

COMPARATIVE PHARMACOGNOSTICAL ANALYSIS OF ROOT AND LEAF OF BHANDIRA (*CLERODENDRUM INFORTUNATUM* LINN.)

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ABSTRACT

Introduction: *Clerodendrum infortunatum* Linn. (*Bhandira*; Family: *Lamiaceae*) is an important medicinal plant used traditionally in the management of bleeding disorders and inflammatory conditions. However, comprehensive Pharmacognostical standards for its root and leaf are limited. The present study was undertaken to establish pharmacognostical, phytochemical and HPTLC profiles of *Bhandira* for its authentication and standardization. **Objective:** To evaluate the Pharmacognostical characters, preliminary phytochemical constituents, and HPTLC profile of *Clerodendrum infortunatum* Linn. **Materials and Methods:** Roots and leaves of *Clerodendrum infortunatum* Linn. were collected from the natural habitats of Udupi district. Pharmacognostical, physicochemical, preliminary phytochemical and HPTLC analysis according to standard

protocols. **Results and Discussion:** Comparative pharmacognostical evaluation of the root and leaf of *Clerodendrum infortunatum* Linn. revealed distinct macroscopic and microscopic characteristics useful for drug authentication. The root was cylindrical, greyish-brown, rough and fibrous, whereas the leaves were opposite-decussate, broadly ovate and pubescent. Microscopically, the root showed well-developed secondary xylem, sclereids and abundant calcium oxalate crystals, while the leaf exhibited a dorsiventral structure with abundant

trichomes and a prominent bicollateral vascular bundle. Physicochemical analysis showed loss on drying of 0.21% and 4.74%, total ash of 6.11% and 9.60%, acid-insoluble ash of 4.37% and 0.74%, alcohol-soluble extractive of 0.03% and 5.32%, and water-soluble extractive of 12.61% and 18.52% for the root and leaf, respectively. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, steroids and triterpenoids in both parts, with saponins and resins present only in the root and tannins confined to the leaves. HPTLC analysis identified gallic acid and rutin in the root extract, whereas no specific constituents were confirmed in the leaf extract. **Conclusion:** The present study establishes diagnostic pharmacognostical and phytochemical standards for *Bhandira*, providing baseline data for its authentication, quality control, and future pharmacological and clinical investigations.

KEYWORDS: *Clerodendrum infortunatum*, Standardisation, Physicochemical, Phytochemical, HPTLC.

INTRODUCTION

Drug evaluation may be defined as the determination of the identity, purity, and quality of a drug. The main reasons behind the need for evaluation of crude drugs are biochemical variation in the drug, the effects of treatment and storage, and the occurrence of adulteration and substitution. The evaluation of a drug is carried out by studying its various properties such as organoleptic, microscopic, physical, chemical, and biological characteristics.^[1] In view of the importance of standardization of crude drugs, it becomes necessary to establish pharmacognostical and phytochemical standards for medicinal plants of therapeutic significance. *Clerodendrum infortunatum* Linn. known as source for the drug *Bhandira*, is important plant in Indian system of Medicine. It was classified under the family *Verbenaceae*. But recently it is categorised into the family *Lamiaceae*.^[2] It is found in degraded forest areas, moist evergreen forests and plain lands. Found all over India, Sri Lanka, Myanmar, Andaman & Nicobar Islands, Thailand, Malaysia and Bangladesh.^[3] It is well known as a medicinal plant because of its wide therapeutic uses. In Indian folk medicine, the plant is used in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy.^[4]

MATERIALS AND METHODS

Clerodendrum infortunatum Linn. was identified and collected from the Udyavara region of Udupi. The roots and tender leaves obtained from the plant were utilized for the study.

PHARMACOGNOSTICAL STUDY^[5]

Macroscopy: The external features of root and leaves were documented using canon IXUS Digital camera. The macroscopic features were compared to local flora for authentication.

Microscopy: The roots and tender leaves were preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Powder Microscopy: Pinch of root and leaf powder of *Clerodendrum infortunatum* previously sieved is put on the slide and mounted in glycerine and powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

HPTLC: 1g of *Clerodendrum infortunatum* Linn. root and tender leaf powder was extracted with 10 ml of alcohol. 4 and 8µl of the above extract was applied on a pre-coated silica gel F₂₅₄ on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate: Formic acid: Methanol (3.0: 6.0: 1.6: 0.4). The developed plates were visualized in under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm and 366nm. R_f, colour of the spots and densitometric scan were recorded.

PHYSICO CHEMICAL ANALYSIS^[6]

The powdered sample of both root and leaf of *Clerodendrum infortunatum* was evaluated for Loss on Drying at 105°C, Total Ash, Acid Insoluble Ash, Water Soluble Ash, Alcohol Soluble Extractive, and Water-Soluble Extractive according to standard procedures.

PHYTOCHEMICAL ANALYSIS^[7]

The powdered sample of both root and leaf of *Clerodendrum infortunatum* was evaluated for the presence of Alkaloid, Carbohydrate, Steroid, Saponins, Tannin, Flavonoids, Phenol, Coumarins, Tri terpenoid, Amino acids, Carboxylic acid, Resins and Quinone.

OBSERVATIONS AND RESULTS

PHARMACOGNOSTICAL STUDY

Macroscopy of Root of *Clerodendrum infortunatum* Linn.

- Type & Shape: It features a well-developed taproot system. The primary roots are cylindrical, slightly tortuous, and gradually taper toward the apex, branching into thinner secondary and tertiary lateral roots.
- Colour: The external surface of the root is generally greyish-brown to dark brown, while the internal fractured surface appears creamy-white to light yellowish.
- Surface Texture: The outer surface is rough and shows longitudinal wrinkles, occasional fissures, and scars of lateral rootlets.
- Fracture: The fracture is short, fibrous, and tough, particularly in older, woody roots.
- Odour & Taste: It has a characteristic, slight aromatic odour and an initially bland taste that turns slightly bitter or acrid.

Macroscopy of Leaf of *Clerodendrum infortunatum* Linn.

- Arrangement: The leaves are opposite-decussate.
- Shape: They are broadly ovate to cordate at the base, with a sharply acute or acuminate apex
- Size: Typically, large and expansive, ranging from 10 to 25 cm in length and 8 to 20 cm in width.
- Margin: Entire to coarsely dentate or serrate
- Venation: Prominently reticulate. The primary and secondary veins are sunken on the upper surface but highly elevated and distinct on the lower surface.
- Surface Texture: Both surfaces are pubescent. The upper surface feels slightly rough or scabrid, while the lower surface feels softly hairy, especially along the prominent vein pathways.
- Petiole: The leaf stalks are quite long (usually 4 to 12 cm), cylindrical, densely hairy, and slightly channelled on the upper side.
- Colour: Dark green on the upper surface; slightly paler green on the lower surface.
- Odour: Distinctly disagreeable, fetid, or heavy aromatic odour when crushed or bruised.
- Taste: Strongly bitter and slightly acrid.

Microscopy of Root of *Clerodendrum infortunatum* Linn.

The transverse section of the root is more or less circular. The margin is prominently wavy.

Cork, cork cambium, and cortex are the tissues present from the periphery to the centre. The cork is thin, brown and made up of few layers of irregular parenchymatous cells. The cork cambium is indistinct. The cortex consists of 10~15 layers of parenchyma. Scattered, isolated sclereids and a continuous well-developed belt of sclereids occur in between the primary cortex and secondary phloem. Each sclereid is more or less rectangular, pitted and thickened with lignin. Some of the parenchymatous cells contain calcium oxalate crystals. The secondary phloem region comprises of mostly phloem. The secondary xylem occupies about three fourths of the transverse section and is transversed regularly by rows of medullary rays whose cells are lignified. The xylem consists of vessels, wood fibres and lignified parenchyma. The vessels are either single or in pairs and show scalariform and bordered pitted thickenings. The xylem fibres appear as rounded or polygonal structures with thick lignified walls. Typical calcium oxalate prisms occur in the wood parenchyma region.

Microscopy of Leaf of *Clerodendrum infortunatum* Linn.

The transverse section of the leaf shows a dorsiventral nature. The section is broadly divided into lamina and midrib region. The lamina of the leaf shows three distinct regions viz, upper epidermis, lower epidermis and mesophyll. The upper epidermis is single layered with more or less rectangular cells covered by a distinct cuticle. Abundant covering and glandular trichomes emerge from the upper epidermal layer. The covering trichomes are lignified or non-lignified, uniseriate, multicellular (2~5 cells) mostly straight and rarely warty with acute tips. The glandular trichomes are unicellular and sessile. The mesophyll is differentiated into palisade and spongy parenchyma. The palisade parenchyma is made up of a single layer of compactly arranged, radially elongated cells. The spongy parenchyma is multi-layered and loosely arranged with intercellular spaces. The lower epidermis which is identical to upper epidermis has numerous trichomes. The epidermal layers of the lamina are continuous in the midrib region also. Strips of collenchyma appear below the upper and above the lower epidermis. A prominent bicollateral vascular bundle occupies the central portion of the midrib with xylem towards the ventral surface and phloem towards the dorsal surface. The vascular bundle is surrounded by sclerenchymatous fibres with calcium oxalate crystals.

Powder characteristics

i) Root - Microscopic examination of the root powder showed fragments of cork cells, which appeared as brownish, rectangular, thick-walled cells arranged in layers. Cortical parenchymatous cells were observed, which were thin-walled and polygonal in shape.

Numerous fibres were present, appearing elongated, thick-walled and lignified, indicating the supporting tissue of the root. Vessel elements with characteristic thickening were also observed, representing the xylem tissue. In addition, crystals of calcium oxalate were found scattered throughout the powder, serving as an important identifying feature of the root drug.

ii) Leaf - Microscopic examination of the leaf powder revealed fragments of epidermal cells with trichomes. The trichomes were observed as elongated hair-like outgrowths arising from the epidermis. Mesophyll tissue fragments were also present, consisting of thin-walled parenchymatous cells. Sclereids were observed as thick-walled, lignified cells providing mechanical support. Tracheids with characteristic thickened walls were also identified, representing the conducting tissue elements of the leaf.



Fig.no.1: Macroscopy of Clerodendrum infortunatum Linn. Root.



Fig. no. 2: Macroscopy of Clerodendrum infortunatum Linn. Leaf.

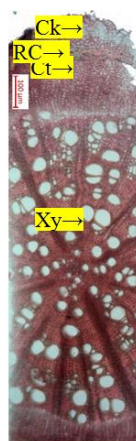
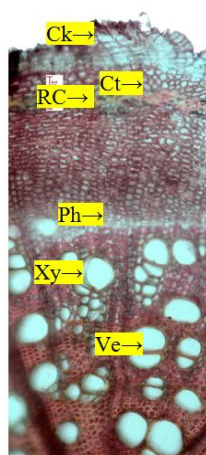
Microscopic view of *Clerodendrum infortunatum* Linn. rootFig. no. 3: TS of root of *Clerodendrum infortunatum* Linn.

Fig. no. 4: A portion enlarged.

Ck - cork; ct - cortex; Ph - phloem; RC - rosette crystals; Xy - xylem; Ve - vessels

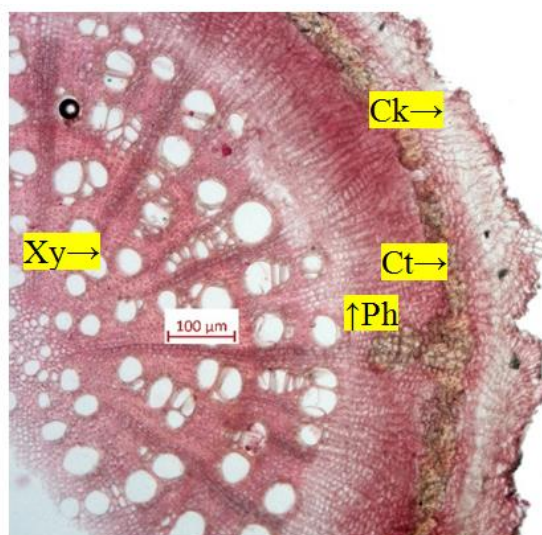


Fig. no. 5: TS extending from periphery to central xylem.

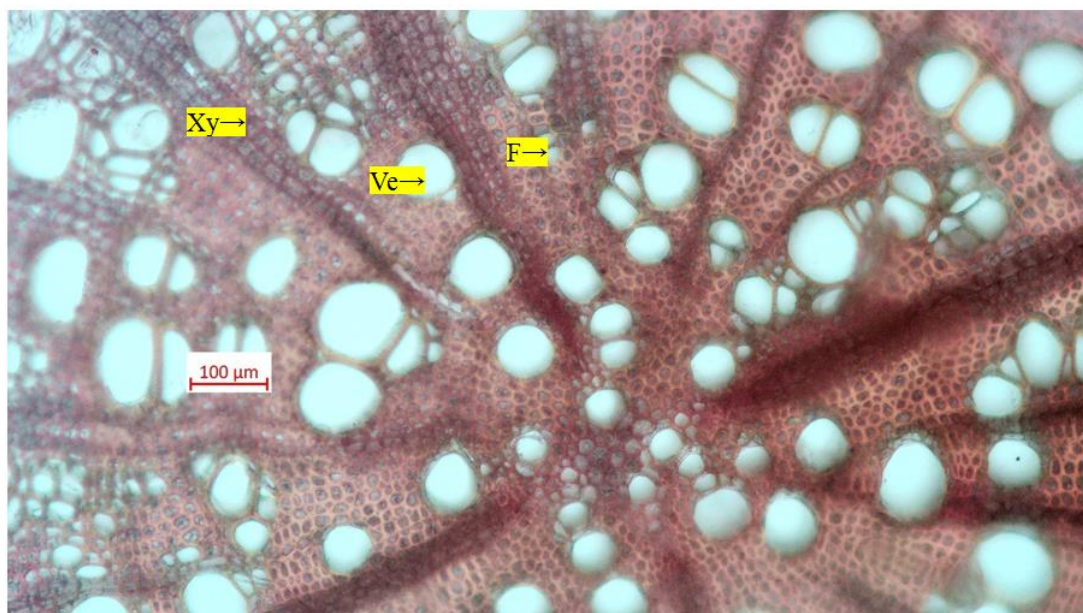


Fig. no. 6: Xylem.

Ck - cork; **ct** - cortex; **F** - fibers; **Ph** - phloem; **RC** - rosette crystals; **Xy** - xylem; **Ve** – vessels

Microscopic view of *Clerodendrum infortunatum* Linn. leaf

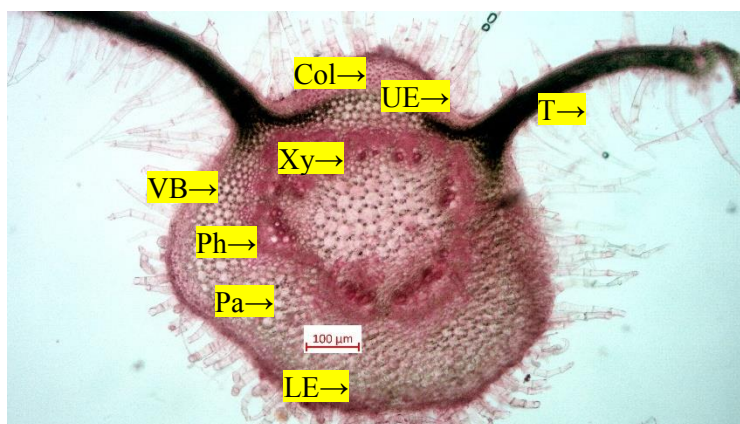


Fig.no. 7: TS of Leaf of *Clerodendrum infortunatum* Linn.

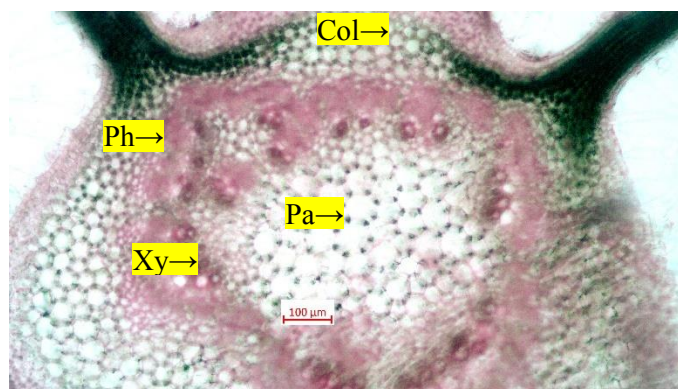


Fig. no. 8: Midrib.

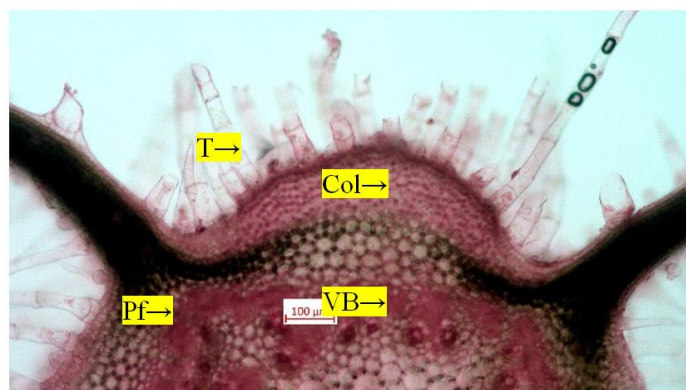


Fig. no. 9: Collenchyma and Vascular bundle.

Col – collenchyma; **T** – trichomes; **LE** – lower epidermis; **Pa** – parenchyma; **PF** –pericyclic fibre; **Ph** – phloem; **SP**–spongy parenchyma; **T**–trichomes; **UE**–upperepidermis; **VB** – vascular bundle; **Xy** – xylem.

Microscopic view of *Clerodendrum infortunatum* Linn. root powder

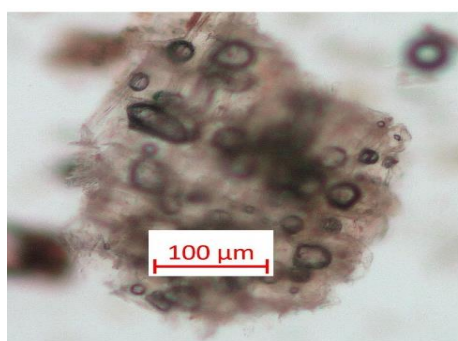


Fig.no. 10: Cork

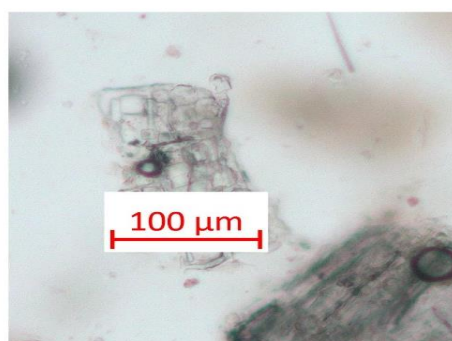


Fig.no. 11: Cortical cells

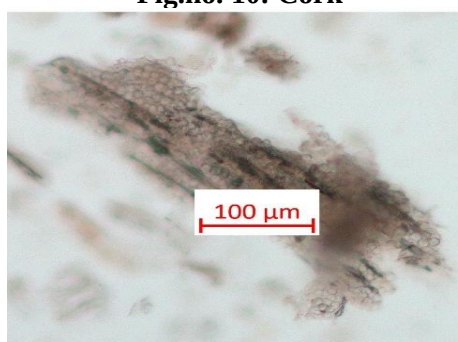


Fig. no. 12: Fibres, vessels, Crystals of calcium

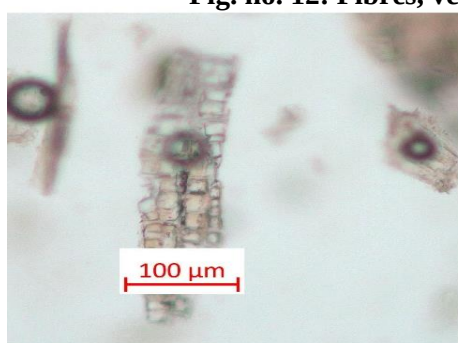
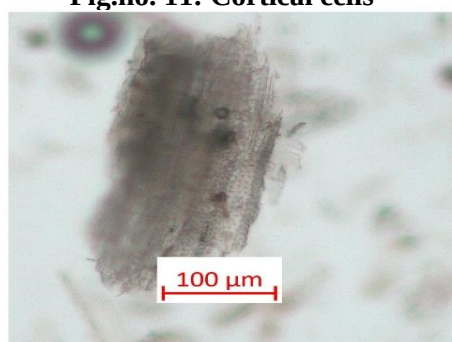


Fig.no. 13: Vessels

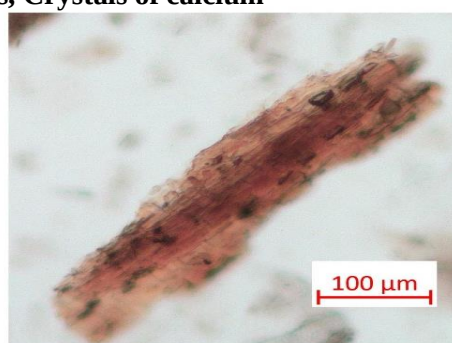
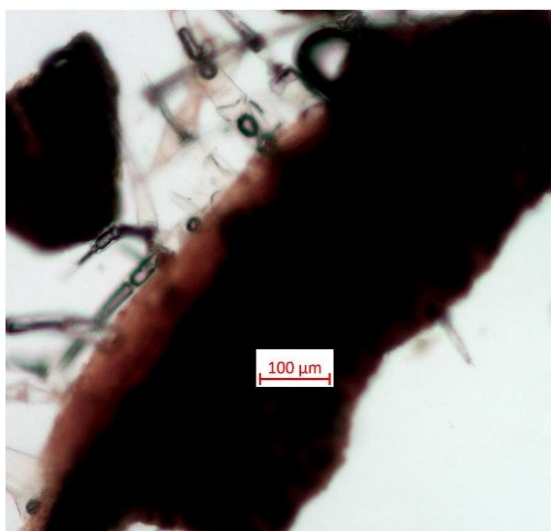
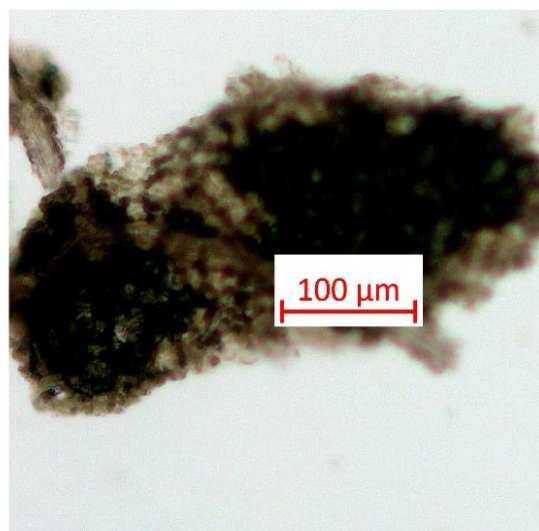
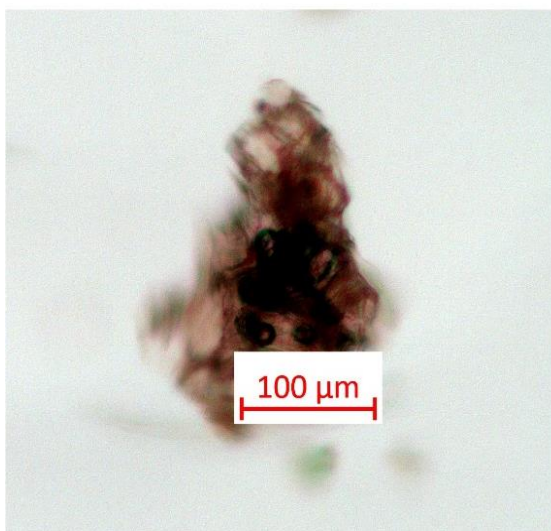
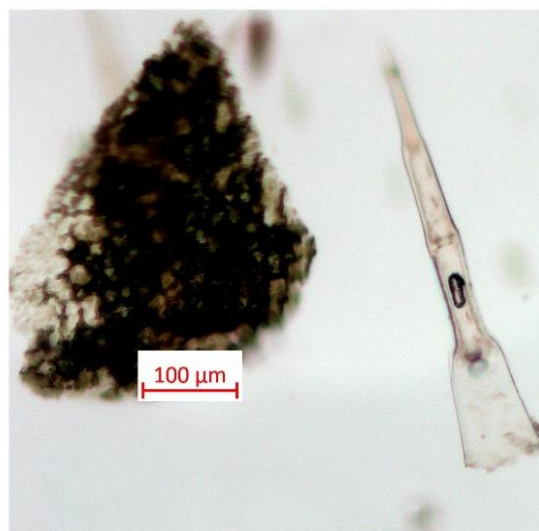
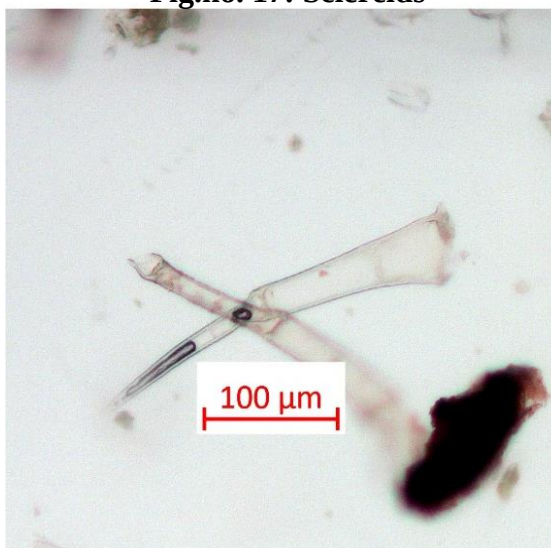


Fig.no. 14: Fibres

Microscopic view of *Clerodendrum infortunatum* Linn. leaf powder**Fig. no. 15: Epidermis with trichomes****Fig.no. 16: Mesophyll****Fig.no. 17: Sclereids****Fig.no. 18: Mesophyll and trichome****Fig.no. 19: Trichomes****Fig.no. 20: Tracheid**

PHYSICO-CHEMICAL ANALYSIS

Table no. 1: Physico chemical standards of *Clerodendrum infortunatum* Linn. root and leaf.

Parameter	Results n = 3 %w/w Avg $\pm$ SD	
	Root of <i>Clerodendrum infortunatum</i> Linn.	Leaf of <i>Clerodendrum infortunatum</i> Linn.
Loss on drying	0.21 $\pm$ 0.01	4.74 $\pm$ 0.02
Total Ash	6.11 $\pm$ 0.21	9.60 $\pm$ 0.84
Acid Insoluble Ash	4.37 $\pm$ 0.02	0.74 $\pm$ 0.01
Water soluble Ash	5.42 $\pm$ 0.03	4.37 $\pm$ 0.02
Alcohol soluble extractive value	0.03 $\pm$ 0.00	5.32 $\pm$ 0.02
Water soluble extractive value	12.61 $\pm$ 0.02	18.52 $\pm$ 0.02

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Table no. 2: Observations of Preliminary Phytochemical tests.

Sl. No	Tests	Colour if positive	Root extract	Leaf extract
1.	Alkaloids			
	Dragendrof's test	Orange precipitate	Orange precipitate	Orange precipitate
	Wagners test	Red precipitate	Red precipitate	Red precipitate
	Mayers test	Dull white precipitate	Dull white precipitate	Dull white precipitate
	Hagers test	Yellow precipitate	Yellow precipitate	Yellow precipitate
2.	Steroids			
	Liebermann-buchard test	Bluish green	Bluish green	Bluish green
	Salkowski test	Bluish red to cherry red	Bluish red to cherry red	Bluish red to cherry red
3.	Carbohydrate			
	Molish test	Violet ring	Violet ring	Violet ring
	Fehlings test	Brick red precipitate	Brick red precipitate	Brick red precipitate
	Benedicts test	Red precipitate	Red precipitate	Red precipitate
4.	Tannin			
	With FeCl ₃	Dark blue or green or brown	Yellow color	Green color
5.	Flavanoids			
	Shinoda's test	Red to pink	Yellow color	Green color
6.	Saponins			
	With NaHCO ₃	Stable froth	Light froth	No froth
7.	Triterpenoids			
	Tin and thionyl chloride test	Red color	Red color	Red color
8.	Coumarins			
	With 2 N NaOH	Yellow	Brownish white	Green color

9.	Phenols			
	With alcoholic ferric chloride	Blue to blue black, brown	Yellowish brown	Greenish yellow
10.	Carboxylic acid			
	With water and NaHCO ₃	Brisk effervescence	No brisk effervescence	No brisk effervescence
11.	Resin			
	With aqueous acetone	Turbidity	Turbidity	No Turbidity
12.	Quinone			
	5% NaOH	Pink/purple/red	Dark brown	Dark green
13.	Amino acids			
	Ninhydrine reagent	Purple color	No Purple color	No Purple color

Table. no. 3: Preliminary phytochemical screening of Aqueous extract of *Clerodendrum infortunatum* Linn. root and leaf.

Test	Inference	
	Root	Leaf
Alkaloid	+	+
Carbohydrate	+	+
Steroid	+	+
Saponins	+	-
Tannin	-	+
Flavanoids	-	-
Phenol	-	-
Coumarins	-	-
Tri terpenoid	+	+
Amino acids	-	-
Carboxylic acid	-	-
Resins	+	-
Quinone	-	-

(+) - Present
(-) - Negative

HPTLC

Colour of the spots and R_f value of *Clerodendrum infortunatum* Linn. in chromatogram is developed in Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4), and root and leaf powder extracted using 10ml ethanol. The photo documentation of HPTLC revealed the presence of phytoconstituents with different R_f value band densitometric scan showed numerous bands under 254nm, 366nm.

Densitometric scan at 254nm showed 9 peak values for leaf extract and 8 peak values for root extract. At 366nm showed 5 peak values for leaf extract and 7 peak values for root extract.

Table no. 4: R_f values of samples.

At 254nm		At 366nm	
0.22 (Green)	-	-	0.75 (F. blue)
-	0.29 (Green)	-	-
-	-	-	0.80 (F. blue)
-	0.85 (Green)	-	-
0.89 (Green)	-	0.88 (F. red)	-

*F – fluorescent; L –light; D – dark

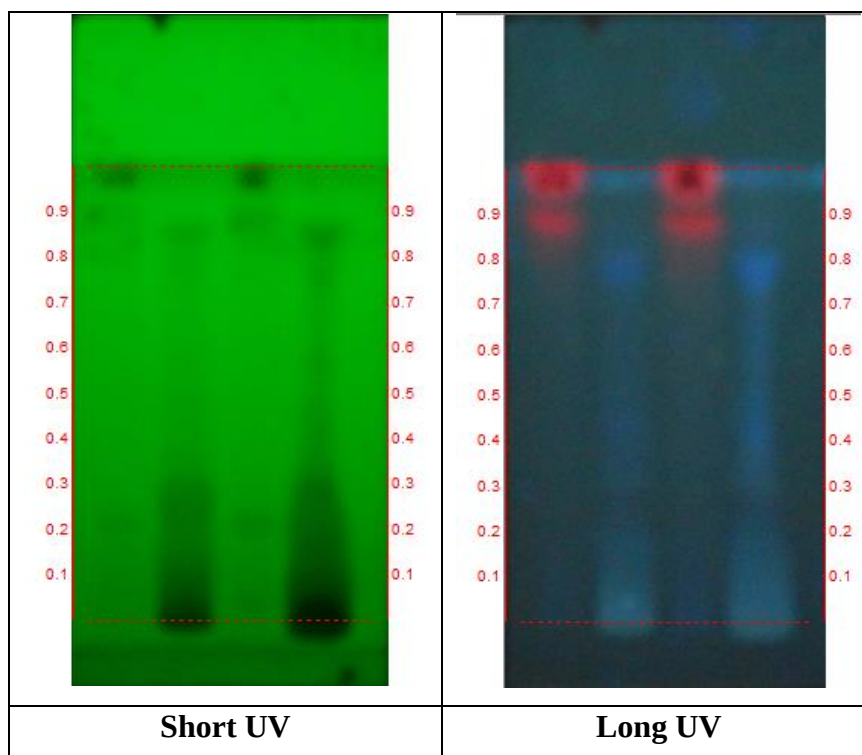


Fig.no. 21: HPTLC photo documentation of ethanolic extract of leaf and Root of *Clerodendrum infortunatum* Linn.

Track 1 – Ethanolic extract of leaf of *Clerodendrum infortunatum* Linn. – 4µl

Track 2 - Ethanolic extract of root of *Clerodendrum infortunatum* Linn. – 4µl

Track 3 - Ethanolic extract of leaf of *Clerodendrum infortunatum* Linn. – 8µl

Track 4 - Ethanolic extract of root of *Clerodendrum infortunatum* Linn. – 8µl

Solvent system – Toluene: Ethyl Acetate: Formic acid: Methanol (3:6:1.6:0.4), 254nm

Solvent system – Toluene: Ethyl Acetate: Formic acid: Methanol (3:6:1.6:0.4), 254nm

R_f- Gallic acid-0.85, Quercetin-0.74 and Rutin-0.18

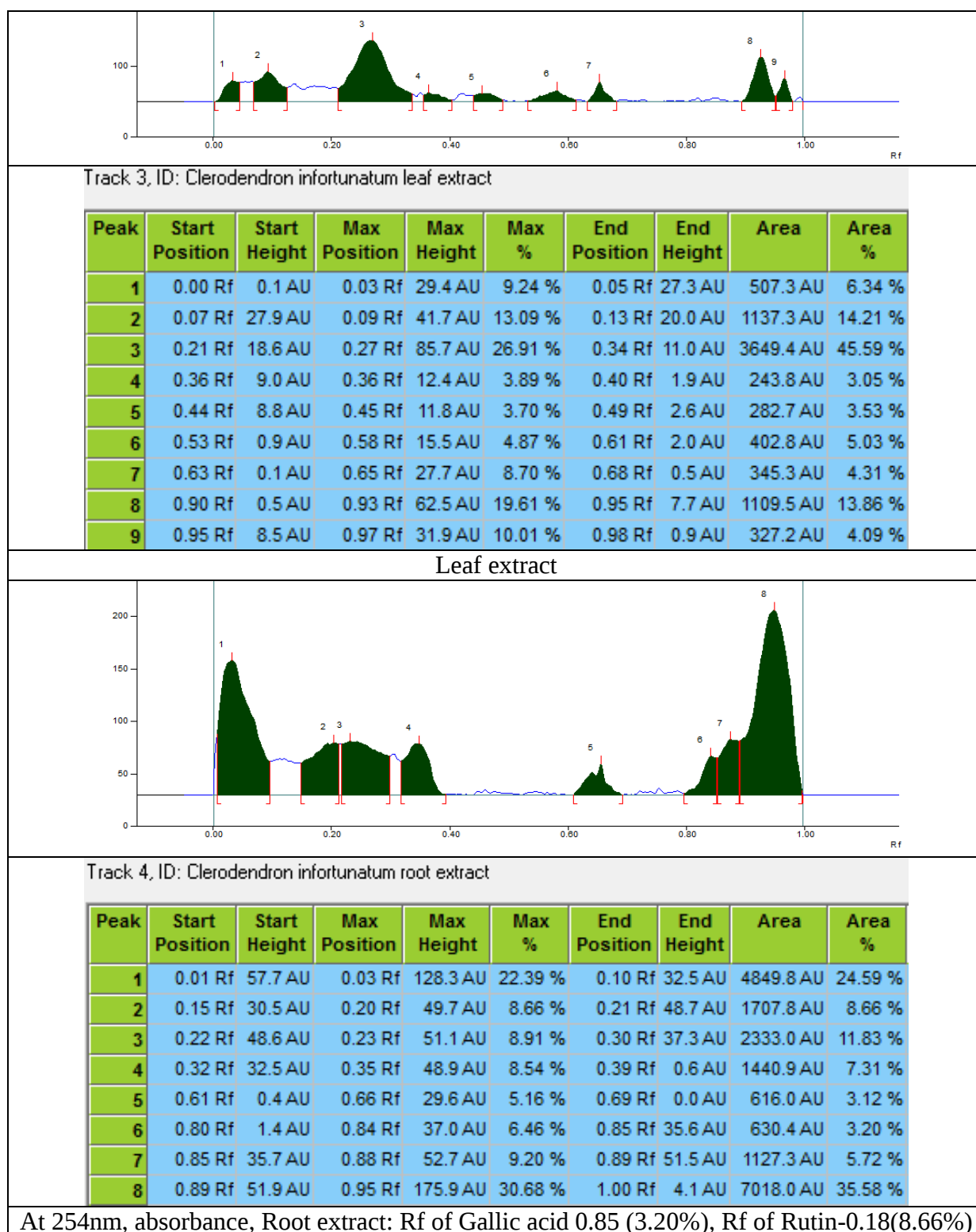


Fig.no. 22: Densitometric scan at 254nm.

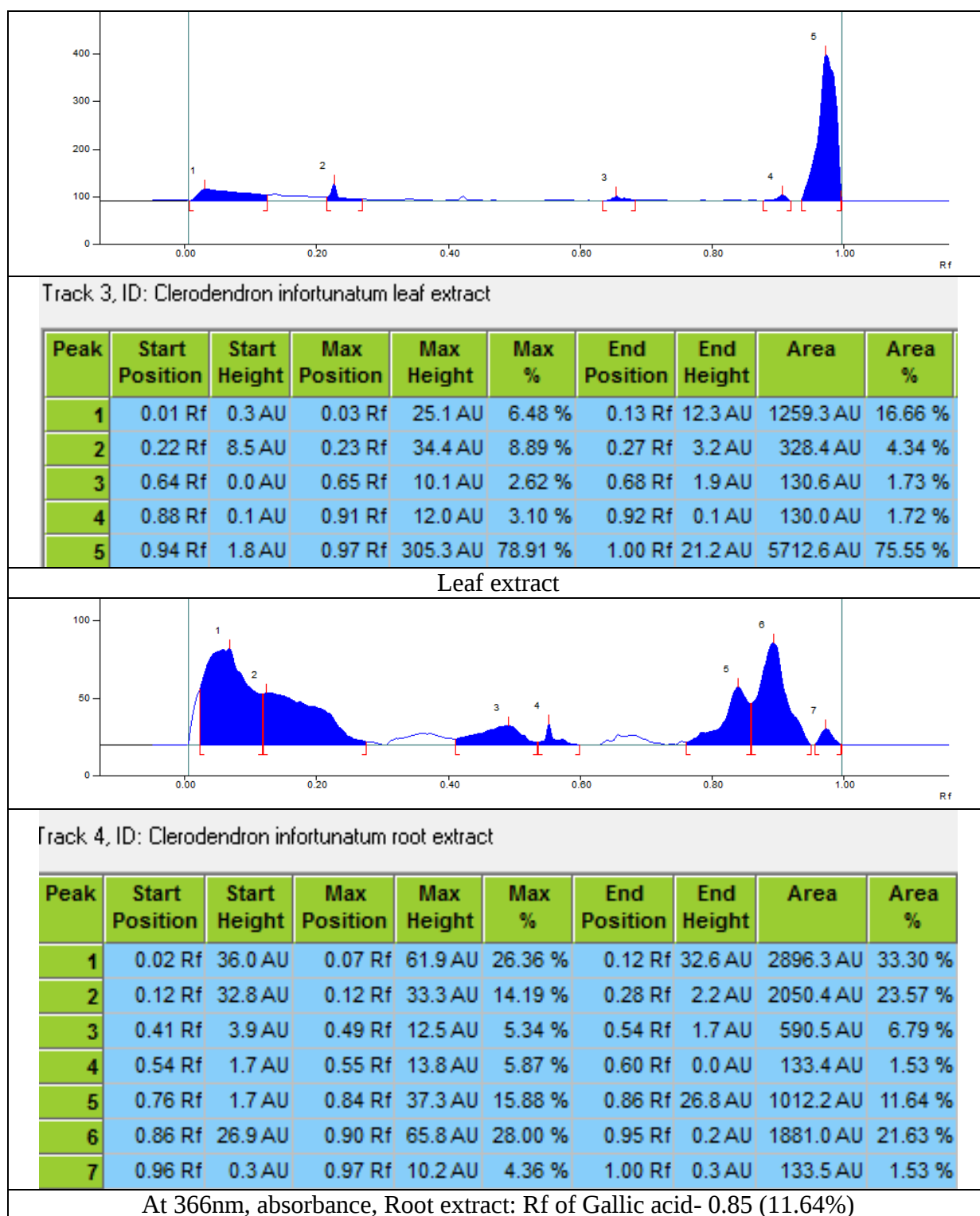


Fig. no. 23: Densitometric scan at 366nm.

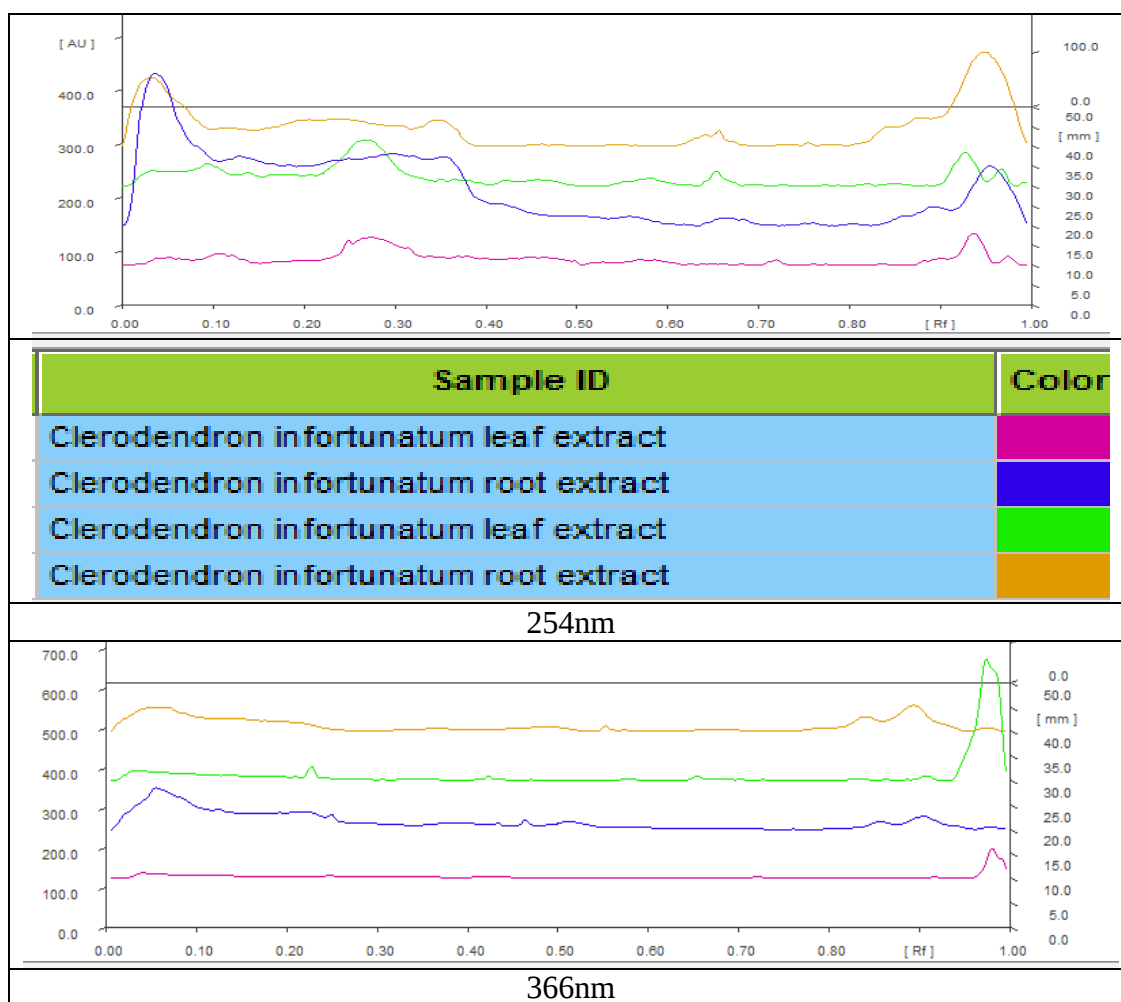


Fig. no. 24: 3D Chromatogram.

DISCUSSION

The present study was undertaken to establish pharmacognostical, physicochemical, phytochemical, and HPTLC standards for *Clerodendrum infortunatum* Linn. Standardization of medicinal plants is essential for ensuring the identity, purity, and quality of crude drugs and serves as an important step in developing reference standards for future research. The macroscopic characteristics observed in the present study were found to be consistent with the botanical description of *C. infortunatum*. The root was characterized by a greyish-brown, rough and wrinkled surface with a fibrous fracture, whereas the leaves were large, opposite, pubescent, and possessed a characteristic fetid odour. These morphological features provide important diagnostic parameters for the identification and authentication of the plant material. Microscopic evaluation further confirmed the identity of the drug. The root showed characteristic tissues such as cork, cortex, sclereids, secondary phloem and xylem, medullary rays, and calcium oxalate crystals. Similarly, the leaf exhibited diagnostic features including covering and glandular trichomes, palisade and spongy parenchyma, bicollateral vascular

bundles, and calcium oxalate crystals. Powder microscopy also revealed the presence of fibres, vessel elements, tracheids, sclereids, and trichomes. These microscopic characters can serve as valuable parameters for identification of the crude drug in powdered form and for detecting adulteration or substitution. The physicochemical parameters generated in the present study provide useful standards for assessing the quality and purity of the drug. The low loss on drying values of both root and leaf samples indicate minimal moisture content and suggest good storage stability of the drug material. The total ash values indicate the presence of inorganic constituents in both plant parts, with the leaf showing comparatively higher ash content than the root. The higher acid-insoluble ash value observed in the root may be attributed to the presence of siliceous matter and extraneous earthy materials commonly associated with underground plant parts. Water-soluble ash values indicate the presence of water-soluble inorganic constituents in both samples. The higher water-soluble extractive values compared to alcohol-soluble extractive values suggest the predominance of water-soluble constituents in both the root and leaf. Preliminary phytochemical screening revealed the presence of various classes of secondary metabolites in both plant parts. The root contained alkaloids, carbohydrates, steroids, saponins, triterpenoids, and resins, whereas the leaf contained alkaloids, carbohydrates, steroids, tannins, and triterpenoids. The variation in the phytochemical profile between the root and leaf indicates differences in the distribution of secondary metabolites within different parts of the plant. HPTLC fingerprint analysis demonstrated distinct chromatographic profiles for the leaf and root extracts of *Clerodendrum infortunatum* Linn. The differences observed in the number of spots and peaks under short UV, long UV, and densitometric scanning conditions indicate the presence of multiple phytoconstituents with varying chemical characteristics. The R_f values ranging from 0.22 to 0.89 further substantiate the chemical complexity of the plant. The presence of gallic acid and rutin was confirmed by matching their corresponding R_f values in the root extract. The chromatographic fingerprints generated in the present study can therefore be utilized as reference standards for the identification, authentication, and quality control of *C. infortunatum* Linn.

CONCLUSION

The macroscopic and microscopic characteristics of root and leaf of *Bhandira* generated in this study provide reliable diagnostic features for the authentication and identification of the plant material. The physicochemical parameters serve as reference standards for assessing the purity and quality of the drug and can be employed for detecting adulteration and ensuring

consistency of the crude drug. Preliminary phytochemical screening revealed the presence of various secondary metabolites in both root and leaf samples, indicating their rich chemical composition and variation in constituent distribution between different plant parts. HPTLC fingerprint profiling demonstrated distinct chromatographic patterns and confirmed the presence of marker compounds such as gallic acid and rutin in the root extract. Overall, the findings of the present investigation provide valuable baseline data for the standardization, authentication, and quality control of *Clerodendrum infortunatum* Linn. The established parameters can serve as reference standards for future Pharmacognostical and Phytochemical investigations on this plant.

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