



Research Article

PHARMACOGNOSTICAL EVALUATION AND HPTLC PROFILING OF YASHTIMADHU (*GLYCYRRHIZA GLABRA* LINN.) ROOTS

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ABSTRACT

Glycyrrhiza glabra Linn., known as *Yashtimadhu*, is a highly valued botanical drug in the Ayurvedic system of medicine. Given its widespread clinical use, establishing rigorous quality control parameters is essential to prevent adulteration and ensure therapeutic efficacy. **Aim:** To systematically evaluate the pharmacognostical, physicochemical, phytochemical, and HPTLC fingerprint profiles of *Glycyrrhiza glabra* roots. **Materials and Methods:** The authenticated root samples were evaluated for macroscopic, organoleptic, and microscopic characteristics. Physicochemical parameters, including loss on drying (LOD), ash values, extractive values, and pH, were determined in accordance with the Ayurvedic Pharmacopoeia of India (API) and WHO guidelines. Preliminary phytochemical screening was conducted on aqueous, alcoholic, and hydroalcoholic extracts. High-Performance Thin-Layer Chromatography (HPTLC) profiling of the hydroethanolic extract was performed using a mobile phase of N-butanol:Water:Acetic acid (7:2:1, v/v/v) and scanned at 254nm and 366 nm. **Results:** Macroscopy and transverse sectioning revealed distinct bark, cork, secondary cortex, broad phloem, and secondary xylem zones. Powder microscopy confirmed the presence of calcium oxalate crystals, abundant starch grains, and pitted xylem vessels. Physicochemical analysis and Phytochemical screening confirmed the presence of vital secondary metabolites like saponins, flavonoids, glycosides, and steroids. HPTLC fingerprinting displayed 7 distinct and well-resolved peaks at both 254nm (max R_f 0.992) and 366nm, validating the complex phytochemical matrix. **Conclusion:** The established macro and microscopic markers, physicochemical constants, and distinct HPTLC fingerprints serve as highly reproducible reference standards for the authentication, routine quality control, and standardization of *Glycyrrhiza glabra* roots.

INTRODUCTION

Glycyrrhiza glabra L., Family: Fabaceae^[1] is a small perennial herb, commonly known as licorice globally and *Yashtimadhu* or *Mulethi* in the Indian subcontinent. It is an erect perennial shrub growing to a height of about four feet. Its principal or primary root does not generally grow deep but gives off several long tuberous secondary roots which may reach a length of four feet or more. The shoot system consists of an erect

stem with a limited number of strong herbaceous branches which bear alternate odd-pinnate leaves with five to seven pairs of ovate-oblong entire pale greenish leaflets. It also produces several long, slender, somewhat succulent, stoloniferous underground branches (rhizomes) which spread out in all directions and reach four to six feet in length. The flowers are medium-sized, sessile, purplish-blue, or pale violet and typically papilionaceous, and the fruits are straight, compressed or flattened oblong to linear echinate glandular pods one half to one and a half inches long, containing several kidney-shaped seeds.^[2]

It holds a prominent position in traditional medical systems, particularly in Ayurveda, where its roots and stolons are extensively utilized. According to *Charak Samhita Sutrasthana* chapter 4, *Yashtimadhu* is

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mentioned in 11 *Mahakashaya*, i.e., *Kandughana*, *Varnaya*, *Jeevneeya*, *Shonithsthapan*, *Sandhaniya*, *Kanthaya*, *Snehopag*, *Vamanopag*, *Asthanopag*, *Mutaviranjneeya*, *Angamardprashman mahakashaya*.^[3] Also classified as a *Rasayana* (rejuvenating herb) and *Medhya* (cognitive enhancer), *Yashtimadhu* is traditionally prescribed for a wide array of ailments, including respiratory disorders (*Kasa* and *Shwasa*), gastric ulcers (*Amlapitta*), eye diseases (*Netraroga*), and skin conditions.^[4]

The profound clinical utility of *G. glabra* described in the *Samhitas* is substantiated by its complex phytochemical matrix. The roots are exceptionally rich in diverse secondary metabolites, predominantly triterpenoid saponins most notably glycyrrhizin (glycyrrhizic acid), which imparts the characteristic sweet taste and yields potent anti-inflammatory properties.^[5] Furthermore, the plant contains an array of bioactive flavonoids and isoflavones, such as glabridin, liquiritin, and isoliquiritigenin. Modern pharmacological evaluations have systematically validated these constituents, demonstrating extensive antioxidant, anti-ulcerogenic, antimicrobial, hepatoprotective, and antitussive activities that closely mirror classical Ayurvedic indications.^[6]

Despite its immense clinical and commercial value, the herbal drug industry faces significant challenges regarding the quality, safety, and efficacy of raw botanical materials. The surge in global demand for *Yashtimadhu* often leads to intentional or accidental adulteration and substitution with morphologically similar but therapeutically inferior species. Such malpractices not only compromise the therapeutic outcomes of Ayurvedic formulations but also pose potential health risks to consumers. Therefore, establishing stringent and reproducible quality control parameters for the raw crude drug is a critical necessity.

In light of this, the present study was undertaken to conduct a comprehensive pharmacognostical evaluation of *Glycyrrhiza glabra* Linn. roots. This encompasses detailed macroscopic and microscopic examinations, physicochemical evaluations, and preliminary phytochemical screening. Furthermore, to establish a robust chemical marker profile, High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting was performed.^[7] Together, these parameters aim to provide authoritative, standardized reference data for the routine identification, quality assurance, and prevention of adulteration of *Yashtimadhu (G. glabra L.)* in the herbal pharmaceutical industry.

MATERIAL AND METHODS

Plant Material Collection and Authentication

Glycyrrhiza glabra Linn. (*Yashtimadhu*) root was procured from a local vendor in Delhi and authenticated as a crude drug. The plant sample was taxonomically identified and authenticated by BGIR - Botanical Garden of the Indian Republic, BSI (Botanical Survey of India), Sector 38A, Gautam Budh Nagar, Noida, with Authentication no. BSI/BGIR/1/TECH./2025/279 at. dated: 19-11-2025.

Plant Sample Processing

The whole and powdered drug samples were stored in airtight, light-resistant containers under ambient conditions in accordance with the standards of the Ayurvedic Pharmacopoeia of India. Finely sieved (60# mesh) and coarsely powdered samples were used for powder microscopy, physicochemical evaluation, phytochemical screening, and chromatographic studies following standard procedures.

Pharmacognostical Study

Macroscopy of Plant Material

The root of *G. glabra* L. was examined for morphological features such as structure, shape, size, and texture using unaided vision and a simple microscope (Olympus OIC DM). Organoleptic properties including colour, odour, and taste were assessed systematically through sensory evaluation of the whole plant powder.^[8]

Sectioning of specimen

The root sample was pre-soaked in water and transversely sectioned using a sharp diamond-edged blade. Temporary mounts of the sections were prepared following standard procedures and examined under a digital trinocular compound microscope (Olympus CX21i fitted with a Magcam DC14 camera). Diagnostic features were observed and photomicrographs were recorded.^[9]

Powder microscopy of root of *Yashtimadhu (G. glabra L.)*

Approximately 2gm of finely dried powder, sieved through 80# mesh, was prepared as wet mounts using 50% glycerine and as dry mounts by the smearing method for uniform distribution. The preparations were examined under a Zeiss AxioCam ICc5 microscope at 10× and 40× magnifications, and photomicrographs were recorded to document the diagnostic microscopic features.

Physicochemical Evaluation

Physicochemical evaluation of the root of *Yashtimadhu (G. glabra L.)*, including loss on drying, ash values, and extractive values, was carried out according to the methods prescribed in the Ayurvedic Pharmacopoeia of India and WHO guidelines. Solvent

extractability was assessed using ethanol and water through conventional extraction methods. ^[10]

Determination of Moisture Content (Loss on Drying)

About 5gm powder of *Yashtimadhu* (*G.glabra* L.) roots were taken in three petri dishes respectively to calculate an average. It was then dried at 105°C for 5 hours and weighed after. It was again dried at the same temperature and weighed at one-hour interval until the difference between two consecutive weights corresponded to no difference between them.

Calculations

Weight of the empty petri dish = W1 gm

Weight of the drug sample = X gm

Weight of the petri dish with drug before drying (W3) = (W1+ X)

Weight of petri dish after drying = W2 gm

Loss on drying in % = $[W3-W2/X] \times 100$

Determination of Extractive Values

Determination of Alcohol-soluble extractive

About 5gm powder of *Yashtimadhu* (*G.glabra* L.) roots were weighed and macerated with 100ml of ethanol. It was then kept in a closed flask for twenty-four hours, shaken frequently during first six hours and then allowed to stand for rest of the eighteen hours. It was then filtered rapidly. Precautions were taken against loss of solvent. Then filtrate was evaporated to dryness in a tarred flat-bottomed shallow petri dish, and dried at 105°C, to constant weight and weighed. The percentage of alcohol soluble extractive with reference to the airdried drug was calculated using the formula.

Calculations

Weight of the drug material = X gm

Weight of the empty petri dish = W1 gm

Weight of the petri dish with dried extract = W2 gm

Percentage of extractive value = $[W2-W1/X] \times 100$

Determination of water-soluble extractive

Proceeded as directed for the determination of water-soluble extractive, using water instead of ethanol.

Determination of Ash values

Determination of Total ash

About 2 gm powder of *Yashtimadhu* (*G.glabra* L.) roots were weighed in tarred silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon. It was then cooled under desiccator and weighed. The percentage of ash with reference to the air-dried drug was calculated using the formula. [Fig.3.3]

Calculation

Wt. of empty silica crucible = A1 gm

Wt. of sample (X) = X gm

Wt. of the crucible with ash = A2 gm

Percentage of total ash = $[A2 - A1/X] \times 100$

Determination of Acid-insoluble ash (AIA)

About 25ml of dilute HCl was added to the respective crucibles containing total ash of *Yashtimadhu* (*G.glabra* L.) roots powder separately. Then these crucibles were kept in heating mantle until they start to boil after which the solution was filtered using the ashless filter paper (Whatman 41). The insoluble matter was collected on the filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried in muffle furnace and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 minutes and weighed without delay. The content of acid insoluble ash with reference to the airdried drug was calculated using formula.

Calculation

Wt. of drug sample = X gm

Wt. of crucible = G1 gm

Wt. of crucible with insoluble ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1

Percentage of acid insoluble ash = $G3/X \times 100$

Determination of pH range

About 3gm of *Yashtimadhu* (*G. glabra* L.) root powder was weighed and immersed in 30ml of distilled water. It was then kept in a closed flask and was shaken frequently for five hours and then allowed to stand overnight. It was then filtered into another beaker, and the pH of the resulting suspension was measured at 27°C using a calibrated digital pH meter.

Preliminary Phytochemical Analysis

Test for Saponins

Foam Test- 2.5ml alcoholic/aqueous extract of powder of *Yashtimadhu* (*G.glabra* L.) roots were taken separately in the test tubes and 10ml distilled water was added to them. The mixture was shaken thoroughly and allowed to stand for two minutes. Formation of honeycomb-like froth indicated the presence of saponins and visa-versa.

Test for Tannins

Ferric Chloride Test- 1ml alcoholic/aqueous extract of powder of *Yashtimadhu* (*G.glabra* L.) Roots were taken separately in the test tubes and 5ml distilled water was added to them. Then, few drops of 10% FeCl₃ solution were added to the respective test tubes. A greenish-black or bluish-black precipitate appears, indicating tannins are present.

Test for Glycosides

Keller-Killiani test- 1ml alcoholic/aqueous extract of powder of *Yashtimadhu* (*G.glabra* L.) Roots were taken separately in the test tubes after which the extracts were dissolved in approximately 1 ml of glacial acetic acid, 10% ferric chloride solution, and concentrated sulfuric acid. A reddish-brown colour ring at the junction of two layers indicated the presence of glycosides.

Test for Flavonoids

Alkaline Reagent Test- In separate test tubes containing 1ml of alcoholic/aqueous extract of *Yashtimadhu* (*G.glabra* L.) Roots, a few drops of 20% sodium hydroxide solution were added, resulting in the formation of a yellow precipitate. A few drops of dilute hydrochloric acid make the solution colourless, thereby confirming flavonoid content.

Test for Alkaloids

Dragendorff's Test- 2ml alcoholic/aqueous extract of powder of *Yashtimadhu* (*G.glabra* L.) Roots were taken separately in the test tubes and few drops of Diluted Hydrochloric Acid (dil. HCl) were added to them respectively. The solution formed was then filtered and 1ml of Dragendorff's reagent (potassium bismuth iodide solution) was added to each. Formation of orange precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Molisch's Test- About 1ml of the aqueous/alcoholic extract of *Yashtimadhu* (*G.glabra* L.) Roots were taken in a test tube, to which 2–3 drops of Molisch's reagent were added. Subsequently, concentrated sulphuric acid was carefully poured along the side of the tube to form a separate layer. The formation of a violet ring at the interface indicated the presence of carbohydrates.

Test for Steroids

Salkowski test- 2ml alcoholic/aqueous extract of powder of *Yashtimadhu* (*G.glabra* L.) Roots, were taken separately in the test tubes to which 2ml of chloroform

along with equivalent quantity (2ml) of conc. H_2SO_4 was added, respectively. The formation of the pink/red ring indicated the presence of steroids.

Fingerprint Analysis by High-Performance Thin Layer Chromatography (HPTLC)

High-Performance Thin-Layer Chromatography (HPTLC) analysis was performed for the hydroethanolic extracts of *Yashtimadhu* roots. The filtrate was concentrated to 1gm per 10ml and then subjected to HPTLC profiling. The extract (4 μ L) was spotted on a pre-coated silica gel GF₆₀ 254 aluminium plate using a Camag Linomat V sample applicator equipped with a 100L Hamilton syringe. The application rate was maintained at 150nL/s under a constant flow of inert nitrogen gas. The plate was developed in a pre-saturated twin-trough chamber using a mobile phase saturated with N-butanol: Water: Acetic acid (7:2:1, v/v/v) to a distance of 90 mm, then dried for 5 min in ambient air. After development, a densitometric scan was done with a Camag TLC scanner III in reflectance absorbance mode at UV detection wavelengths of 254 nm and 366 nm using Win CATS Software. (V 1.2.1. CAMAG)

RESULTS

Macroscopic characters

Root is cylindrical, straight, or with bends, elongated, 0.2-1.0cm thick, and with few secondary branches. Root is yellowish-brown, surface smooth with longitudinal cracks in young roots. Mature root surface rough and corky. Cut root circular in outline with light brown colored, thin, corky, bark region. Internal to the cork zone, cut root appeared of three main zones. The outermost zone formed of creamish white zone having alternating reddish-brown rays, followed by inner creamish white porous region alternated with light brown spoke like rays and central circular reddish-brown pith region. (Fig.1)

Table 1: Organoleptic evaluation of *Yashtimadhu* roots

S.No.	Character	<i>Yashtimadhu</i> roots	<i>Yashtimadhu</i> powder
1.	<i>Shabda</i> (Sound)	Fibrous & splintery	-
2.	<i>Sparsha</i> (Touch)	Rough	Smooth / Fine
3.	<i>Roopa</i> (Colour)	Yellowish or pale brown	Yellowish
4.	<i>Rasa</i> (Taste)	Sweet	Sweet & mucilaginous
5.	<i>Gandha</i> (Odor)	Faint, characteristic	Sweet smell

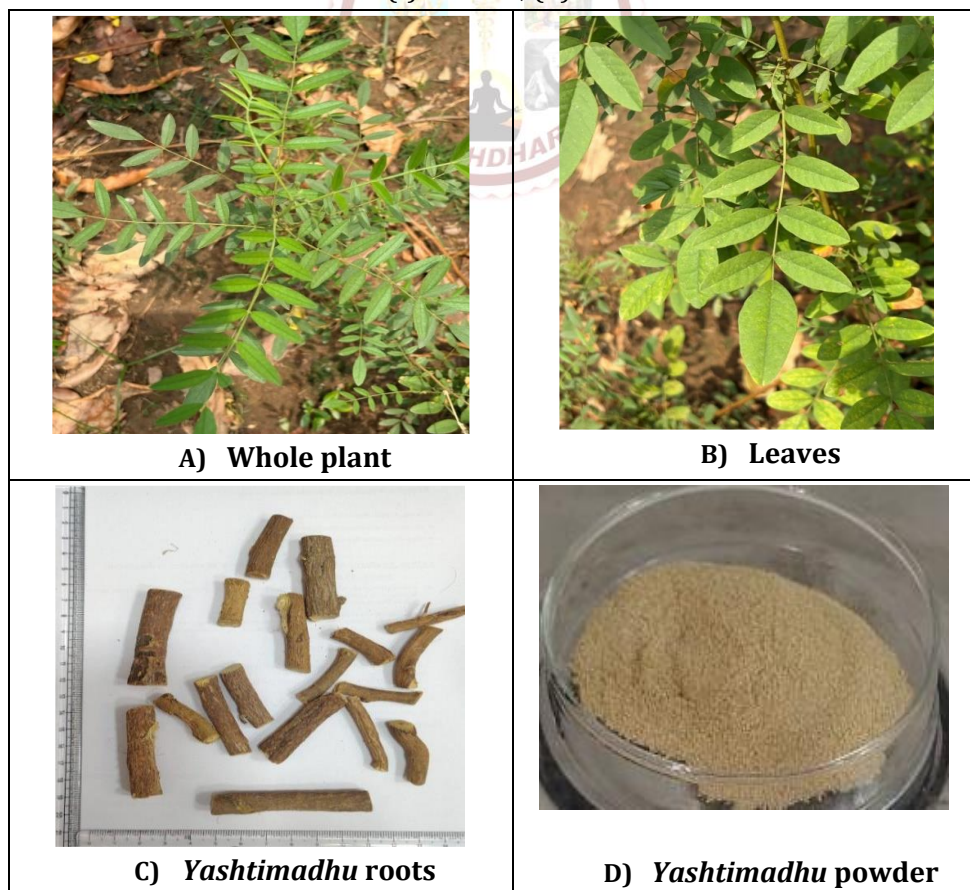
Table 2: Physicochemical parameters of Yashtimadhu (*Glycyrrhiza glabra* Linn.) roots

S.No.	Test	Observations
1.	Foreign matter	Nil
2.	Moisture content (LOD)	5.0%
3.	Extractive value (alcohol soluble)	34.5%w/w
4.	Extractive value (water-soluble)	23.5%w/w
5.	Total ash	5.57%w/w
6.	Acid insoluble ash	0.72%w/w
7.	pH value	5.4

Table 3: Phytochemical analysis of Yashtimadhu (*Glycyrrhiza glabra* Linn.) roots

S.No.	Phytochemical	Aqueous extract	Alcohol extract	Hydroalcoholic extract
1.	Alkaloids	-	-	-
2.	Saponins	+	+	+
3.	Tannins	+	-	-
4.	Glycosides	-	+	+
5.	Proteins	-	+	-
6.	Flavonoids	+	+	+
7.	Carbohydrates	+	-	+
8.	Phenols	-	+	+
9.	Steroids	+	+	+

Note: (-): Absent, (+): Present

**Figure 1: Yashtimadhu (*Glycyrrhiza glabra* Linn.)**

Microscopic characters

Transverse section of *Yashtimadhu* (*G.glabra* L.) Roots

The microscopic transverse section of the root is characterised by the outermost region being the periderm, multi-layered phellem (cork), the phellogen (cork cambium), and the phelloderm (secondary cortex). Beneath this lies the broad secondary phloem, which contains sieve elements, phloem parenchyma, and distinct groups of phloem fibres. A continuous ring of vascular cambium separates the phloem from the expansive secondary xylem, which is made up of large xylem vessels, xylem parenchyma, and xylem fibres. the xylem and phloem regions, there is the presence of crystal sheaths surrounding the lignified fibres to form distinctive crystal fibres. Multiseriate medullary rays intersect the secondary vascular tissues, appearing narrow in the xylem before characteristically expanding in the phloem, and are associated with starch grains and prismatic calcium oxalate crystals. (Fig.2)

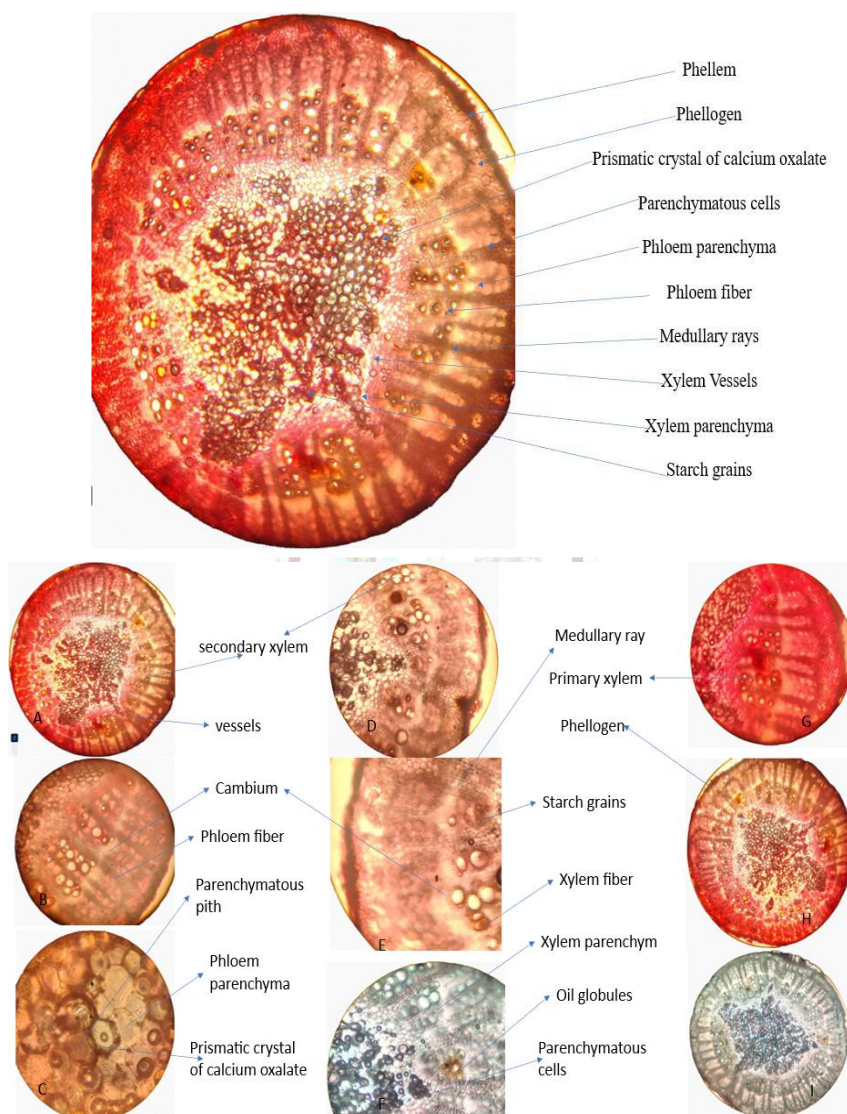


Figure 2: T.S. of *Yashtimadhu* (*G.glabra* L.) showing at 10x & 40x

Powder Microscopy of *Yashtimadhu* (*G.glabra* L.) Roots

The powdered of *Yashtimadhu* roots was examined under the microscope at 40X magnification after staining with safranin. The diagnostic characters revealed the presence of few prismatic crystals, few golden-brown fragments, fragments of pitted xylem vessels with reticulate thickenings, cork cells, parenchyma cells with starch grains, prismatic crystals, and abundant spheroidal to elongated starch grains. Additionally, pigmented cells were recorded, which contribute to the characteristic appearance of the *Yashtimadhu* roots powder. Prominent phloem fibers were found along with rosette crystals of calcium oxalate embedded in the parenchymatous matrix. (Fig.3)

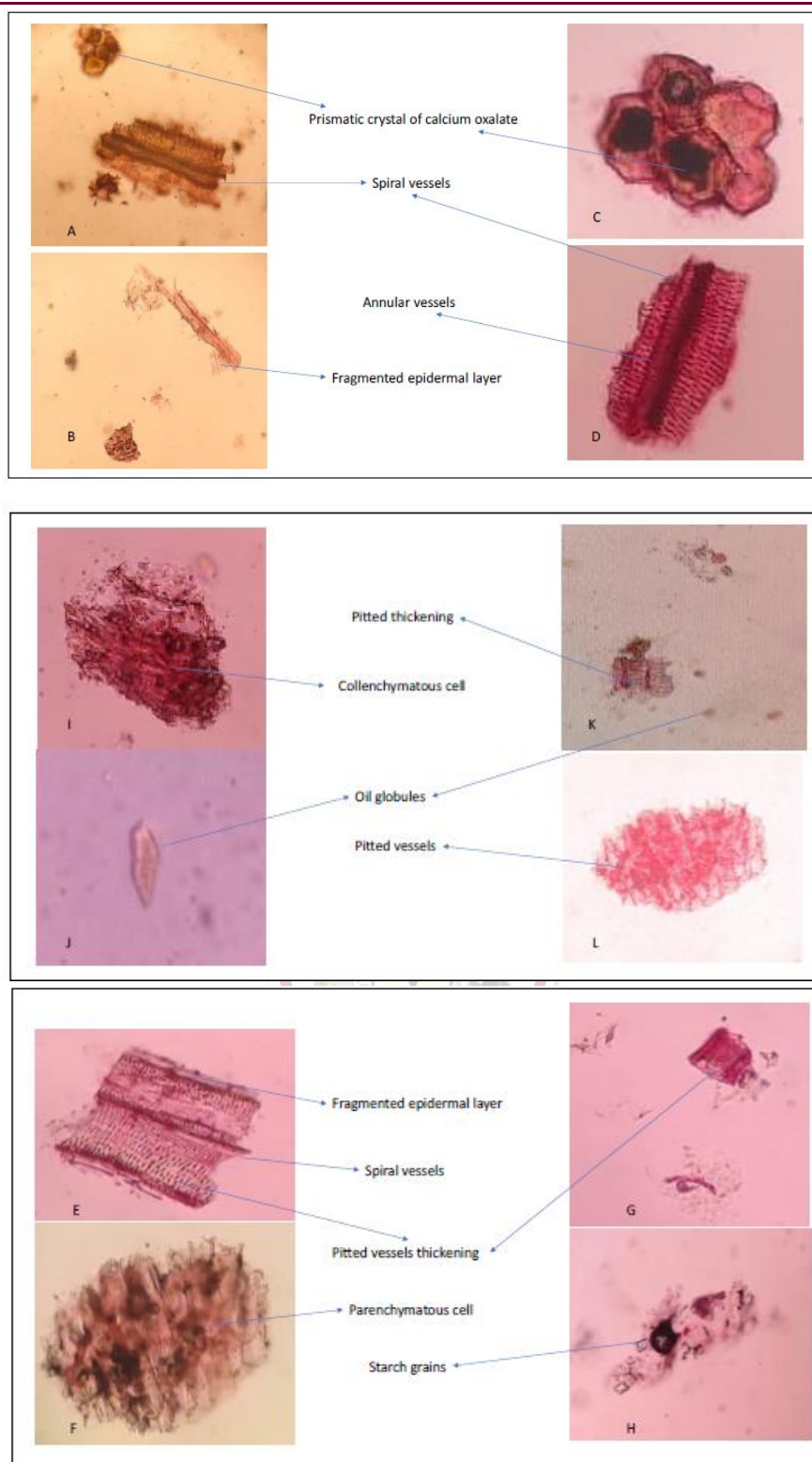


Figure 3: Powder microscopy of Yashtimadhu (*Glycyrrhiza glabra* Linn.) roots powder stained in safranine at 40X showing

HPTLC Analysis of Yashtimadhu (*Glycyrrhiza glabra* Linn.) roots

HPTLC analysis of Yashtimadhu (*Glycyrrhiza glabra* Linn.) extract was performed using pre-coated silica gel 60 F₂₅₄ plates. The sample was applied using an automatic applicator, and the plates were developed in a saturated chamber using an optimized mobile phase before being scanned at 254nm and

366nm using a TLC scanner (Fig.4). The HPTLC fingerprint profile of the extract revealed the presence of multiple phytoconstituents at both wavelengths, confirming the chemical complexity of the botanical drug.

At 254nm, the chromatogram showed a total of 7 distinct peaks, indicating the presence of various secondary metabolites. These phytoconstituents were well-resolved at specific R_f values of 0.36, 0.40, 0.54, 0.71, 0.87, 0.95, and 0.99. The clear separation and distribution of these peaks confirm the presence of several major and minor UV-absorbing compounds within the extract. (Fig.5)

At 366nm, the chromatogram similarly exhibited 7 fluorescent peaks, highlighting the presence of distinct UV-active compounds. These bands were observed at R_f values of 0.04, 0.40, 0.46, 0.53, 0.68, 0.85, and 0.99. The prominent peaks observed under this wavelength may be attributed to triterpenoid saponins or flavonoid-type

phytoconstituents characteristic of the plant. Previous phytochemical studies on *Glycyrrhiza glabra* have reported the abundant presence of bioactive compounds such as glycyrrhizin, liquiritin, and glabridin, which could contribute to this observed chromatographic profile; however, further confirmation using standard marker compounds is required. (Fig.6)

Overall, the HPTLC analysis confirms the presence of multiple bioactive constituents. The developed fingerprint profile, mapped to these precise R_f values, can serve as a highly reliable reference standard for the identification, standardization, and rigorous quality control of *Yashtimadhu* (*G.glabra* L.) Roots.

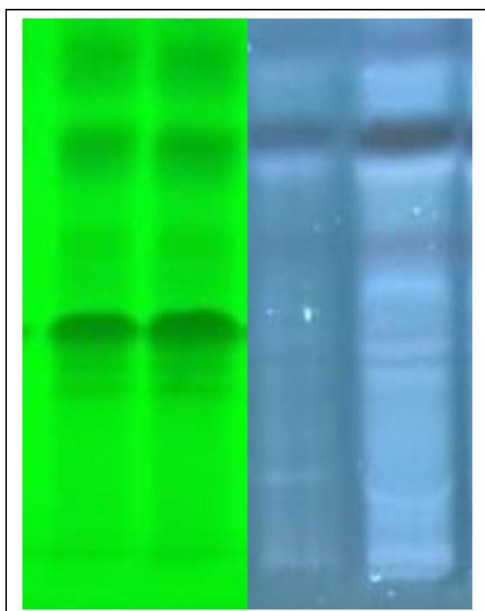


Figure 4: Photography of HPTLC plate *Yashtimadhu* roots visualization at 254 nm and at 366nm

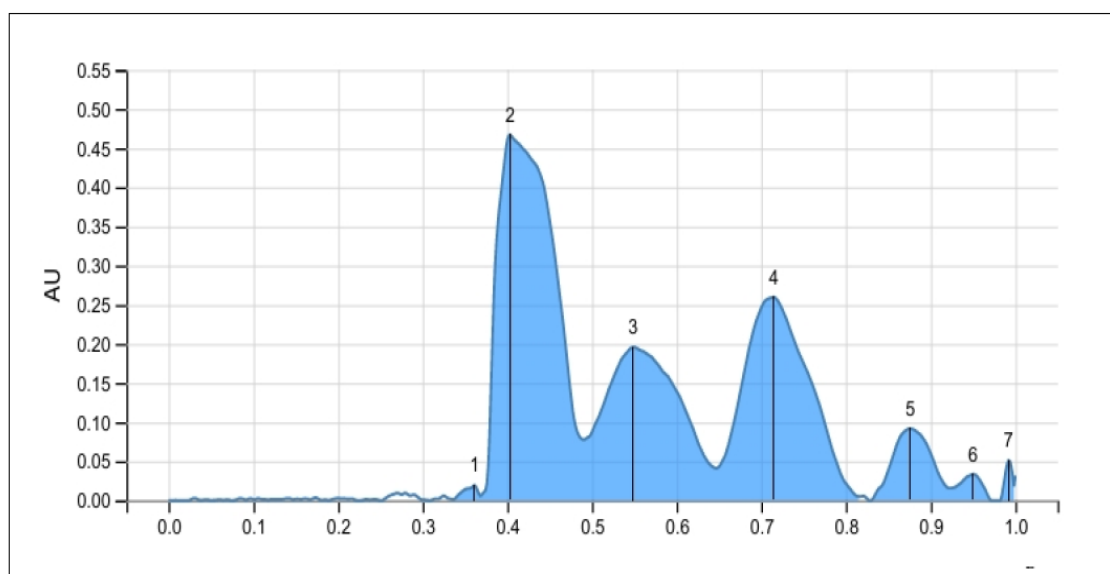


Figure 5: Chromatogram at 254 nm of *Yashtimadhu* roots

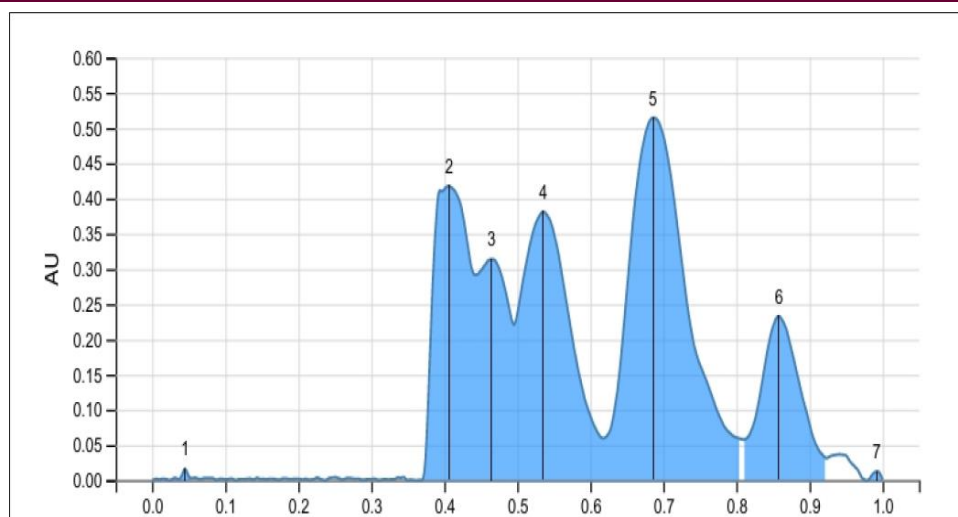


Figure 6: Fig. 4.1.8: Chromatogram at 366 nm of Yashtimadhu roots

CONCLUSION

The present study establishes a comprehensive pharmacognostical and phytochemical profile for the roots of *Glycyrrhiza glabra* Linn. (*Yashtimadhu*). Diagnostic microscopic features such as specific calcium oxalate prisms, distinct medullary rays, thick pitted xylem vessels, and abundant starch grains provide factual primary markers for accurate botanical identification. Phytochemical screening confirms a robust presence of potent secondary metabolites, particularly saponins, flavonoids, glycosides, and steroids, which are the fundamental drivers behind licorice's highly prized anti-inflammatory, antiviral, and anti-ulcerogenic therapeutic benefits. This chemical complexity is quantitatively validated through HPTLC analysis, producing a reproducible chromatographic fingerprint comprising 7 distinct peaks at both 254 nm and 366 nm.

Moving forward, the macro-microscopic data and documented R_f values compiled in this article serve as definitive reference standards with several practical future applications. Primarily, they provide the herbal pharmaceutical industry with a framework for routine quality control, rapid authentication of raw materials, and the accurate detection of adulterants in crude or powdered drugs. Furthermore, the established HPTLC fingerprinting offers a foundational baseline for future pharmacological research aimed at identifying and isolating targeted bioactive compounds, such as glycyrrhizin and glabridin,

thereby ensuring the safety, purity, and clinical efficacy of commercial Ayurvedic formulations.

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