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# Pharmacognostic Analysis of *Euphorbia hirta, Cissus quadrangularis,* and *Ruellia tuberosa*

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**ABSTRACT**
*Plants are a major natural source of therapeutic chemicals and are used as medication to sustain human health. Authentication is the first stage in recording the quality of the herbal material, and it can be followed by the creation of standard numerical values for evaluation. Euphobia hirta, often called Pill pods and mat, is a plant with a slender, upright stem that can reach a height of 80cm. It has an oblong, elliptical, opposite arranged leaf with a thick, hairy stalk. The plant, which has historically been used to treat diabetes, fever, and asthma, is typically found in meadows, walkways, roadside areas, and water-rich places. The climbing cactus is the common name for the stem of Cissus quadrangularis. This plant is frequently found in the arid habitat of tropical and subtropical climates. Typically used to treat diabetes joint pain, reduce pain, and act as an antioxidant. The craker plant, Ruellia tubeosa L, has long been used as an emetic, diuretic, and anti-hypertensive for kidney and gall bladder disorders. The goal of the current study was to compare the pharmacognostic and physico chemical characteristics of the stem of Cissus quadrangularis, the leaves of Euphorbia hirta. And Ruellia tuberosa.*
**Keywords**: *Euphorbia hirta,* leaves*, Cissus quadrangularis, stem, Ruellia tuberosa.*

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## INTRODUCTION

The Greek terms "pharmacon," which means drug or medicine, and "gnosis," which means knowledge, are the roots of the phrase "pharmacognosy." C.A. Seydler originally used this term in his 1895 dissertation, "Analectapharmacognosia." Traditional medicine has utilized herbal remedies as its main treatment for thousands of years, and they have greatly aided in preserving human health [1].

Since ancient times, people in India have used the different parts of different medicinal plants to treat specific illnesses. The traditional medical systems of Siddha, Unani, and Ayurveda have been used for many millennia. There are already certain Ayurvedic drugs on the market that cure modern ailments. Because they are the source of some important drugs, plants play a crucial role in modern medicine [2]. Millions of individuals cannot afford synthetic pharmaceuticals, despite the fact that they are helpful in treating a wide range of conditions. It is estimated that some 70,000 plant species have been used for medical purposes. The herbs provide the raw ingredients for traditional medicines [3].

In the current paper, an overview of the current knowledge and information about *E. hirta, Cissus quadrangularis and Ruellia tuberosa* is presented for the study of its pharmacognistic study.

## MATERIAL AND METHODS

### Collection of Plant Specimens

The plant parts like *Euphorbia hirta* leaves, *Cissus quadrangularis* stem and *Ruellia tuberosa* leaves were collected from several habitats at Yadadri bhuvanagiri district, Telangana in the month of Dec 2023. They are authenticated by Dr. Madhava Chetty Professor, Sri Venkateswara University, Tirupati, A.P.

Anatomical Studies of Plants

Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5mL+ Acetic acid-5mL + 70% Ethyl alcohol-90mL). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per the schedule given by Sass, et al., 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60$^{o}$C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. De waxing of the sections was done by customary procedure. The sections were stained with Toluidine blue. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained [4,5]. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections were also stained with safranin and Fast-green and IKI (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jefferey's maceration fluid were prepared. Glycerin mounted temporary preparations were made for macerated/ cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component was studied and measured.

Microscopic descriptions of tissue are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Digital microscope. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bi-fringent property under polarized light, they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.

**Powder Microscopy**

Coarsely powdered *Euphorbia hirta leaves*, *Cissus quadrangularis stem* and *Ruellia tuberosa* leaf were viewed under the microscope at different magnifications using mountains including chloral hydrate, phloroglucinol in concentrated HCl, water and iodine. Photomicrographs of the different cellular structures and inclusions were taken [6,7].

**Physicochemical Parameters**

Powderd *Euphorbia hirta* Leaf, *Cissus quadrangularis* Stem*, Ruellia tuberosa* Leaf were subjected to physicochemical analysis. Their solvent soluble extractives, ash values, loss on drying, swelling and foaming index were determined according to the official methods described in the Indian pharmacopeia and WHO guidelines on quality control methods for medicinal plant materials [8,9](1) .

Metal Analysis

Powdered *Euphorbia hirta* Leaf, *Cissus quadrangularis* Stem*, Ruellia tuberosa* leaf was determined by following standard procedure using Atomic Absorption Spectroscopy [10].

**Fluorescent Analysis**

The Powdered Material *Euphorbia hirta* leaves, *Cissus quadrangularis* stem and *Ruellia tuberosa* leaf were examined as such and then treated with freshly prepared acids, alkaline solutions and different solvents like $FeCl_3$, Concentrated HCl, 10% $HNO_3$, NaOH, Conc. $HNO_3$, Bromine water, 5% $H_2O_2$, $CCl_4$, methanol, acetic acid, and iodine solution. They were subjected to fluorescence analysis under UV chamber and viewed in daylight, shortwave (254 nm) and long (365) nm wave length [5,11].

**RESULTS AND DISCUSSION**

The organoleptic features of three plants were shown in the table 1.

**Table 1: Organoleptic and Macro-Morphological Studies**

| Organoleptic characters | EPH Leaves | CSQ Stem | RT Leaves |
|---|---|---|---|
| Colour | Green | Green | Dark Green |
| Odour | Characteristic | Characteristic | Characteristic |
| Taste | Bitter | Characteristic | Bitter |
| **Macroscopic characters** | | | |
| Shape | Ovate | Quadrangularis | Elliptic oblong |
| Length | 3.30cm | Varying | 4-7cm |
| Wide | 1.12cm | **Inter nodes** | 1.2-2.5cm |
| Petiole | Short | internodes 4-15 cm long and 1-2 cm thick | Ovate to oblong |
| Arrangement | Opposite | | Opposite |
| Apex | Asymmetrical | | Obtuse |

***Euphorbia Hirta Linn***

**Macroscopical Characters**

*E.hirta* plant leaf was carried by length, width and leaf area index. The leaves are generally green (but also have reddish or purplish in color) with short petiole. Leaves were completely covered with sparsely appressed pubescent. The porosity was observed little denser along with veins (3 to 5 pairs of lateral veins arising base) and scattered at axial side. The average length of leaf was 3.30 cm, width was 1.12 cm, and a leaf area index was 2.82 cm2. The base was asymmetric and apex was acute having margined in serrate to serrulate as shown in figure 1 A and B.

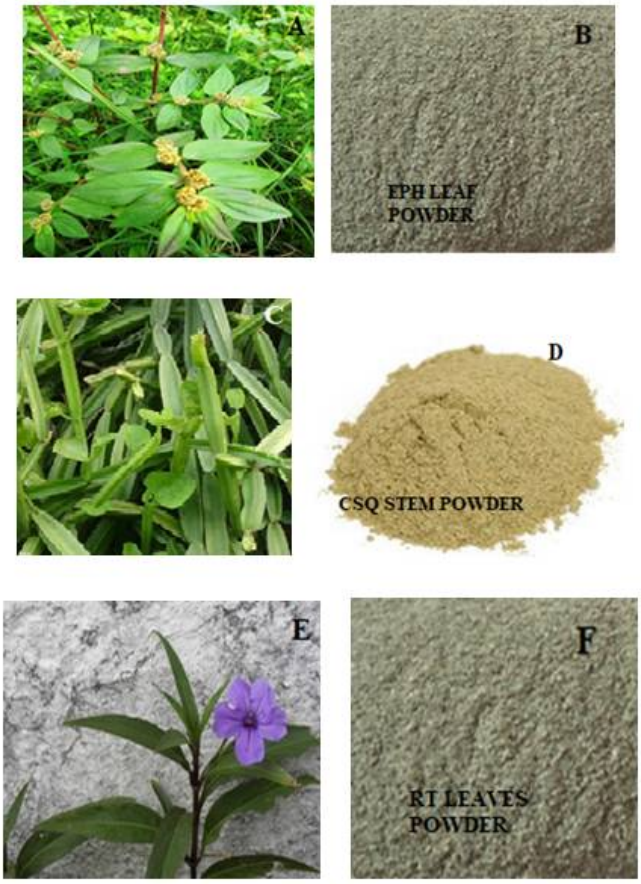


**Figure 1: A.** *Euphorbia hirta* leaves **B.** Powder **C.** *Cissus quadrangularis* stem **D.** Powder **E**. *Ruellia tuberosa* leaves **F.** Powder

**Anatomy of *Euphorbia Hirta* Leaves**

As shown in figure 2 the cross section of the lamina has two areas: a slightly curved midrib at the upper face and deeply bent at the lower side and thinner at the lamina. In the middle of the blade, cylindrical midrib, shows two epidermises (upper and lower) and a mesophyll which includes various tissues (basic and spinal cord parenchyma, primary wood and primary phloem collenchyme).

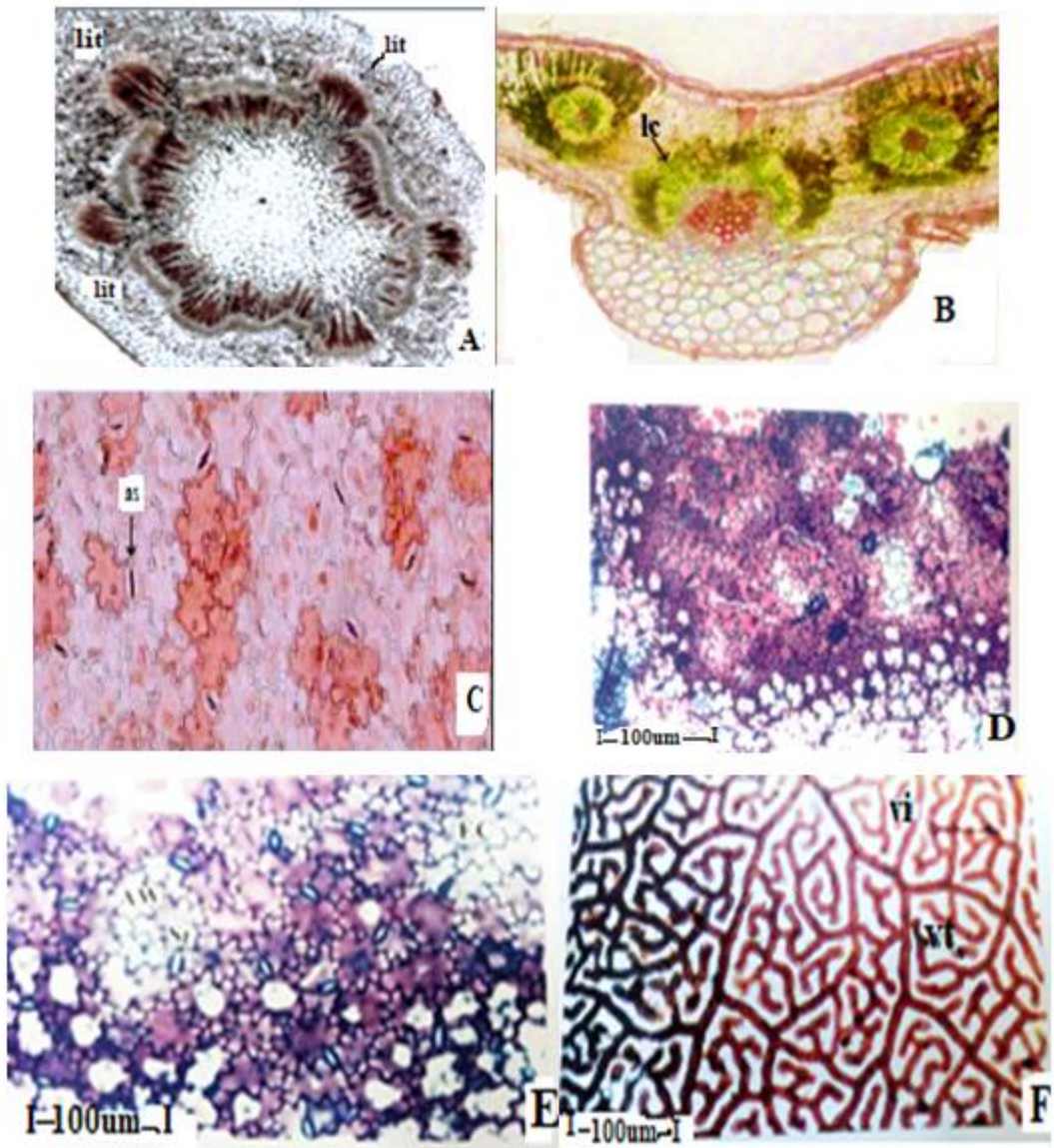


**Figure 2: A. Node (800X) lit -lateral leaf trace B. Lamina with midrib(480X) lc- latex cell C. Surface view of epidermis of Lamina(480X) as-anomocytic stomata D. Epidermis and stomata E. Stomata enlarged F. Venation pattern: vi-vein islets, vt-Vein termination**

***Cissus quadrangularis* STEM**

**Macroscopical Characters**

Leaves on the stem of the plant are simple ovate or reniform; entire or cordate; serrulate dentate or crenate-serrate; 3–7 lobed; terminal lobe triangular or sub-spathulate; subacute or cuspidate; membranous; glabrous on both sides; 3–5 × 5–3 cm; and stipules ovate or cuneate, obtuse, and deciduous shown in figure 1 C and D.

**Anatomy of CSQ Stem**

As shown in the Figure 3 TS of the stem shows rectangular - pentagonal, 1-2 layered, epidermis covered by thin cuticle, followed by 3-4 layered, circular-polygonal, chlorenchymatoushypodermis deposited more near the angle; cortex very narrow, cortical parenchymatous, 5-7 layered; pith very large, parenchymatous similar to that of region surrounded by discontinuous band of numerous, small, conjoint, collateral vascular bundles, each shielded with sclerenchymatous sheath, stele near the angle formed into strip, capped with collenchymatous band, few starch grains and rosette crystals and abundant large cells of mucilage, clusters and bundles of acicular crystals of calcium oxalate scattered throughout the section.

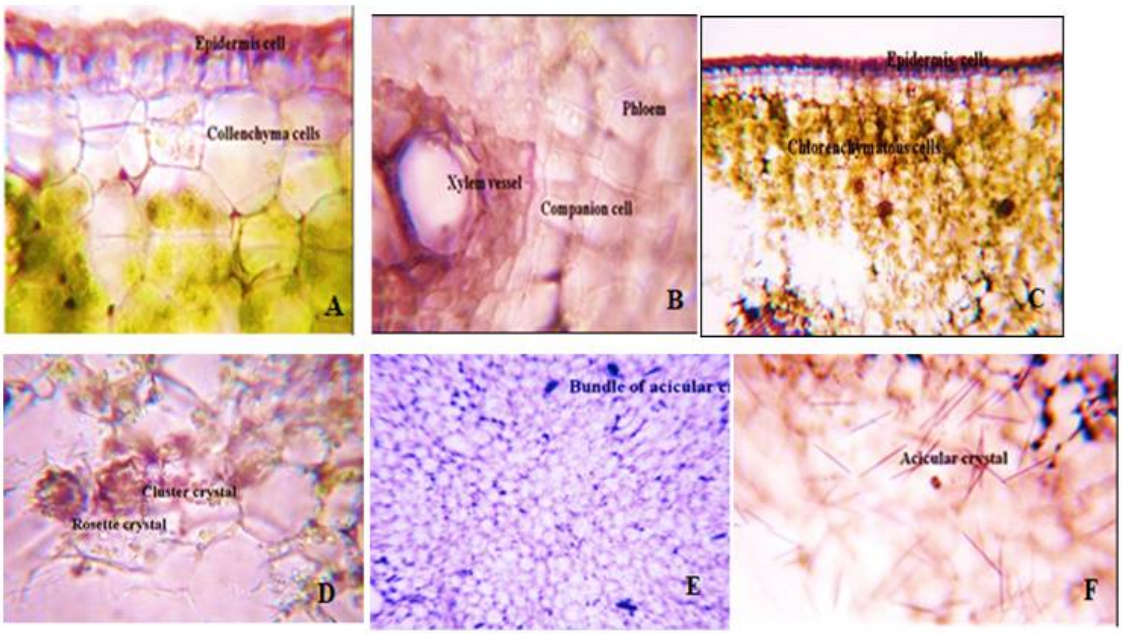


**Figure 3: A. Epidermis and Hypodermis B. Cortical Region with Vascular Bundle C. Ts with Overall Arrangement of Tissues D. Cortex in Enlarge View Shows Cluster and Rosette Crystals of Calcium Oxalate E. Pith Consisting Bundles of Acicular Crystals of CalciumOxalate F. Crystals in scattered form**

***Ruellia tuberosa***

**Macroscopical Characters**

Leaves are oblong-obovate and hairless on both sides (4-8 cm long, 1.5-4.2 cm wide). Leaf margin is undulate to approximately entire (smooth edges). Leaves are arranged oppositely along the stem as shown in Figure No.1 E &F.

**Anatomy of RT Leaves**

As shown in Figure 4 Dorso ventral T.S through lamina shows single-layered upper and lower epidermis with thin cuticle, diacytic stomata on lower epidermis, glandular sessile trichomes and few covering trichomes, collenchymatous hypodermis, ground cortical parenchyma with prominent double-layered compact palisade and loosely arranged spongy mesophyll tissue zone with chlorophyll and oil globules; spiral xylem vessels associated with sclerenchymatous fibers present discontinuing the mesophyll tissue. In midrib, a crescent-shaped collateral vascular zone consisting of xylem strands, phloem, and sclerenchymatous fibers, etc. embedded in ground tissue.

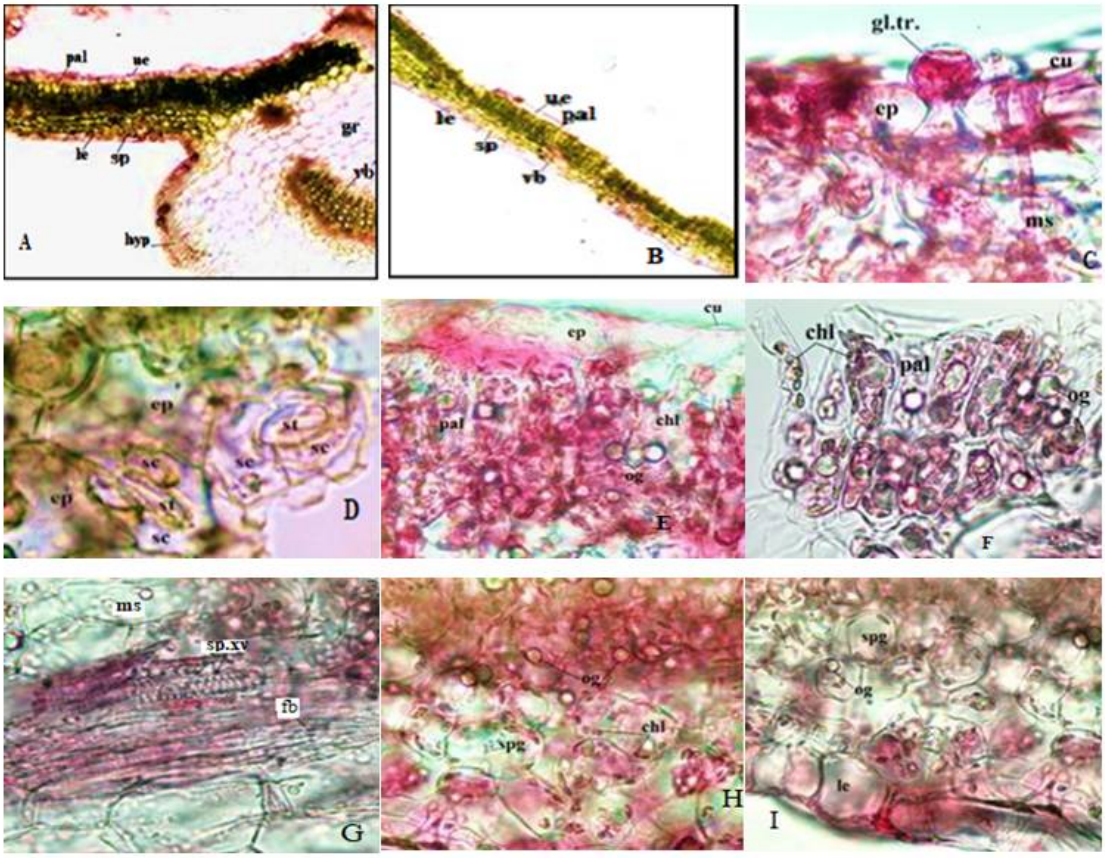


**Figure 4: A and B: T.S. through midrib and lamina showing upper (ue) and lower epidermis (le), hypodermis (hyp) , palisade (pal) and spongy parenchyma(sp), vascular bundle (vb) and ground tissue (gr) C:epidermis (ep) with thin cuticle (cu) showing sessile glandular trichome (gl.tr)mesophyll tissue (ms) D:epidermis (ep) showing diacytic stomata (st) with subsidiary cells (sc) , E and F :Palisade parenchymatous (pal) zone showing profuse oil globules (og) and chlorophyll (chl), G: Vascular zone showing spiral xylem vessels (sp.xv.) With a tuft of fibers (fb), H and I: Spongy tissue (spg) having oil globules (og) and chlorophyll (chl) attached with lower epidermis (le).**

**Powder Microscopy of EPH**

Powdered material consists of fragment of parenchyma cells, testa is formed by lignified, thick-walled macro sclereid, in surface view, trichome simple, uniseriate and multicellular; fragments of vascular tissue with spiral vessel, reticulate or bordered-pitted thickenings; starch granules are mostly simple and numerous, isolated fibres, fragment of epidermis cells shown in figure 5.

**Powder Microscopy of CSQ**

As shown in the figure 6, the powder of the stem shows plenty of cluster, rosette and acicular crystals of calcium oxalate scattered as such throughout or embedded with parenchymatous cells. Simple and compound with 2-celled starch grains scattered as such or embedded in parenchyma. Fragments of epidermis in surface view embedded with anisocytic stomata. The fibers isolated or in groups, thin walled, occasionally exhibiting dentate margin, vessels with annular, reticulate and boarded pitted thickening. Cellsof the medullary rays with pitted thickening [11,12].

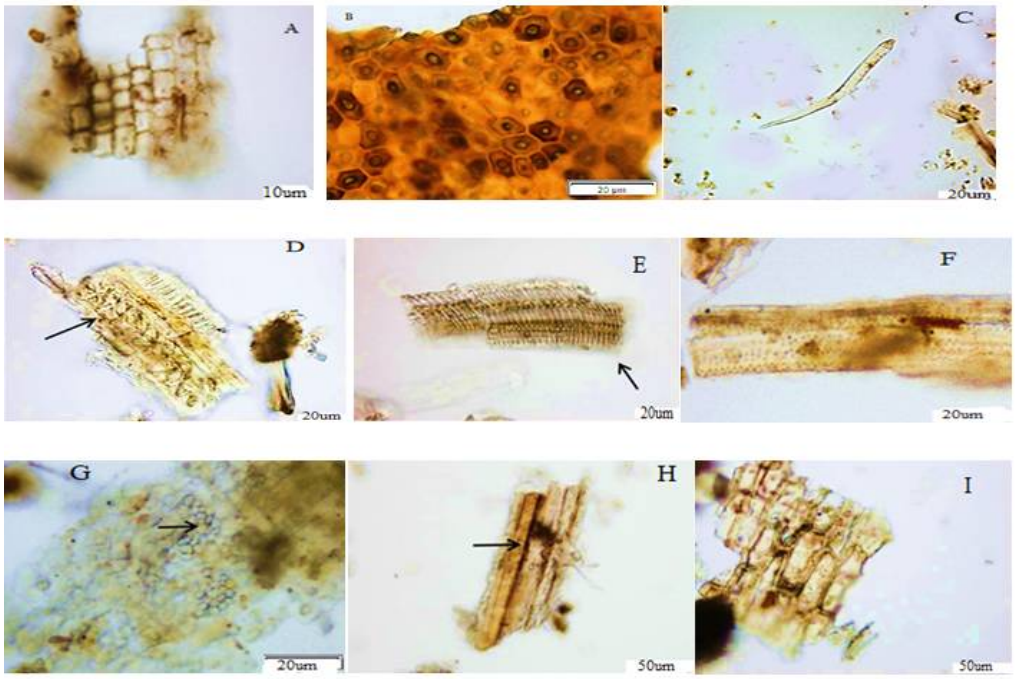


Figure 5: A) parenchyma cells (10X)10µm B) macrosclereid of testa (20X) 20µm C) multicellular trichome (arrow)(20x) D) spiral vessel (arrow) (20x) E) reticulate vessel (arrow) (20X) F) pitted vessel (20X) G) starch granules (20X) H) fibre (arrow) (10X)(50 µm) I) epidermis cells (10X) )(50 µm)

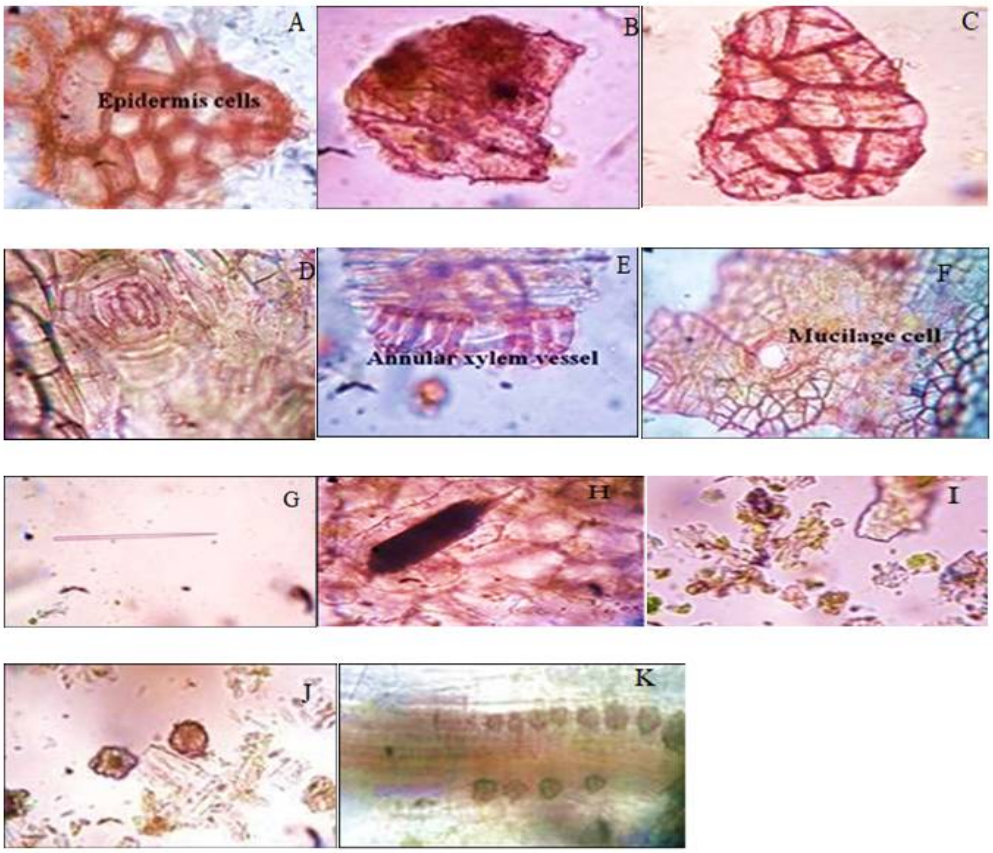


Figure 6: A) epidermal cells in surface view B) epidermal cells fixed with clusters of calcium oxalate C) epidermis overlapped with hypodermis D) epidermal cells with anisocytic stomata E) xylem vessels with annular thickening F) cells of mucilage in parenchyma G) acicular crystals H) bundle of acicular crystals I) starch grain J) clusters and rossettes of calcium oxalate K) Idioblasts-vascular strands surrounded by clusters androssettes of calcium oxalate.

**Powder Microscopy OF RT**

Fine powder dark green in colour with no salient taste and odour, shows the presence of groups of an epidermal cell with diacytic stomata, uniseriate nonglandular trichome, sessile and stalked glandular trichome, aseptate fibres with reticulate striations on thick fiber wall, parenchyma with dense cell contents and chlorophyll, few fragments of dark reddish-brown crystalline mass and prismatic crystals of Ca-oxalate [13,14] (Figure 7).

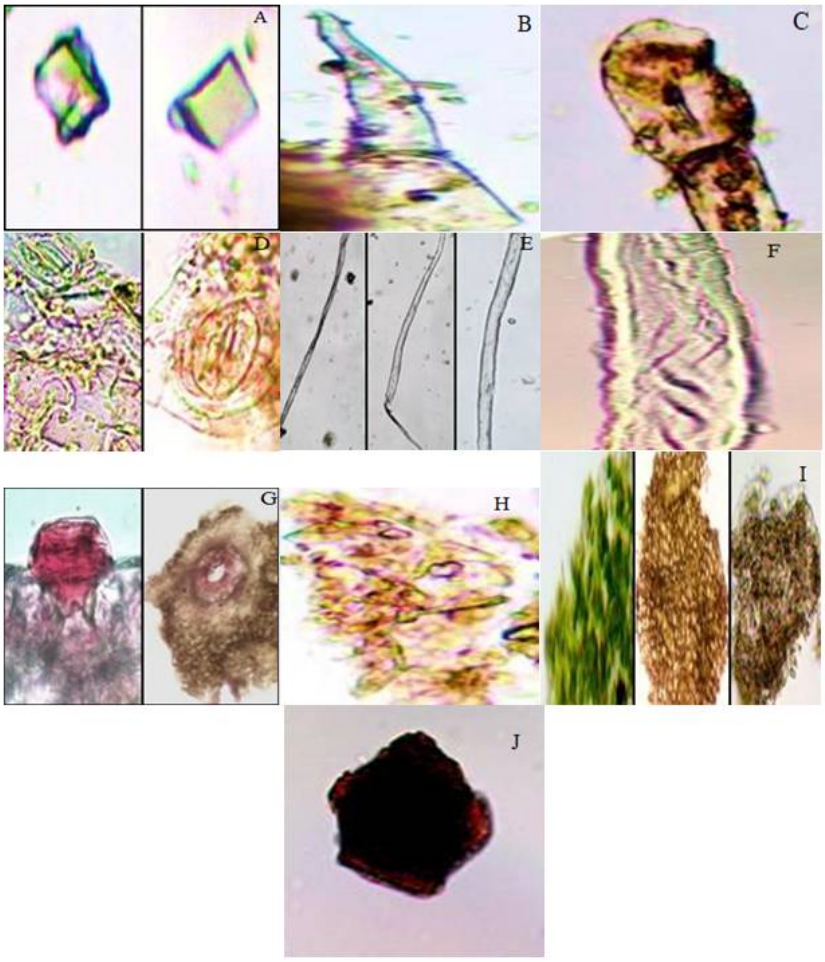


**Figure 7: A) Prismatic crystals of Ca-oxalate B) Uni seriate non glandular trichome C) Glandular trichome with oval gland and stalk D) Groups of epidermal cell with diacytic stomata E) Aseptate fiber F) Reticulate striations on thick fibre wall G) Sessile glandular trichome on an epidermal layer (longitudinal & dorsal view) H and I) Parenchymatous cells with cell contents and chlorophyll J) Reddish-brown crystalline mass**

**Physiochemical Analysis**

The physiochemical parameters are mainly used in detecting adulteration or improper handling of drugs. Extractive values are useful in the detection of adulterated drugs and also give information about the chemical constituents present in the drug. Ash values reveal about inorganic composition or earthy matter or other impurities. The results of the present study are shown in Table 2. Loss on drying infers about the percentage of moisture present in the drug. The moisture content of dry powder of *Euphorbia hirta, Cissus quadrangularis and Ruellia tuberosa* was 8.5, 7 and 4 % respectively, these results suggest that, it would discourage bacteria, yeast or fungi growth. Low total ash and acid insoluble ash in all the three plants suggest that the inorganic and non-physiological matter is less. High alcohol soluble extractive values infer the presence of polar substance like glycosides, phenols and tannins the metal analysis of three plants were as shown in table 3 [15-20].

**Table 2: Physical Parameters of plants *Euphorbia hirta* leaves, *Cissus quadrangularis* stem, *Ruellia tuberosa***

| Physical Parameters(%) | *EPH* | *CSQ* | *RT* |
|---|---|---|---|
| Total ash | 15 | 7.75 | 17.05 |
| Acid-insoluble Ash | 7.84 | 17 | 7.67 |
| Alcohol soluble extractive | 36.1 | 52.6 | 14.7 |
| Water soluble extractive | 3.2 | 2.8 | 9.34 |
| Loss on drying | 8.5 | 7 | 4 |
| Swelling Index | 5 | 7 | 2 |
| Foaming Index | 8 | 10 | 8 |

**Table 3: Metal Analysis**

| METAL | Con./ 100 g*EPH* (ppm) | Con./ 100 g *CSQ* (ppm) | Con./ 100 g *RT*(ppm) | PERMISSIBLE LIMIT (ppm) |
|---|---|---|---|---|
| Arsenic | NIL | NIL | NIL | 1.1 |
| Mercury | NIL | NIL | NIL | 1.0 |
| Lead | 0.15 | 0.55 | 0.355 | 10 |
| Cadmium | NIL | NIL | NIL | 0.3 |
| Chromium | NIL | NIL | NIL | 2 |
| Nickel | NIL | NIL | NIL | 0.1 |
| Manganese | 0.0010 | 0.0013 | 0.0045 | 0.3 |
| Zinc | 12.54 | 10.65 | 15.44 | 50 |
| Iron | 24.75 | 21.5 | 22.95 | 25 |
| Copper | 0.001 | NIL | 0.002 | 1.5 |
| Aluminium | 0.0001 | 0.0002 | 0.0001 | 2 |
| Barium | 0.0003 | 0.0002 | 0.0003 | 0.025 |
| Selenium | 0.00013 | 0.0012 | 0.00013 | 1.3 |

**FLUORESCENCE ANALYSIS**

Fluorescence analysis of *Euphorbia hirta, Cissus quadrangularis and Ruellia tuberosa* plant powders was carried out individually after treating with several solvents. Fluorescence was observed at 254 and 365 nm relating its change of colour in visible light. The observations showing the variation in colour of *Euphorbia hirta, Cissus quadrangularis and Ruellia tuberosa* are presented in Table 4, 5 and 6 respectively [21,22].

**Table 4: Fluorescence characteristics of *Euphorbia hirta***

| Chemical treatment | Visible light | UV Short 254nm | UV Longer 365nm |
|---|---|---|---|
| Dry powder | Green | Green | Green |
| Powder+1N NaOH (aqueous) | Light Green | Light Green | Dark Green |
| Powder+1N NaO(alcoholic) | Yellowish orange | Light Green | Dark Green |
| Powder+1N Hcl | Yellowish Green | Fluorescent Green | Dark Green |
| Powder+50% $H_2SO_4$ | Light Green | Dark Green | Dark Green |
| Powder+ $HNO_3$ | Yellowish orange | Light Green | Dark Green |
| Powder+$CH_3COOH$ | Light Green | Light Green | Dark Green |
| Powder+ 5%$Fecl_3$ | Dark Green | Dark Green | Dark Green |
| Powder +$HNO_3$+$NH_3$ | Light Green | Yellowish Green | Dark Green |

**Table 5: Fluorescence Characteristics of *Cissus quadrangularis***

| Chemical treatment | Visible light | UV Short 254nm | UV Longe365nm |
|---|---|---|---|
| Powder +$Fecl_2$ | Pale Green | Black | Pale Green |
| Powder +Iodine | Brown | Black | Black |
| Powder +NaOH | Light Green | Pale Brown | Pale Green |
| Powder +Hcl | Light Brown | Deep Brown | Pale Green |
| Powder +Acetic acid | Pale Green | Pale Brown | Fluorescent Green |
| Powder +$HNO_3$+Ammonia | Pale Green | Pale Brown | Pale Green |
| Powder +Methanol | Fluorescent Green | Pale Brown | Fluorescent Green |
| Powder +$H_2SO_4$ | Blakish Green | Reddish Brown | Brown Green |
| Powder +NaOH+Methanol | Fluorescent Green | Yellowish Brown | Pale Green |
| Powder +Nitric acid | Yellowish Red | Reddish Brown | Pale Green |

**Table 6: Fluorescence Characteristics of *Ruellia tuberosa***

| Chemical treatment | Visible light | UV Short 254nm | UV Longe365nm |
|---|---|---|---|
| 1N Hcl | Pink | Brownish greenish | Light pinkish |
| 1N NaOH | Greenish brown | Light pinkish | No color |
| 1N NaOH + Methanol | Light green | Brownish | Light pinkish |
| 50% KOH | Yellowish brown | Brownish black | Blackish grey |
| 50%$H_2SO_4$ | Black | No color | Fade bluish |
| Conc $H_2SO_4$ | Black | Fade pinkish | No color |
| Conc $HNO_3$ | Yellow straw | Fade pinkish | Pale bluish |
| Acetic acid | Light green | Pinkish | Bluish |
| 50%$HNO_3$ | Light pink | Fade bluish | No color |
| Acetone | Green | Pale brownish | Bluish |
| Toluene | Yellowish green | No color | No color |

## CONCLUSION

The pharmacognostic study of *Euphorbia hirta*, *Cissus quadrangularis,* and *Ruellia tuberosa* provides valuable insights into their anatomical, physicochemical, and fluorescence characteristics. The macroscopic and microscopic analyses revealed distinctive features for each plant, including leaf morphology, stem structure, and cellular components. Powder microscopy highlighted specific elements like crystals, starch grains, and trichomes, aiding in identification. Physicochemical parameters such as ash values, extractive values, and moisture content indicated the quality and purity of the plant materials. Metal analysis ensured the absence of harmful contaminants. Fluorescence analysis under different wavelengths and with various reagents offered additional means of authentication. These comprehensive findings contribute to the standardization and quality control of these medicinal plants, supporting their potential use in traditional medicine and modern drug development. The study underscores the importance of thorough pharmacognostic evaluation in ensuring the safety, efficacy, and proper identification of herbal materials for therapeutic applications.

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