

## MICROSCOPIC AND MACROSCOPIC INVESTIGATION ON AERIAL PARTS OF CENTRATHERUM PUNCTATUM CASS. A TRADITIONAL DRUG SOURCE

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<p><b>*For Correspondence:</b> Department of Biotechnology, Srimad Andavan Arts &amp; Science College (Autonomous), Trichy- 5.</p>	<p><b>ABSTRACT</b> Objective: The present study deals with the microscopical and macroscopical investigation on leaves, petioles and stems of <i>Centratherrum punctatum</i> Cass. Materials and methods: Fresh leaf sample and dried powder of aerial part of <i>Centratherrum punctatum</i> Cass were studied macroscopically and microscopically. Results: The microscopical studies revealed the presence of epidermal layer covered with thick cuticle, cortex, pith, vascular region with xylem and phloem, glandular trichomes surrounded by rosette cells, Anisocytic stomata. The quantitative microscopical studies of leaf content such as stomatal number, stomatal index, epidermal cells, vein islet number, vein termination and palisade ratio in leaf were also carried out. Conclusion: The microscopic analysis of the <i>C.punctatum</i> leaf is useful in standardization for quality, purity and sample identification. <b>KEY WORDS:</b> <i>Centratherrum punctatum</i> Cass. Asteraceae. Microscopy and leaf constant.</p>
<p><b>Received: 23.04.2018</b> <b>Accepted: 22.09.2018</b></p>	
<p><b>Access this article online</b></p>	
<p><b>Website:</b> www.drugresearch.in</p>	
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### INTRODUCTION

An authentication and quality assessment of herbal material deals with the Pharmacognosy that is based on macroscopic and microscopic characters (Heinrich, 2000). *Centratherrum punctatum* a traditional drug belonging to the family Asteraceae. *Centratherrum punctatum* Cass. is one among 33 species of the type genus *Centratherrum* and is a perennial bushy plant of 45-60 cm height. It has a well branched stem with revitalizing scented foliage and purple flower heads. Recently an vital oil containing nearly 59 different compounds has been isolated from the leaves of this plant (Globade et al. 2009). *Centratherrin*, sesquiterpene lactone, has been isolated from *C. punctatum* but its medicinal properties have not yet been established conclusively (Bevelle et al. 1981]. An interrelated species *C. anthelminticum* is known for anti-filarial (Nisha et al. 2007) and anti-hyperglycemic properties (Ani and Naidu; 2008). Previous phytochemical studies on *Centratherrum punctatum* Cass. Asteraceae included studies on preliminary phytochemical screening of different extracts of *Centratherrum punctatum* cass. micromorphological and histochemical localisation studies on aerial parts of *Centratherrum punctatum* Cass were also carried out.(Chitra and Brindha; 2014 & Chitra et al. 2014) . For standardization and quality oath purpose, the following three attribute must be verified: authenticity, purity and assay (Torey et al. 2010). Literature survey did not provide sufficient information about pharmacognostical studies of this plant. The current work aims to contribute in solving the problems of controversial drugs prevalent in Ayurveda besides helping in laying down pharmacopoeial standards.

Therefore keeping above view in mind various macroscopic and microscopic studies on aerial parts of *C.punctatum* were carried out in present study.

## **MATERIALS AND METHODS**

Authentication of the plant material: Aerial parts of the chosen plants were scatterbrained from in and around Trichy, identified using Flora of Presidency of Madras and authenticated by comparing with the sampling deposited at RAPINAT Herbarium, St. Joseph`s College, Trichy, Tamil Nadu, India **(Plate.1)**



Plate 1 - Macroscopic characteristics of *Centratherum punctatum* Cass.

Macroscopic and microscopical characters were studied as described in quality control method. Thin projection microscope. Photograph at different magnification were taken by using Nikon digital camera.

**Microscopic studies:** Microscopic studies were carried out by preparing thin section of leaf, stem, and petiole. The thin section was collected in watch glass and bleach with bleaching agent with little boiling, after that thin section were washed with water. Stained with safrannin and mounted in glycerin for observation. Thin sections were observed under binocular and projection microscope. Photograph at different magnification were taken by using Nikon digital camera 12 megapixel.

### **Quantitative Microscopy**

A piece of leaf was cut in the middle portion and boiled in chloral hydrate solution. Upper and lower epidermis were peeled out and mounted on glycerine on a glass slide. The slide was observed under microscope. The quantitative microscopic data were calculated according to the procedures given by (Salisbury, 1927 & Kokate, 1997) and Photo micrographs were taken using Carl Zeiss microscope with the help of Prog Res digital camera and some photos were taken with Nikon labophoto 2 micrographic unit. Following quantitative microscopic data were determined. i) Number of stomata present in  $1\text{mm}^2$  ii) Total number of epidermal cells in  $1\text{mm}^2$  iii) Total number of vein – islet present in  $1\text{mm}^2$  iv) Total number of vein termination in  $1\text{mm}^2$  v) Palisade ratio.

## **RESULTS**

### **Microscopic features**

#### **Anatomy of the leaf**

In cross sectional view the leaf appears undulate due to prominently projecting midrib and lateral veins **(Plate 2.1)**. The midrib is planoconvex with flat adaxial side and prominent semicircular abaxial side **(Plate 2.2)**. The mid rib is  $550\mu\text{m}$  thick and  $700\mu\text{m}$  wide. The epidermal layer of the midrib is prominent comprising fairly large radially oblong thick-walled cells with prominent cuticle. There were four or five layers of thin walled circular parenchymatous ground tissue surrounding the vascular strand. On the adaxial part of the midrib about four layers of collenchymatous cells seen beneath the adaxial epidermis. The vascular strand is single fairly wide and collateral. It consists of adaxial long, thin rows of xylem elements and fibres and abaxial masses of phloem elements **(Plate 2.2)**.

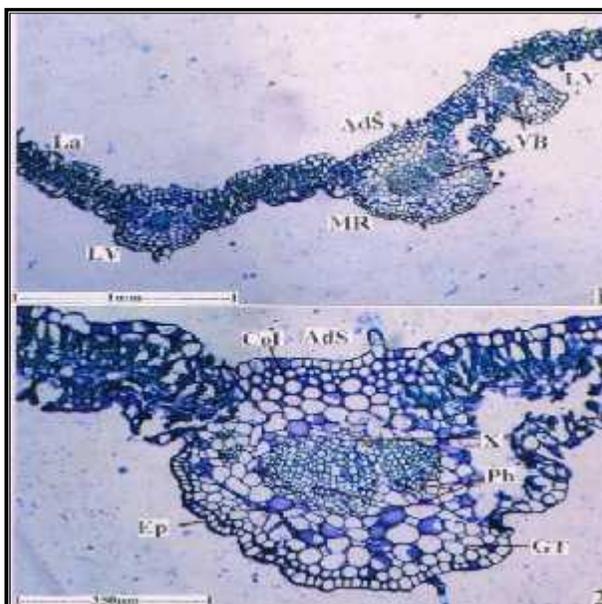


Plate 2.1 - T.S. of leaf through midrib and lateral vein.

**La- Lamina, LV – Lateral Vein, MR- Midrib, VB- Vascular Bundles, ADS-Adaxial Side.**

Plate 2.2 - T.S. of Leaf Midrib enlarged.

**Ep- Epidermis, Col- Collenchyma, X- Xylem, Ph – Phloem, GT – Ground Tissue.**

### Lamina

The lamina is smooth on both surfaces and exhibit dorsiventral symmetry. The adaxial epidermis consists of fairly large spindle shaped cells (**Plate 3.1**). Non glandular epidermal trichomes may be seen on the adaxial epidermis. The abaxial epidermis is thin and includes small, squarish, thick walled cells. The mesophyll tissue includes adaxial band of vertical cylinders of palisade cells and abaxial band of similar type of palisade cells. Inbetween these two layers there is a narrow median layer of small, lobed, loosely arranged spongy parenchyma. Stomata are common on the axial epidermis. Usually the stomata are slightly raised above the surface of the lamina (**Plate 3.3**). The lamina is 170µm thick.

### Leaf margin

The marginal part of the leaf is slightly dilated into semicircular, club shaped outline. The epidermal cells of the margin are uniformly rectangular which are radially oriented. There is a thick cuticle on the surface of the marginal cells. Palisade parenchyma radiate towards the inner boundary of the epidermal cells (**Plate 3.2**).

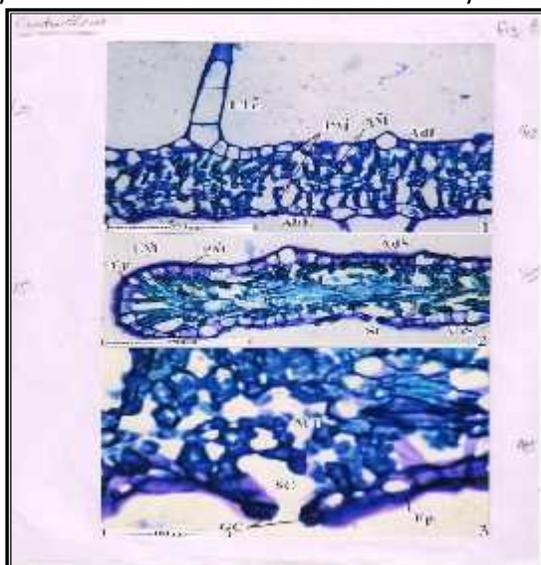


Plate 3.1 - T.S. of lamina showing dorsiventral symmetry and non-glandular trichome.

**AbE- Abaxial Epidermis, AdE – Adaxial Epidermis, ETr – Epidermal Trichome, PM – Palisade Mesophyll, SM – Spongy Mesophyll.**

Plate 3.2 - T.S. of marginal part of the lamina.

**LM – Leaf Margin, Ep – Epidermis, PM - Palisade Mesophyll, ADS – Adaxial side, ABS- Abaxial side, St- Stomata.**

Plate 3.3 - T.S. of lamina showing stomata with raised guard cells.

**GC – Guard Cell, SC – Subsidiary Cell, Ep- Epidermis, MT- Mesophyll Tissue.**

#### **Epidermal cells and stomata**

The epidermal cells are polygonal with wavy thick anticlinal walls. The anticlinal walls have sharp folding at certain places (**Plate 4.1 & 4.2**). The stomata are anisocytic having two unequal subsidiary cells adjoining the guard cells and three larger subsidiary cells encircling the guard cells and two inner subsidiary cells (**Plate 4.2 & 5.2**). The guard cells are broadly elliptical measuring  $25 \times 35\mu\text{m}$  in size (**Plate 4.1**).

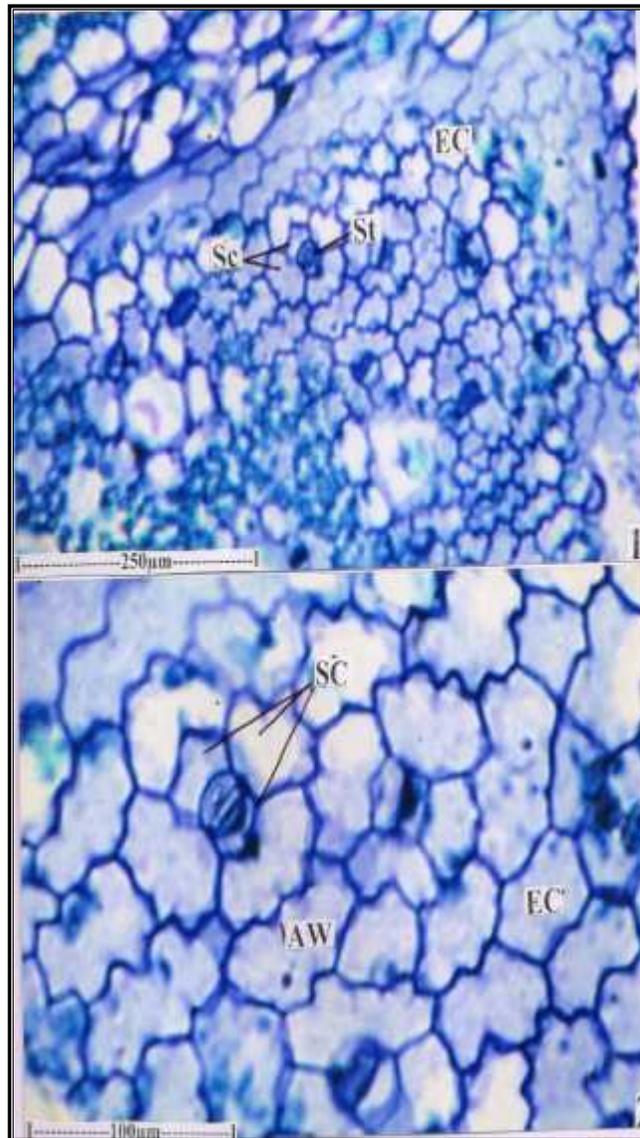


Plate 4.1 - Paradermal Section of the epidermis in surface view showing epidermal cells and stomata.

**Sc – Subsidiary cell, St - Stomata, EC- Epidermal Cell.**

Plate 4.2 - Anisocytic type of stomata.

**Sc – Subsidiary cell, AW – Anticlinal Wall, EC- Epidermal Cell.**

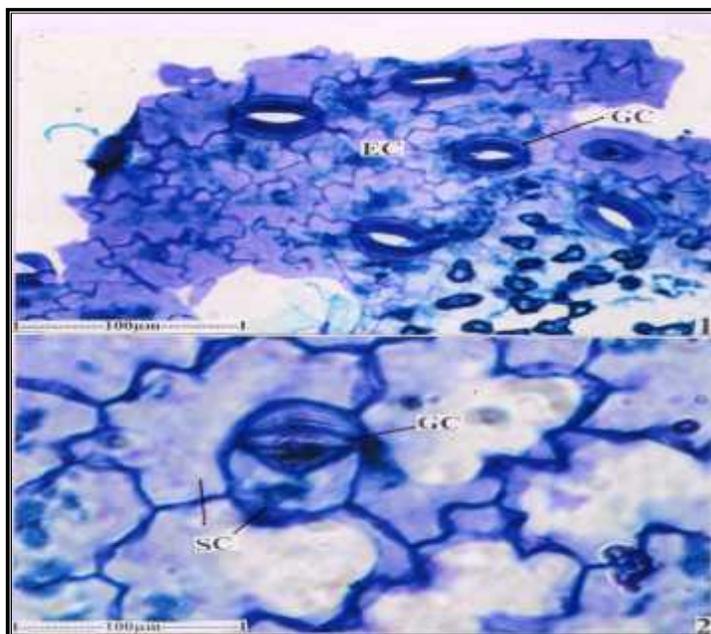


Plate 5.1 - Guard cells of the stomata enlarged.

**EC – Epidermal Cells, GC- Guard Cells.**

Plate 5.2 - A stomata with subsidiary cells.

**GC- Guard Cells, SC – Subsidiary Cells.**

Epidermal glandular trichomes are seen in abundance in the paradermal section (**Plate 6.1 & 6.2**). The glands are peltate type. It has a short one celled stalk and a circular horizontal semicircular plate of secretory head. The gland arises from a circular thick walled epidermal cell which is surrounded by about eight radiating wedge shaped rosette cells (**Plate 6.2**). The glandular head is 25µm in diameter.

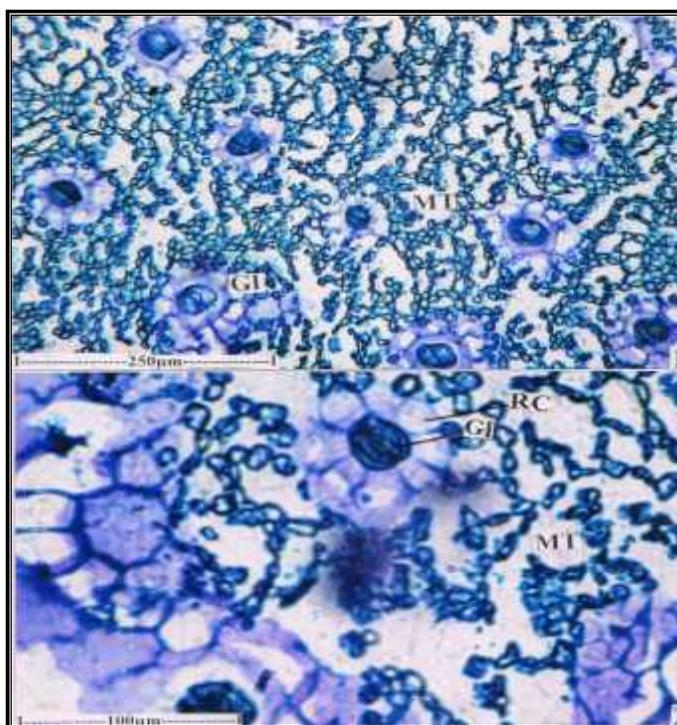


Plate 6.1 - Surface section of the lamina showing distribution of glandular trichomes.

**GI - Gland, MT – Mesophyll Tissue**

Plate 6.2 - A glandular trichome surrounded by rosette cells.

**GI - Gland, MT – Mesophyll Tissue, RC – Rosette cells.**

### **Venation pattern**

The lamina was cleared and made transparent for the study of venation pattern. The venation is densely reticulate with wide polygonal vein islets which are surrounded by prominent vein boundaries. Almost all vein islets have vein terminations (**Plate 7.1 & 7.2**). The vein terminations may be branched (**Plate 7.3**) or simple (**Plate 7.4**). The terminations are long and slender, either straight or wavy.

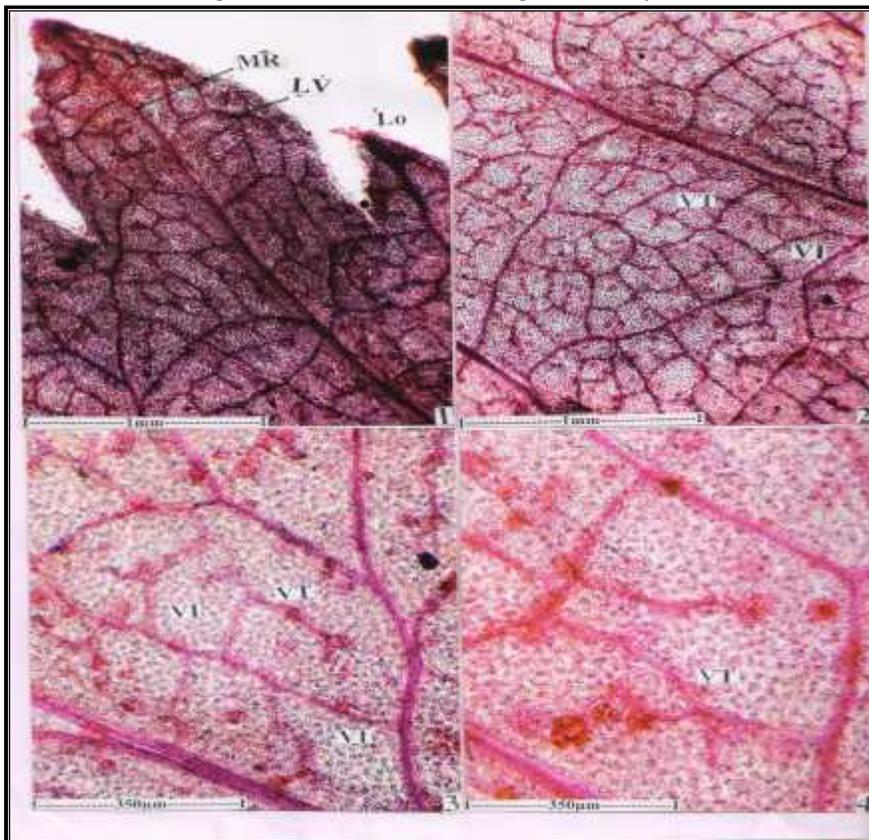


Plate 7.1 - Lobes of a leaf showing venation pattern.

**MR – Midrib, LV – Lateral Vein, Lo- Lobes**

Plate 7.2 - Venation pattern in the inner part of the lamina.

**VT – Vein Termination, VI- Vein**

Plate 7.3 & 7.4 - Vein islets and Vein terminations enlarged.

**VT – Vein Termination, VI- Vein**

### **Petiole**

The petiole can be differentiated into proximal region and distal region. The proximal petiole is semicircular with flat adaxial side and semicircular abaxial side. It has distinct layer of small squarish epidermal cells and homogenous, angular, compact parenchymatous ground tissue. The vascular system consists of a horizontal arc of three large, circular vascular bundles and one small bundle. The bundles are medullary and lateral in position. They have thick, horizontal segment of xylem elements and semicircular mass of phloem. The proximal petiole is 1mm thick and 2mm wide (**Plate 8.1**). The distal petiole is similar to the proximal petiole in general profile except that there are two small adaxial lateral wings. Moreover in addition to three major bundles there is additional wing within each wing (**Plate 8.2 & 8.2**). The bundles are thick and collateral with dense several lines of xylem elements and thick arc of prominent phloem elements. The distal petiole is 850  $\mu$ m thick and 1.8 mm wide (**Plate 9.1 & 9.2**).

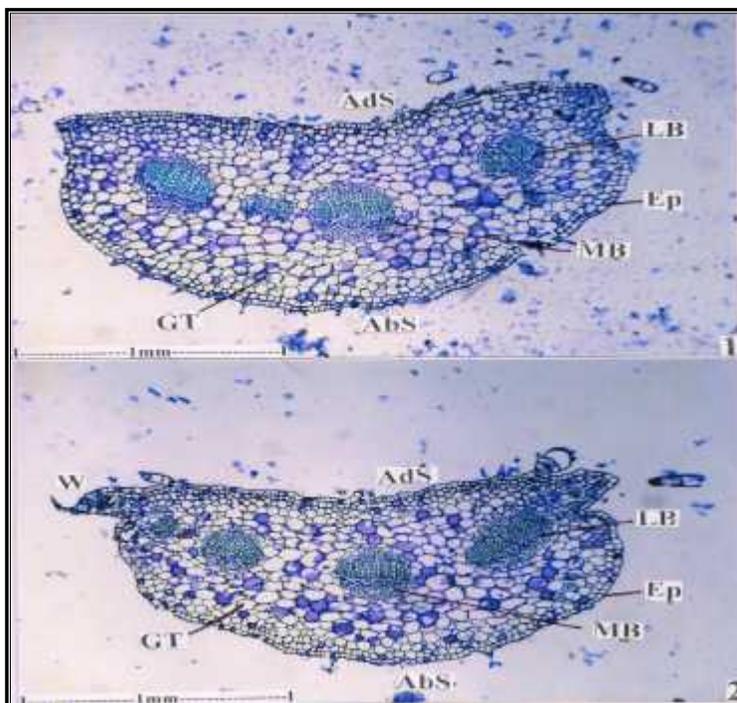


Plate 8.1 - T.S of proximal part of the petiole.

GT - Ground Tissue, AdS- Adaxial Side, Abs – Abaxial Side, MB – Median Bundle, LB – Lateral Bundle, Ep - Epidermis

Plate 8.2 - T.S of distal part of the petiole.

W-Wing, GT Ground Tissue, AdS- Adaxial Side, Abs – Abaxial Side, MB – Median Bundle, LB – Lateral Bundle, Ep – Epidermis.

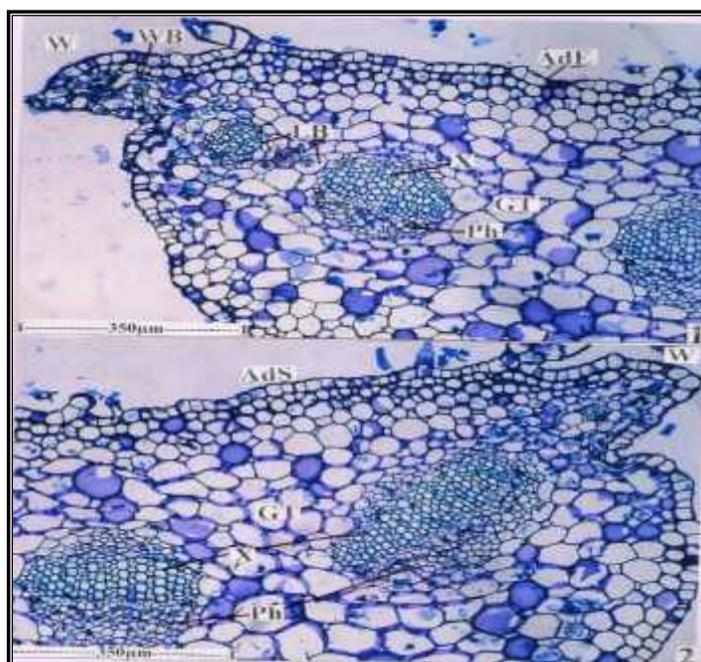


Plate 9.1 - T.S. of distal part of the petiole one sector enlarged.

W – Wing, WB – Wing Bundle, LB – Lateral Bundle, X- Xylem, Gt – Ground Tissue, Ph-Phloem, AdE - Adaxial Epidermis.

Plate 9.2 - T.S. of distal part of the petiole opposite sector enlarged.

**X- Xylem, Gt – Ground Tissue, Ph-Phloem, AdS – Adaxial Side.**

**Stem**

Young stem measuring 2.5mm thick was studied. It is circular in outline. It consists of fairly distinct epidermal layer of small, thin walled squarish cells. The cortical zone includes seven layers of thin walled parenchymatous tissue. The pith is wide homogenous and parenchymatous. The vascular system is eustele type comprising several discrete wedge shaped collateral bundles. The medullary rays are narrow and parenchymatous. The circle of vascular bundles includes both cauline bundles and leaf trace bundles (**Plate 10.1**). The leaf trace bundles are slightly larger and located just outside the cylinder of Cauline bundles (**Plate 10.2**). The vascular bundle consists of a thick sclerenchymatous cap, four or five parallel lines of angular, thick walled multiples of xylem elements. Phloem occurs in between the sclerenchyma cap and xylem strand. A small mass of sclerenchyma cell is also located at the inner end of vascular bundle (**Plate 10.3**).

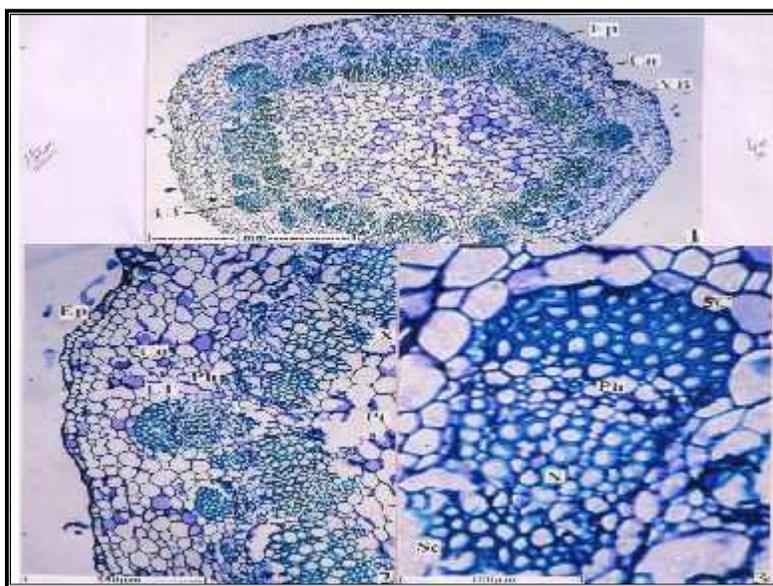


Plate 10.1 - T.S. of stem entire.

**Ep- Epidermis, Co- Cortex, LT – Leaf Trace, VB- Vascular Bundle**

Plate 10.2 - T.S. of stem a sector enlarged.

**Ep- Epidermis, Co- Cortex, LT – Leaf Trace, Pi- Pith, Phloem, X-Xylem, Pi - Pith**

Plate 10.3 - One vascular bundle of the stem enlarged.

**Ph- Phloem, X- Xylem.**

**Quantitative microscopy**

The stomatal number, stomatal index, epidermal cells, vein islet number, vein termination and palisade ratio in leaf are constant for species and can be used to differentiate closely related species. The results are presented in **Table.1**

**Table. 1 : Physicochemical constants of *Centratherum punctatum***

S. No.	Parameter	Range/mm <sup>2</sup>
1.	Stomatal number	24±5.84
2.	Stomatal index	71±9.7
3.	Epidermal cells	142±16.95
4.	Vein islet number	11±1.97
5.	Vein termination	12±3.31
6.	Palisade ratio	5±1.09

## DISCUSSION

Nowadays sophisticated modern investigate tools for evaluation of the plant drugs are available but microscopic method is still one of the simplest and cheapest methods to commence for establishing the correct identity of the source materials (Singh et al. 2010). In the present work microscopy and macroscopic evaluation of *C.punctatum* leaf were carried out. Morphological and macroscopical studies of the leaf will enable to identify the crude drug. The macroscopical characters of the leaf can serve as diagnostic parameters. The microscopical studies of the transverse section showed presence of thick cuticle, cortex, pith, vascular region with xylem and phloem. Leaves consist of glandular trichomes surrounded by rosette cells. The stomata are Anisocytic having two unequal subsidiary cells adjoining the guard cells and three larger subsidiary cells encircling the guard cells and two inner subsidiary cells. The petiole can be differentiated into proximal region and distal region. The stem consist of consists of fairly distinct epidermal layer of small, thin walled squarish cells and vascular bundles. In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Microscopic study and macroscopical standards can be useful to substantiate and authenticate the drug.

## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

## ACKNOWLEDGEMENTS

The authors are grateful to SASTRA university for providing necessary facilities to carry out the present study. We also acknowledge Dr. Jayaraman, Assistant professor, Department of Botany, Madras University for his valuable help and guidance to conduct this research work.

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