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Research Article

STANDARDIZATION AND HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHIC FINGERPRINT PROFILING OF *MANJISHTHA* (RUBIA CORDIFOLIA L.) ROOTS

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ABSTRACT

In Ayurvedic medicine, *Manjishtha* (Rubia cordifolia L.), a perennial climbing herb, is well recognized for its *Raktashodhana* (blood-cleansing) activity and its therapeutic use in *Kuṣṭha* (skin disorders), *Vraṇa* (wounds), and *Prameha* (urinary ailments). Its roots contain anthraquinones responsible for diverse pharmacological effects. **Objective:** To evaluate the pharmacognostic, physicochemical, and phytochemical properties of *Manjishtha* roots and develop a High-Performance Thin Layer Chromatographic (HPTLC) fingerprint for authentication and standardization. **Methods:** Collected roots were authenticated and were analysed as per Ayurvedic Pharmacopoeia standards through macroscopic, transverse and powder microscopic examinations. An analysis of physicochemical parameters such as moisture loss, ash values, solvent extractives, and pH was carried out. Alcoholic and aqueous extracts were screened for phytochemicals. High-Performance Thin Layer Chromatographic profiling was performed on silica gel GF 60 254 plates using Toluene: Ethyl Acetate: Formic Acid (7:3:1), scanned densitometrically at 254 and 366nm. **Results:** Roots were cylindrical, reddish-brown, and brittle, with microscopic features showing cork, cambium, phloem, xylem, and pith. Powder microscopy showed the presence of xylem elements, phloem fibres, lignified vessels and fibres. Physicochemical analysis indicated 0.39% foreign matter, 10% total ash, 24.53% alcohol-soluble extractives, 10.70% water-soluble extractives, and pH 6. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, phenol and steroids. The HPTLC chromatogram revealed 12 bands (Rf 0.006–0.958) at 254nm and 11 bands (Rf 0.006–0.976) at 366nm. **Conclusion:** The study establishes comprehensive pharmacognostic, physicochemical, and HPTLC data for *Manjishtha* (*Rubia cordifolia* L.) root, providing a valuable standard for identification and future research applications.

INTRODUCTION

Manjishtha (Rubia cordifolia L.), a medicinal plant of the Rubiaceae family, is a perennial climbing herb commonly known as Indian Madder. In Ayurvedic medicine, it is widely recognized for its therapeutic applications and is also known by several regional names, including *Manjitha*, *Manjit*, and *Majith* in Hindi; *Manjistha*, *Manjith*, and *Manjit* in Bengali; *Manjihtha* and *Manjeshta* in Gujarati; *Manjishth* in Marathi; *Manjiste* and *Citravalli* in Kannada; *Poont* in Malayalam; and *Manjatte*, *Manjitti*, *Shevelli*, and *Sevvelli* in Tamil.[1] This perennial herb is further distinguished by its slender, four-angled climbing stems, occasionally bearing fine prickles, and by leaves arranged in whorls of four, with two leaves typically larger than the others. The lanceolate to ovate leaves exhibit prominent venation, while small, pale yellow to greenish-white flowers occur in clustered inflorescences [Fig 1] and mature into red to black berries. The plant's long, cylindrical, reddish roots have historically served as a natural source of red dye. Native to the Indian subcontinent, it grows at

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elevations up to 3750m in the Himalayas and is widely distributed across tropical Asia including China, Japan, and Southeast Asia as well as parts of Africa and Australia. [2]

In classical texts, it is extensively described as a potent *Raktashodhaka* indicated in conditions arising from vitiated *Pitta* and *Rakta doṣas*. It is traditionally prescribed for *Kuṣṭha*, *Vraṇa*, *Prameha*, *Raktapradara* (menorrhagia), and as a *Garbhashaya-sankochaka* (uterine contractive) owing to its detoxifying, astringent, and haemostatic actions. It is also documented in Siddha and Unani medicine for rheumatism, jaundice, urinary disorders, and as a natural dye source.[3] *Manjishtha* demonstrates broad pharmacological potential, including antioxidant, immunomodulatory, anti-inflammatory, and anti-PAF effects. Its solvent-free alcoholic extract inhibits lipid peroxidation and preserves glutathione levels,[4] while rubiadin, a dihydroxyanthraquinone, shows superior dose-dependent antioxidant activity compared to standard agents.[5] The alcoholic extract hydrogel promotes wound contraction, accelerates closure, enhances tissue regeneration,[6] and suppresses *P. acnes*-induced ROS and pro-inflammatory cytokines. [7]

Members of the Rubiaceae family, particularly *R. cordifolia*, are rich in root-derived anthraquinones. Key constituents include purpurin, manjistin, alizarin, mollugin, lucidin primeveroside, ruberythric acid derivatives, and various methylated or glycosylated anthraquinones, along with naphthohydroquinones and their dimers.[1] This study aims to investigate the pharmacognostic features of the plant through powder microscopy and transverse section examination, alongside establishing its chemical profile by phytochemical screening, physicochemical evaluation, and HPTLC fingerprinting.

MATERIAL AND METHODS

Plant Materials Collection and Authentication

Roots of *Manjishtha* (*Rubia cordifolia* L.) were procured from a local vendor in Medak, Telangana. Herbarium specimen was prepared for identification and authentication of drug. The plant sample were taxonomically identified and authenticated by BGIR-Botanical Garden of Indian Republic, BSI (Botanical Survey of India), Sector 38A, Gautam Budh Nagar, Noida with Authentication no. BSI/BGIR/1/TECH./2024/159 at. dated: 9-12-2024.

Plant Sample Processing

The whole and powdered drug samples were kept in sealed, light-protected containers under ambient conditions, as per the standards outlined in the Ayurvedic Pharmacopoeia of India. [8] Finely sieved (60# mesh) and coarsely ground samples were employed for powder microscopy, physicochemical evaluation, phytochemical screening, and chromatographic analysis as per standard protocols.

Pharmacognostical Study

Macroscopy of Plant Material

The root of *R. cordifolia* was examined for morphological features such as structure, shape, size, texture using unaided vision and a simple microscope (Olympus OIC DM), while its organoleptic properties such as colour, odour, taste were systematically evaluated through sensory analysis. [9,10]

Transverse Section Microscopy

The root sample was pre-soaked in water before being transversely sectioned using a sharp diamond-edged blade. Multiple temporary mounts of the sections were prepared and examined under a digital trinocular compound microscope (Olympus CX21i equipped with a Magcam DC14 camera) and photomicrographs were subsequently recorded for the diagnostic features. [9,10]

Powder Microscopy of Plant Material

Finely dried powdered root sample approximately 2 g, sieved through 80# mesh, was mounted as both wet (with 50% glycerine) and dry preparations using a smearing technique for uniform distribution. These mounts were examined under a Zeiss AxioCam ICc5 microscope at 10X, and 40X magnifications, and photomicrographs were captured to document the diagnostic microscopic features.[9,10]

Physicochemical Evaluation

Physicochemical evaluation of R. cordifolia root, including loss on drying, ash values, and extractive content, was performed in accordance with the procedures described in the Ayurvedic Pharmacopoeia of India and WHO guidelines. [11,12] Solvent extractability was evaluated using ethanol and water, employing conventional extraction techniques.

Determination of Moisture Content (Loss on Drying)

This procedure estimates the proportion of volatile matter, primarily the moisture content, present in the drug. About 5g each of powder of *R. cordifolia* root 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. The percentage of loss on drying with reference to the airdried drug was calculated using the formula

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] × 100

Determination of Extractive Values

- **Determination of Alcohol-Soluble Extractive**

Approx 10gm each of powder of *R. cordifolia* root 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.

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] × 100

- **Determination of Water-soluble Extractive**

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

Determination of Ash Values

- **Determination of Total Ash**

Approximately 2 g of *R. cordifolia* root powder was accurately weighed into a pre-weighed silica crucible and incinerated at the required temperature. 500-600°C until free from carbon. It was then cooled under desiccator and weighed. The ash content was determined as a percentage of the air-dried drug using the standard formula.

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] × 100

- **Determination of Acid-Insoluble Ash (AIA)**

Each crucible containing the total ash of *R. cordifolia* root was treated with 25ml of dilute HCl. 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. After collecting the insoluble matter on filter paper, it was transferred to the same crucible, dried in the muffle furnace, and subjected to ignition until a stable weight was obtained. The residue was cooled in a desiccator for 30 minutes, weighed immediately, and the percentage of acid-insoluble ash relative to the air-dried sample was calculated using the standard formula.

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 × 100

Determination of pH range

Approx. 3gm each of powder of *R. cordifolia* root were 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 formulation was determined using a pH paper by noticing the colour change.

Preliminary Phytochemical Analysis

Approximately 5g of powdered *R. cordifolia* root was taken in conical flask, and solvents (ethanol and distilled water) were added in a 1:10 ratio (50mL per sample). The samples were subjected to rotary shaking for six hours, allowed to settle, and subsequently passed through Whatman filter paper to yield the extracts. The filtrate was taken for screening of phytochemicals by using standard chemical tests. [13] [14]

Test for Alkaloids

Dragendroff's Test – 2ml alcoholic/aqueous extract of powder of *R. cordifolia* root 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 Dragendroff's reagent (Potassium bismuth Iodide Solution) was added to each. Formation of orange precipitate indicates the presence of Alkaloids.

Test for Saponins

Foam Test– 2.5ml alcoholic/aqueous extract of powder of *R. cordifolia* root were taken separately in the test tubes and 10 ml 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 *R. cordifolia* root 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 *R. cordifolia* root 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 R. cordifolia root, 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 Carbohydrates

Molisch's Test- About 1ml of the aqueous/alcoholic extract of *R. cordifolia* root was 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 Phenols

Ferric Chloride Test- A few drops of 5% ferric chloride solution were added to approximately 1ml of aqueous or alcoholic extract of R. cordifolia root. A bluish-green to deep blue color appeared, indicating the presence of phenolic compounds.

Test for Steroids

Salkowski test– 2ml alcoholic/aqueous extract of powder of *R. cordifolia* root, were taken separately in the test tubes to which 2ml of chloroform along with equivalent quantity (2ml) of conc. H2SO4 was added, respectively. The formation of the pink/red ring indicated the presence of steroids.

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

The filtrate was concentrated to 1 g per 10ml and then subjected to HPTLC profiling. The extract (4µL) was spotted on a pre-coated silica gel GF 60 254 aluminium plate using a Camag Linomat V sample applicator equipped with a 100 L Hamilton syringe. The plate was developed in a pre-saturated twin trough chamber using the mobile phase as Toluene, Ethyl Acetate, Formic Acid (7:3:1) (v/v/v) to a distance of 90 mm, 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: The roots of *R. cordifolia* are cylindrical in shape and are often topped with a knotty crown derived from the root stock. The size ranges from 2 to 9cm in length and 0.2 to 0.6cm in width. The surface is typically smooth, featuring fine longitudinal striations and occasional grooves, with noticeable scars from detached lateral roots. Both the outer and inner surfaces present a dark reddish-brown coloration. On breaking, the root shows a short, brittle fracture. [Table 1; Fig 2]

Table 1: Organoleptic features of *Manjishtha* (*Rubia cordifolia* L.) Root and its Powder

S.No.	Character	*Manjishtha* Root	Root Powder
1.	*Shabda* (Sound)	-	-
2.	*Sparsha* (Touch)	Smooth	Smooth/fine
3.	*Roopa* (Colour)	Dark reddish brown (Both internally and externally)	Reddish brown
4.	*Rasa* (taste)	*Tikta* (Bitter)	*Tikta* (Bitter)
5.	*Gandha* (Odor)	None	None

Transverse Section microscopic view of *Manjishtha* (*Rubia cordifolia* L.) Root

Under the microscope, the transverse section (T.S.) of *Manjishtha* root shows a cylindrical and flexuous structure with a smooth, reddish surface. The outermost layer consists of 5 to 7 layers of cork tissue, occasionally containing tannin. The phellogen is not clearly distinguishable. The secondary cortex is made up of thin-walled, polygonal cells that appear red. The secondary xylem forms a continuous reddish cylinder and primarily consists of vessels and tracheid. The vessels are numerous and uniformly distributed. The secondary phloem appears as a broad reddish region, consisting of thin-walled sieve elements and phloem parenchyma, while phloem fibres are absent. The cambium can be observed distinctly and is characterized by the absence of medullary rays. The overall reddish coloration throughout the root structure indicates the presence of anthraquinone compounds. [Fig. 3.]

Powder Microscopy

Fine powder of root appears dark reddish-brown in colour and exhibits numerous fragments of cork. Powder microscopic examination of *Manjishtha* roots revealed lignified xylem vessels, xylem fibres, phloem fibres,

scalariform vessels, pitted vessels, and other fibres with distinct thickenings. Xylem parenchyma contained red-coloured inclusions. These features serve as reliable diagnostic markers for the identification and standardization of the plant material [Fig. 4].

Physicochemical Parameters

The root powder was analysed for foreign matter, loss on drying, total ash, acid-insoluble ash, pH, and water and alcohol extractive values. [Table 2]. All tests were performed using the standard protocols recommended by the Ayurvedic Pharmacopoeia of India and the WHO. [11,12]

Table 2: Physicochemical observations of *Manjishtha* (*Rubia cordifolia* L.) Root

S.No.	Test	Observations
1.	Foreign matter	0.39%
2.	Extractive value (alcohol soluble)	24.53%
3.	Extractive value (water-soluble)	10.70%
4.	Total ash	10%
5.	Acid insoluble ash	0.56%
6.	Moisture content (LOD)	9.85%
7.	pH value	6

Preliminary Phytochemical Analysis

Aqueous and ethanolic extracts, prepared as outlined in the preceding section, were subjected to preliminary phytochemical screening using approximately 2ml of each extract for individual tests. The findings are presented in Table 3.

Table 3: Phytochemical Observations of *Manjishtha* (*Rubia cordifolia* Linn.) Root

S.No.	Phytochemical	Alcohol extract	Aqueous extract
1.	Alkaloids- Mayer's test (in dil. HCl)	+	+
2.	Saponins- Foam test	+	-
3.	Tannins- $FeCl_3$ test	+	+
4.	Glycosides- Keller–Killiani test	+	+
5.	Flavonoids- Alkaline Reagent Test	+	+
6.	Carbohydrates- Molisch's test	+	+
7.	Phenols- $FeCl_3$ test	+	+
8.	Steroids- Salkowski Test	+	-

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

FIGURES

Fig 1. Flowering Twig of *Manjishtha* (*Rubia cordifolia* L.)

Fig. 2 A. Crude *Manjishtha (Rubia cordifolia L.)*. root B. Root powder

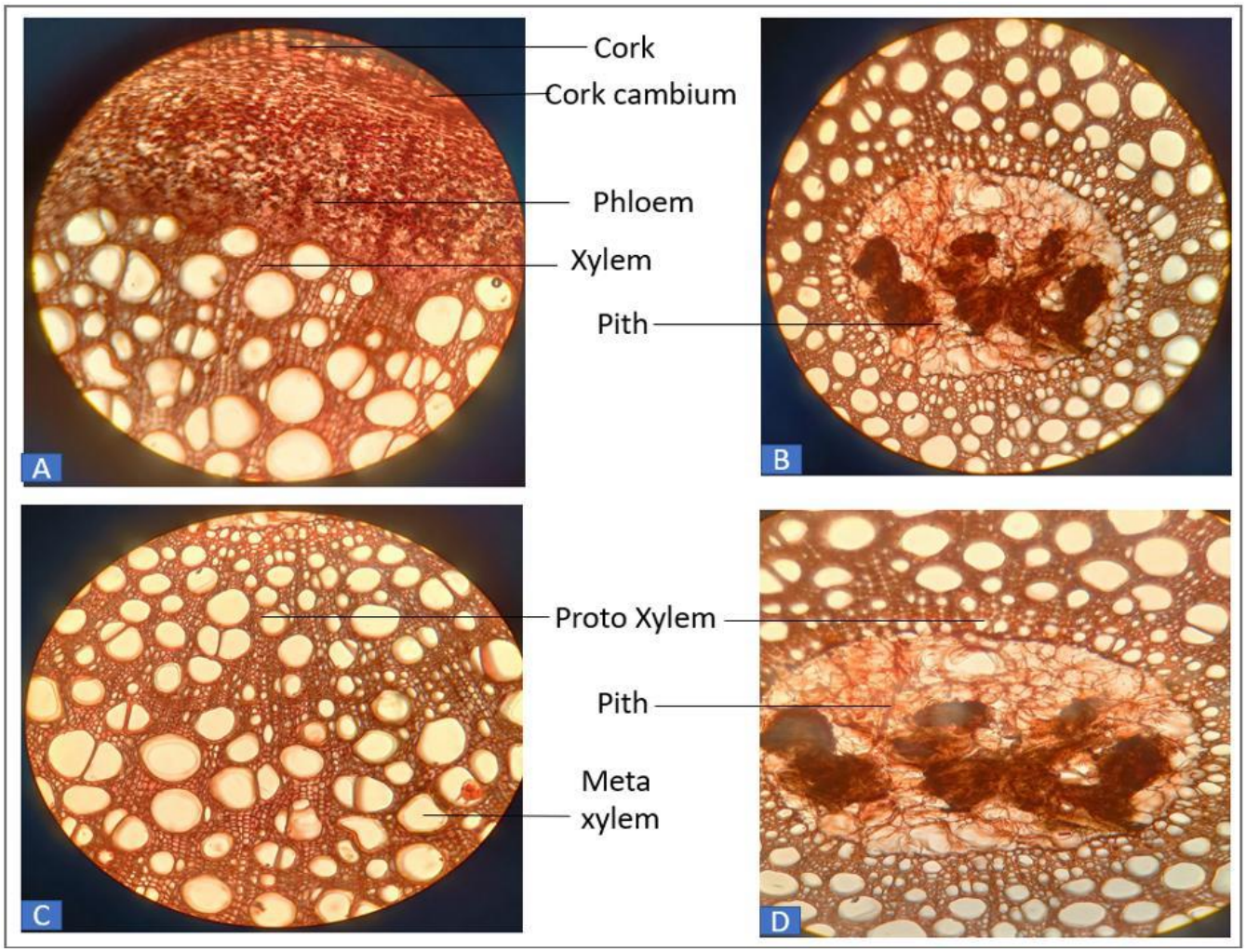


Fig 3: T. S. of Safranin-Stained *Manjishtha* Root At 10x and 40x Showing A. Cork, Cork Cambium, Phloem & Xylem B. Pith C. & D. Protoxylem & Metaxylem

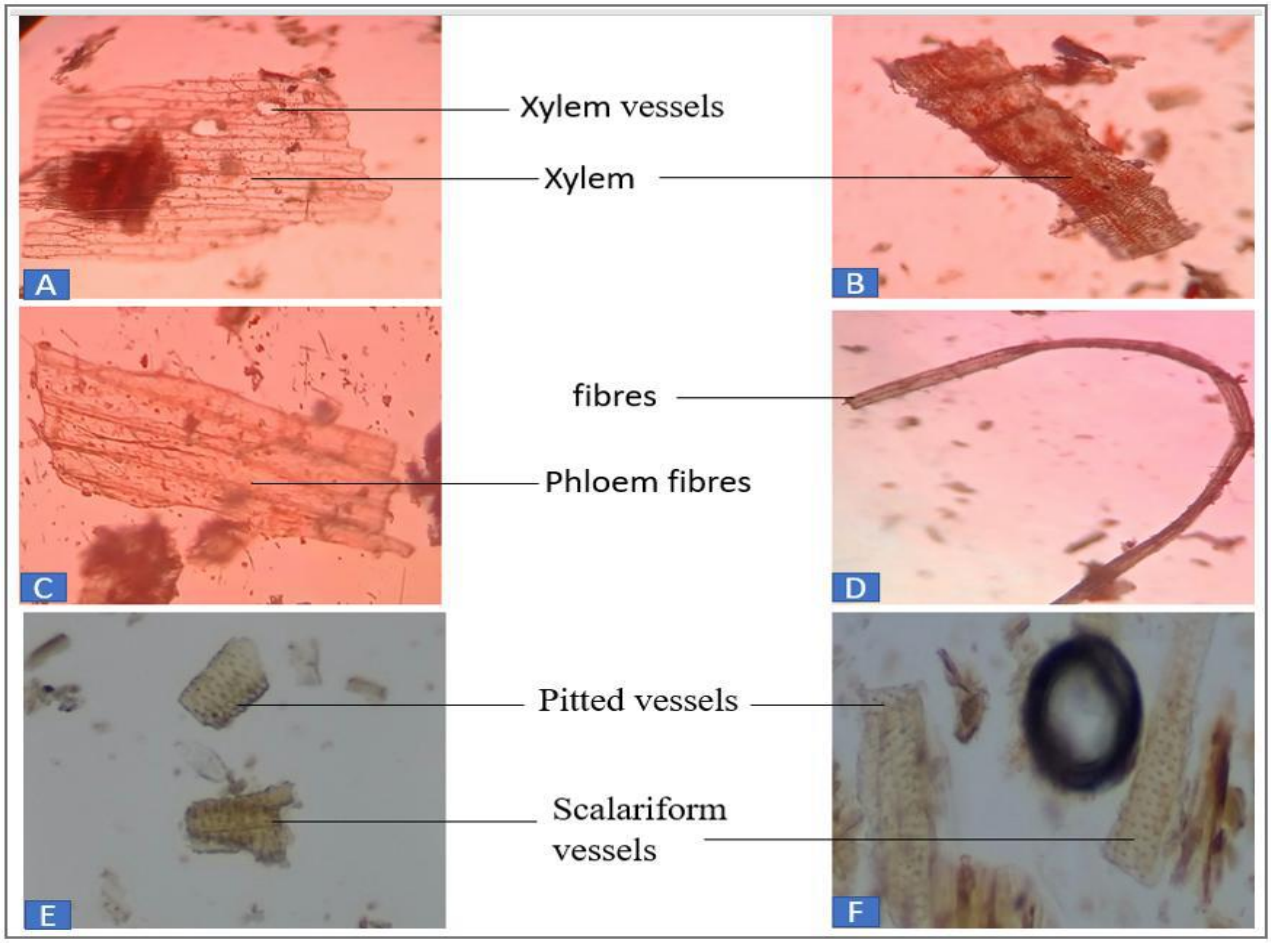
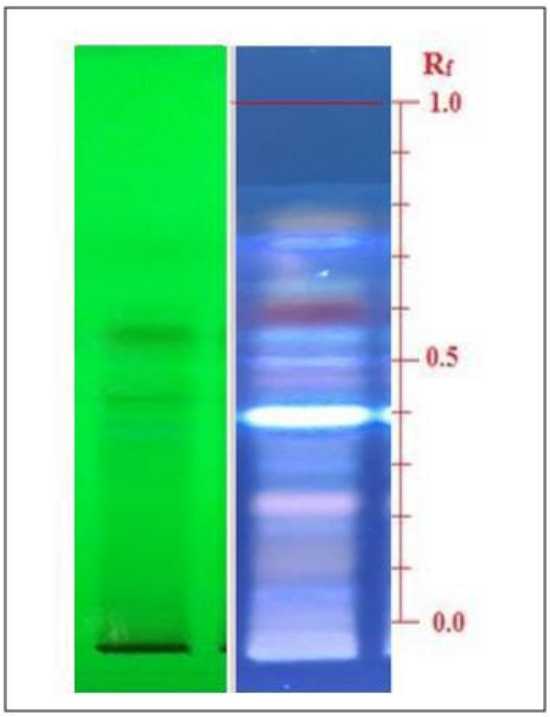


Fig 4: *Manjishtha* Root powder at 10X and 40X showing A. & B. Xylem Vessels & Xylem, B. Xylem C. Phloem fiberes, D. Fibres E. & F. Pitted vessels & Scalariform vessels.



(A) (B)

Fig. 5: Photography of HPTLC Plate- (A) Visualization at 254 Nm; (B) Visualization At 366 nm

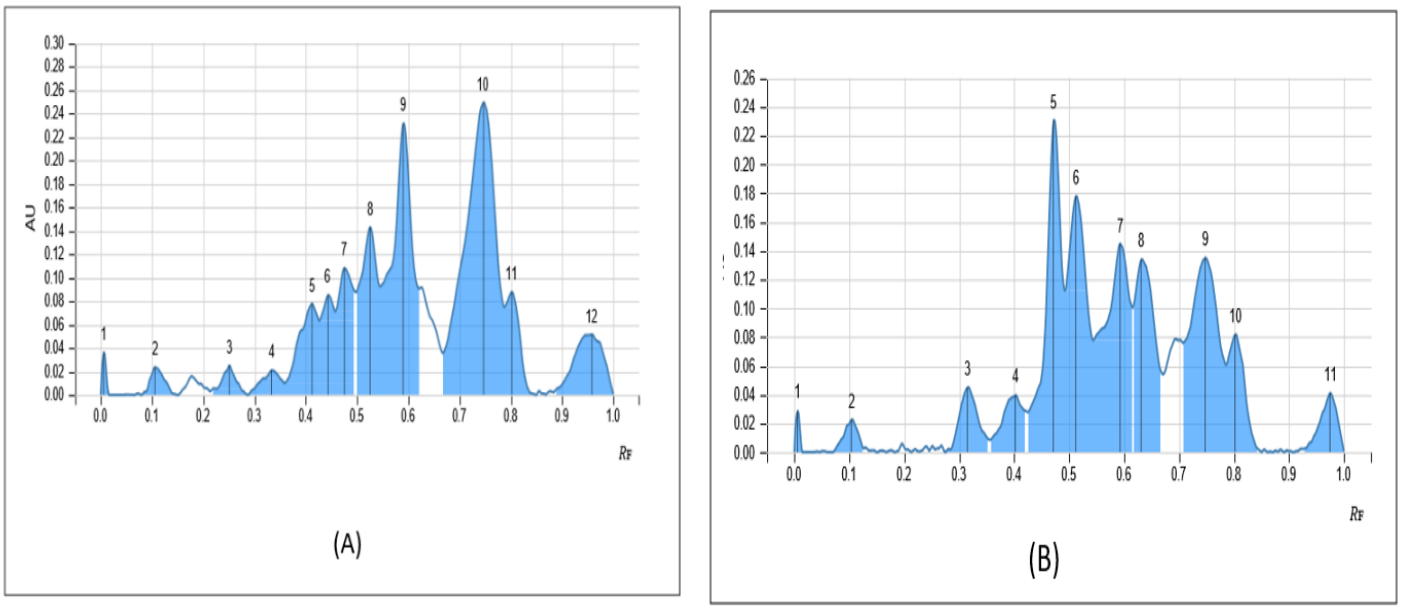


Fig. 6: Densitometric Finger Print Profiles At (A) 254 Nm, (B) 366 Nm of Root of *Manjishtha* (*Rubia cordifolia* L.)

HPTLC Finger-printing Profile of Root of *Manjishtha* (*Rubia cordifolia* L.)

The HPTLC procedure was standardized using pre-activated, precoated silica gel GF 60 254 aluminium plate along with various combinations of polar and non-polar solvent systems as mobile phases. Optimal resolution was achieved with Toluene, Ethyl Acetate, Formic Acid in the ratio of 7:3:1 (v/v/v). Under these conditions, the root extract of *Rubia Cordifolia* L. exhibited 12 distinct bands at Rf values of 0.006, 0.106, 0.252, 0.334, 0.413, 0.444, 0.476, 0.526, 0.590, 0.747, 0.802 and 0.958 under UV light at 254nm; 11 bands at 0.006, 0.105, 0.316, 0.403, 0.473, 0.513, 0.594, 0.632, 0.748, 0.803 and 0.976 at 366 nm respectively.

The pictorial representation of the developed plate of root methanolic extracts are given in Fig.5. Densitometric scanned pictures at 254nm and 366nm of the developed plates as fingerprint profile are shown in Fig.6.

CONCLUSION

The present study establishes comprehensive pharmacognostic, physicochemical, and HPTLC fingerprint data for the dried roots of *Manjishtha (Rubia cordifolia* L.). Microscopic characteristics and physicochemical parameters provide reliable diagnostic criteria for the development of a reference monograph. Preliminary phytochemical screening of aqueous and ethanolic extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, phenolic compounds and steroids, as indicated by characteristic reactions with specific reagents. These secondary metabolites are recognized for their medicinal value and distinct physiological activities. Moreover, the phytochemical profile highlights the potential of *R. cordifolia* root extracts for further pharmacological evaluation to explore their therapeutic efficacy. The chromatographic fingerprint developed in this work may be employed as a reference tool for ensuring quality, identity, and authenticity of herbal preparations formulated with *Manjishtha*. This study strengthens the scientific basis for the standardization and quality assurance of *Manjishtha (Rubia cordifolia* L.) roots, thereby supporting their safe and effective use in herbal medicine.

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