudinally

wrinkled, with occasional small buds, encircling scale leaves, transversely smooth, cut surface shows a cambium ring about one-third of radius from outer surface and a small central pith. Odour is faint and characteristic, taste is sweetish.



Figure 1: Crude and Powdered drug used for the study

Microscopic evaluation: T.S. of stolon was circular in outline, showing the following structures –

(As shown in figure no. 1)

Cork – Numerous layers (around 10-20) of tubular cells, outer layers with reddish-brown amorphous contents, inner 3 or 4 rows having thicker, colourless walls.

Phellogen and Pheloderm – Not separately distinguish, 3-5 rows of cells having thicker wall. It may a part of secondary cortex.

Secondary phloem- It is part of secondary cortex, it is abroad band which contains -

- **Phloem Fibers** – About 10-20 fibers are radially arranged, inner cells are cellulosic and outer part of phloem fibers is lignified. Intercellular spaces present.
- **Phloem parenchyma** – Phloem fibers are surrounded by a sheath of parenchyma cells. It may contain calcium oxalate crystals.

- **Medullary rays**

Cambium – Cambium is distinct, form of 3 to 5 layers of cells.

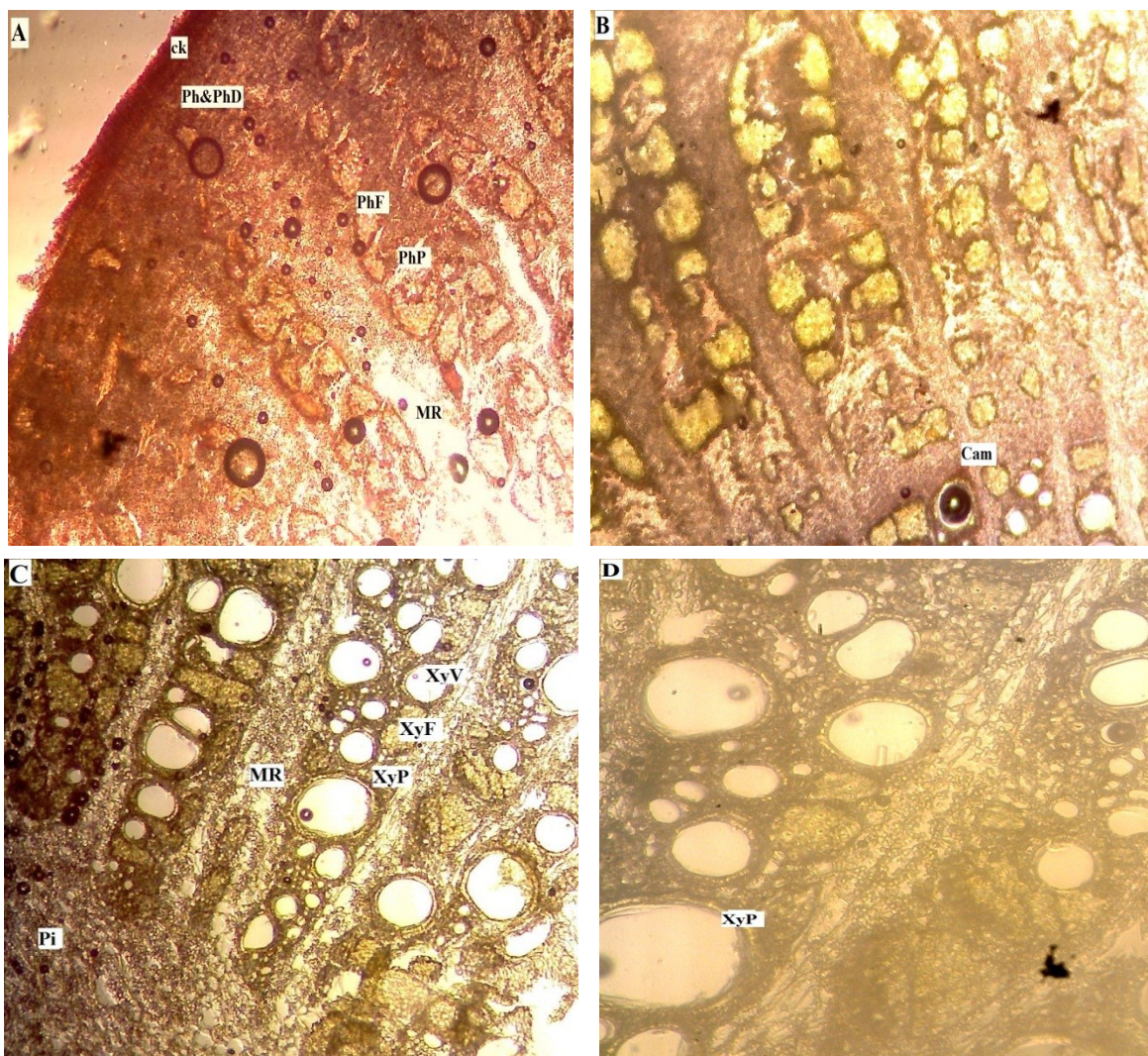
Secondary xylem – It contain -

- **Xylem vessels** – Vessels are round with thick, yellow, pitted, reticulately thickened walls.
- **Xylem fibers** - Lignified with crystal sheaths similar to those of phloem.
- **Xylem parenchyma** - Parenchyma of two kinds, those between the vessels having thick pitted walls without inter-cellular spaces, the remaining with thin walls
- **Medullary rays**

Medullary rays – Distinct, radially arranged 3-5 rows of parenchymatous cells from xylem to phloem, narrow in the xylem region and wider in the phloem region.

Pith – form of thin walled parenchymatous cells with intercellular spaces.

Figure 1a: (A, B, C, D) Microscopy of Stolon of Glycyrrhiza glabra Linn.

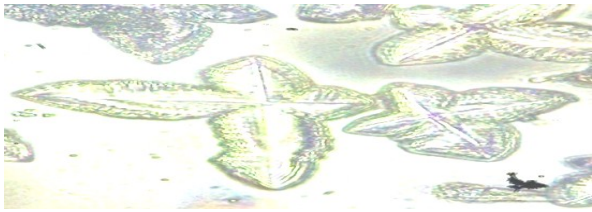
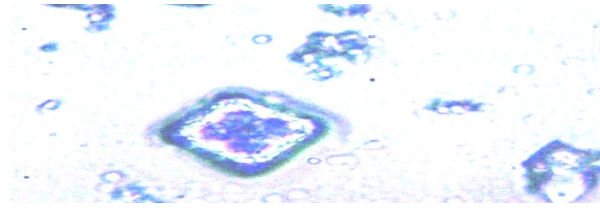
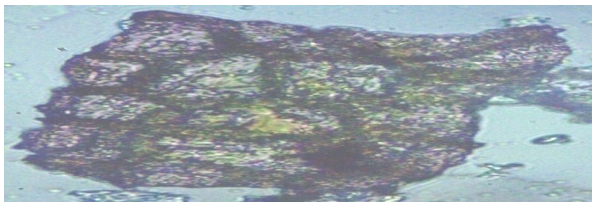
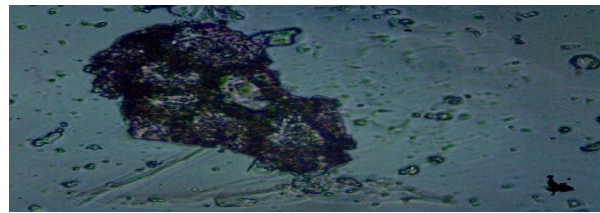
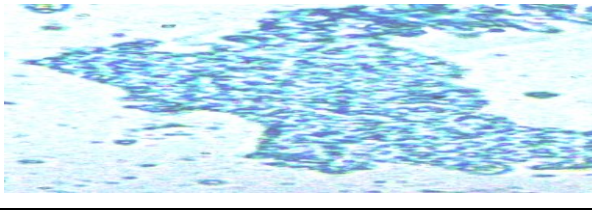
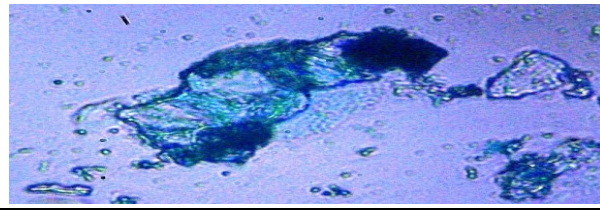


ck - cork, Ph & PhD – Phellogen & Phelloderm, PhF – Phloem Fibers, PhP – Phloem Parenchyma, MR – Medullary Rays, Cam- Cambium, XyV – Xylem Vessels, XyF – Xylem Fibers, XyP – Xylem Parenchyma, Pi – Pith,

Powder microscopy – Powder microscopy of Glycyrrhiza shows presence of Calcium Oxalate

Crystals, starch grains, surface view of cork, tangentially cut medullary ray cells with associated parenchyma, Fragment of vessel with elongated bordered pits, deep blue colour of mucilage. (As shown in figure no. 2)

Figure 2: Powder Microscopy of *Glycyrrhiza glabra* Linn. Powder

	
Calcium Oxalate Crystals	Violet Colour of Starch Stain: Iodine Solution
	
Cork in surface view	Tangentially cut medullary ray cells with associated parenchyma
	
Fragment of vessel with elongated bordered pits	Deep Blue Colour of Mucilage (Stain: Methylene Blue)

Physicochemical analysis: Detailed results of physicochemical analysis are given in Table 1.

Table 1: Physicochemical analysis of *Glycyrrhiza glabra* (stolon) powder

Physicochemical parameter	Result (w/w)
Moisture content	5.525%
pH	5.73
Ash Value	6.131 %
Acid Insoluble Ash	0.79 %
Water Soluble Ash	1.819 %
Water soluble Extractive value	25.0064 %
Alcohol Soluble Extractive value	12.7398 %

Phytochemical analysis: The qualitative phytochemical test revealed presence of carbohydrate, alkaloids, amino acids, proteins, saponins and tannins in aqueous

extract. In alcoholic extract carbohydrate, alkaloids, glycosides, flavonoids and tannins are present. The results of tests performed are shown in Table 2.



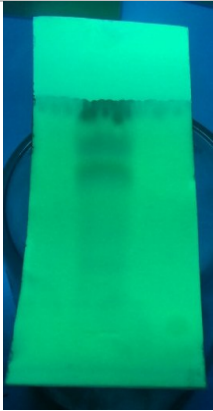
Table 2 Qualitative analysis of *Glycyrrhiza glabra* (stolon) in aqueous and alcoholic extract

Name of Test	Aq. Ext.	Alco. Ext.
Carbohydrate		
Molish's test	-ve	-ve
Benedict test	+ve	+ve

Fehling test	+ve	-ve
Barfoed's test	-ve	-ve
Alkaloids		
Dragendorff test	+ve	-ve
Wagner's test	-ve	+ve
Mayer's test	-ve	+ve
Hager's test	+ve	+ve
Amino acids		
Ninhydrine test	+ve	-ve
Protein		
Biuret test	-ve	-ve
Xanthoproteic test	-ve	-ve
Millon's test	+ve	-ve
Saponin		
Foam test	+ve	-ve
Glycosides		
Keller kiliani test	-ve	+ve
Borntrager's test	-ve	+ve
Phenolic compound		
Phenolic compound	-ve	-ve
Flavonoids		
Shinoda test	-ve	+ve
Steroids		
Salkowski tests	-ve	-ve
Tannins		
FeCl ₃	-ve	-ve
Lead acetate	+ve	+ve
Pot. Dichromate	-ve	-ve

Chromatography: Result of TLC Screening Ethanolic Extract of *Glycyrrhiza glabra* Linn was shown in Table 3.

Table 3: TLC Screening of Ethanolic Extract of *Glycyrrhiza glabra* Linn.

After derivatization by vanillin-sulphuric acid reagent	in UV spectrophotometer (long waves)	in UV spectrophotometer (short waves)
R _f Value – 0.23, 0.58, 0.71	R _f Value– 0.69, 0.80	0.29, 0.64, 0.70, 0.80
		

DISCUSSION

In Macroscopic study, test sample of *madhuka* that is *Glycyrrhiza glabra* Linn. has its peculiar characteristics like sweetish taste, faint and characteristic odour, externally dark brown in colour and internally golden yellow, externally longitudinally wrinkled, with occasional small buds etc. *Glycyrrhiza glabra* Linn. Root was similar, without a pith, fracture, coarsely fibrous in bark and splintery in wood. On the basis of macroscopic characters stolon part is differentiated with root of *Glycyrrhiza glabra* or any other adulterations.

In microscopic study, structures which were found from outer to inner were Cork, Phellogen and Phello-derm, Secondary phloem, Cambium, Secondary xylem, Medullary rays, Pith. These findings match with the standards that are given in Quality Standards of Indian medicinal plants, ICMR, vol. 9. and API, vol. 1.

In **Powder microscopy** structures like cork (in surface view), Tangentially cut medullary ray cells with associated parenchyma, Fragment of vessel with elongated bordered pits were seen. Other than these structures Calcium Oxalate Crystals, Starch, Mucilage were also seen in powder microscopy. These findings match with the standards that are given in Quality Standards of Indian medicinal plants, ICMR, vol. 9. and API vol. 1.

pH value of test sample was 5.73 that shows its acidic nature.

Alcohol Soluble Extractive value of tests ample has been found 12.7398 % and **Water-Soluble Extractive value** has been found 25.0064%, which matches with the standards mentioned in the API indicates the probability of the presence of high water-soluble constituents than the alcohol-soluble constituents.

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water-soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material¹⁵.

Total ash value of test sample is **6.131 %**. Acid insoluble ash value is **0.79 %**, Water soluble ash value is **1.819 %**. These values match with the standards men-

tioned in the API. It is suggested that no impurities in our sample.

Thin layer chromatography is a technique for separation and identification of chemical constituent present in the herbs or chemical mixtures. With mobile solution of n-Butanol: Water: Glacial acetic acid with 7: 2:1 ratio, Ethanolic extract of root of *Glycyrrhizaglabra* Linn. shows R_f values 0.23, 0.58, 0.71 after derivatization with vanillin – Sulphuric acid reagent which are matches with the standard given in Quality Standards of Indian medicinal plants, ICMR, vol. 9. In UV spectrophotometer (long waves) R_f Values – 0.69, 0.80 and in UV spectrophotometer (short waves) R_f Values 0.29, 0.64, 0.70, 0.80

In **phyto-chemical analysis** of test sample, it was reported that carbohydrate, alkaloid, amino acid, tannin, protein, saponin, glycosides, flavonoids and tannin were present in the sample. This means test sample contains both primary & secondary metabolites which are required for plant's growth & are present in a plant when plant is fully grown. It means sample was authentic one.

CONCLUSION

On the basis of these pharmacognostical and phytochemical findings it was observed that the test sample *madhuka* which is used for the study complies all standard when matches with the reference standard book. It suggests that test sample is authentic and without any impurities and adulteration. The result of the present study can be considered as the reference values for similar research works in future.

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