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RESEARCH ARTICLE

LEAF ANATOMICAL STUDIES OF *ATALANTIA RACEMOSA* WIGHT EX HOOK., AN IMPORTANT MEDICINAL PLANT OF TIRUVANNAMALAI, TAMIL NADU, INDIA

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ABSTRACT

Atalantia racemosa (Rutaceae) is a small tree or shrub, differing from the other species of *Atlantia* and it is always unarmed, found in the lower mountain regions of Ceylon, in Southern India and in the western peninsula from the Concan to Travancore. Plant fruit pulp paste works as an antidote to Cobra bite while the leaf juice is taken internally to treat acidity. Leaf decoction is externally applied on itching skin. Seed oil of this plant is used to cure paralysis and chronic rheumatism. Anatomical characters are potentially useful to identify small fragments of commercially important herbal products as well as toxic plants that may be of relevance in forensic investigations. In this investigation, we screen the taxonomical characters execute the identification of *Atalantia racemosa* with anatomical studies of leaf in order to provide a scientific basis for this important medicinal plant, which these observations on microscopical characters confirmed that *A. racemosa* has apparent anatomical distinctiveness and peculiarities which differentiate with any adulterant taxa.

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INTRODUCTION

Plant anatomy or phytotomy is the general term for the study of the internal structure of plants, which provides characters such as trichomes, stomata, cuticular pattern, leaf venation, wood anatomy, growth rings etc. to aid in species identification and in performing physical matches of evidence. An increasing number of indigenous medicinal plants are being used both in the formal and informal economy so that the positive identification of species and infraspecific taxa has become important. Unfortunately very few of these plants have been studied anatomically. Plants are often the cause of fatalities due to the inappropriate use of herbal medicines or the use of misidentified plants (or sometimes intentionally used in homicide and suicide). Anatomical characters can be useful as supportive evidence in forensic work to help identify the plant species in question/anatomical evidence may often give additional clues as to the identity of the plant when medical diagnosis/ chemical forensic studies turn out to be inconclusive. Anatomical characters of potential diagnostic value will be presented for a selection of herbal medicine. *Atalantia racemosa* is a small tree or shrub, differing from the other species of *Atlantia* in that it is always unarmed. It is found in the lower mountain regions of Ceylon, in Southern India and in the western peninsula from the concan to Travancore 1-3.

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In February the plant produces its white flowers which are arranged in short but distinct racemes with the peduncles a little less than a quarter of an inch in length. The berry is globular- ovoid, three- fourths of an inch wide with a long epiculus, four-celled, four seeded". (Trimen, Handbook of the flora of Ceylon and Hooker Flora of British India) introduced for the Citrus breeding work of the office of crop Physiology and Breeding Investigations for distribution later Jhon De Britto and Mahes, 2017; Ranade and Nair, 2009). *Atalantia racemosa* (Rutaceae) plants material collected from Kannalam, Villupuram District, Tamil nadu, India. Habit : Shrubs or small trees ca. 4m tall. Trunk/bark : Bark brownish, smooth; blaze yellowish. Branchlets : Branches with long spines; Branchlets terete, glabrous. Leaves : Leaves simple, alternate, spiral; petiole 0.5-1 cm long, planoconvex in cross section, glabrous, articulate; lamina 4.5-9 x 2.5-5 cm, elliptic to elliptic-ovate, apex emarginate, base acute to rounded, margin entire, coriaceous, glabrous, glandular punctate, drying olive green; midrib raised above; secondary nerves 10-18 pairs; tertiary nerves medially ramified. Flowers: Inflorescence axillary short cymes; flowers white; pedicel 0.4 cm long; stamens connate. Fruit& seed: Berry globose, to 1.9 cm long; seeds 4. Ecology: Undergrowth in evergreen forests up to 1000 m. Distribution: Peninsular India and Sri Lanka; in the Western Ghats- South, Central and south Maharashtra Sahyadris. Oral administration of *Atalantia racemosa* fruit pulp paste works as an antidote to Cobra bite (Sekar *et al.*, 2011). The leaf juice is taken internally to treat acidity (Devi Prasad *et al.*, 2013).

Leaf dodoction is externally applied on itching skin . oil from fruits is used to cure paralysis and chronic rheumatism (Sukumaran and Raj, 2010 & 2017). The leaves contain rich antioxidant molecules on natural basis. Also new antimicrobial drug can be developed for treating various disease from the selected plants (Saraswathy *et al.*, 2017). Fruit pickles by tribes (Kudavul and Dixi, 2009). Antiviral activity has been observed in Ranikheit disease virus in the crude extracts of the plant excluding the root (Bhakuni *et al.*, 1971). Leaf, rhizome and seeds are used inflammation diarrhea paralysis and chronic rheumatism arome therapy (Jayapal *et al.*, 2014). Leaf, bark and fruit are cure digestive and cough (Symydurai *et al.*, 2012). The leaf essential oil of *Atlantia racemosa* shows maximum (Larvicidal agents) activity against the three selected mosquito species namely *Culex quinque fasciatus*, *Anopheles stephenst* and *Aedes aegypti* (Arun *et al.*, 2015).

MATERIALS AND METHODS

ANATOMICAL STUDIES

Collection of plant materials: Leaves of *Atlantia racemosa* (Rutaceae) for the proposed study were collected from Kannalam, Villupuram District, Tamil nadu, India and care was taken to select healthy plants and for normal organs. The identity of the plant specimens was confirmed by the use of local Floras (Gamble, 1957; Henry *et al.*, 1987). The botanical identify was authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil anadu, India.. Herbarium specimens of this plant (Fig.1) was deposited at PG and Research Department of Botany, Government Arts College, Tiruvannamalai, Tamil nadu, India. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5 ml + Acetic acid – 5 ml + 70 % Ethyl Alcohol – 90 ml) as per the schedule given by Sass (1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) Tertiary Butyl Alcohol (TBA) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning: 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 (Johansen, 1940). The sections were stained with Toluidine blue as per method published by O'Brian *et al* (1964). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. 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 stomatal morphology and venation pattern paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of the leaf with 5 % sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were employed. Glycerine mounted temporary preparations were made for macerated materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs: Microscopic descriptions of tissues are supplemented with micrographs wherever necessary.

Photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was used as they appear bright against dark background. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esau, 1960, 1964).

RESULTS AND DISCUSSION

According to Metcalf and Chalk (1957) microscopical characters are necessary to establish the botanical identity of commercial samples of medicinal plants and play an important role in checking adulteration and substitution. Anatomical and Histochemical studies of leaves of

Atlantia racemosa: The leaf is bifacial comprising distinct adaxial and abaxial sides. In sectional view, the lamina is smooth and even. The midrib is thick and wide assuming semicircular adaxial part and slightly convex abaxial part (Fig.1.1). The midrib is 600µm thick. The adaxial dome shaped part is 550µm wide. The epidermal layer along the adaxial part consists of vertically oblong small cells with thick cuticle. The abaxial epidermal cells are small less prominent, thick called and darkly stained (Fig.1.2). The ground tissue of midrib consists of small, polyhedral the thick compact parenchyma cells. The vascular system of the midrib double starved, these an adaxial slightly curved thick plate vascular strand and an abaxial wide arch of vascular strand. Both adaxial and abaxial vascular strands are collateral. The phloem units are located on the outer (adaxial and abaxial) ends of the xylem. The xylem segments comprise several long, parallel, vertical rows of thick walled lignified angular xylem elements. The protoxylem cells are towards the center (Fig.3.3). The phloem strands are thick and dense and it includes the strands of compact parallel lines of angular cells. Thick and wide segments sclerenchyma cells are abutting closely the phloem strands. The vascular system, excluding sclerenchyma layers, measures 300µm both along the vertical plane and horizontal plane wide, circular lysigenous secretory cavities common the ground tissue. The cavities be 130µm wide.

Lamina: Lamina is smooth and even on to the adaxial and abaxial surfaces. The lamina is 250µm thick. The adaxial epidermis includes thick squarish cells with thick cuticle. The epidermis is 20µm thick (Fig 2.1). The abaxial epidermal cells are small, thick walled with their cuticle. The mesophyll tissue is differentiated into adaxial band of two layers of pillar like thin and compact palisade cells and tick abaxial zone of about eight layers of irregular and circular loosely arranged spongy parenchyma cells. Lateral vein are often seen in the lamina. The vein are more or less vertically elliptical and are composed of small xylem and phloem stands capped by very thick upper and lower sclerenchyma masses.

Leaf margin (Fig. 2.2): The marginal part of the leaf is thick and conical. It is 150µm thick. The structure of the leaf margin is similar to the mid part of the lamina. There is a thick double strands of palisade cells and thick compact spongy mesophyll layers. Calcium oxalate druses are abundant in the mesophyll tissues of the leaf. The druses are solidar; they are diffuse in distribution. The cell bearing the druses are not modified in size and shape (Fig. 2.3) The druses are 20µm thick.

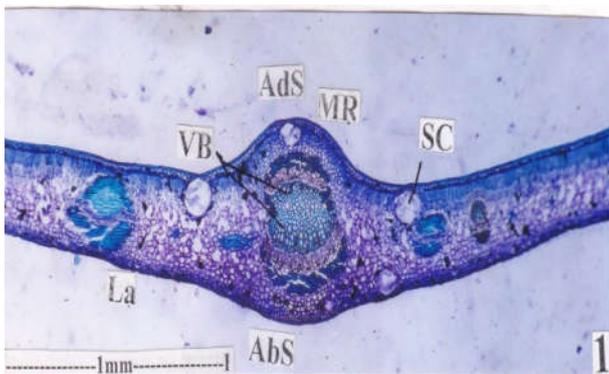


Fig.1.1. T.S of leaf through midrib

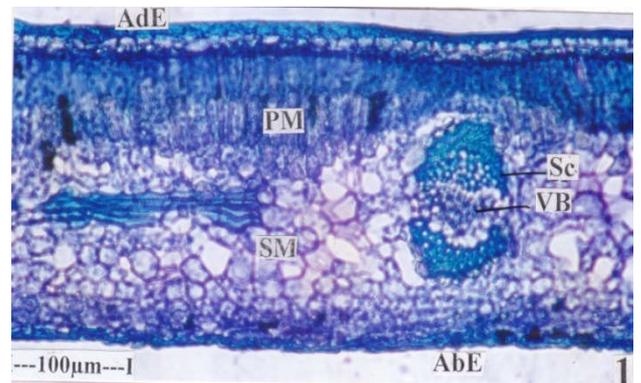


Fig.2.1. T.S of lamina

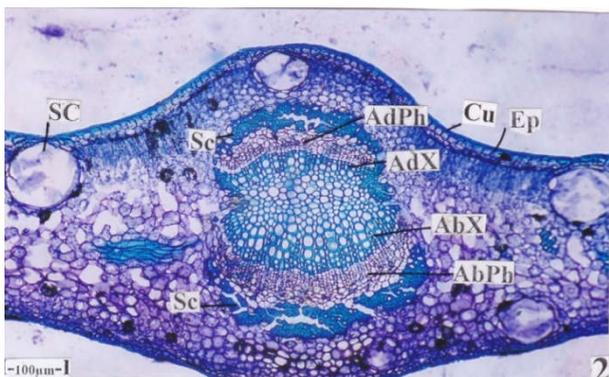


Fig.1.2. T.S of leaf midrib enlarged

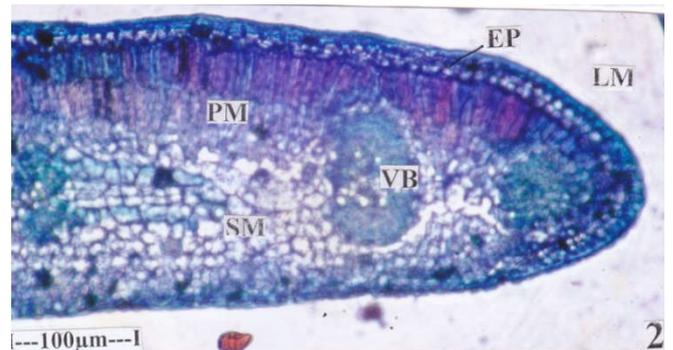


Fig.2.2. T.S of lamina – marginal part

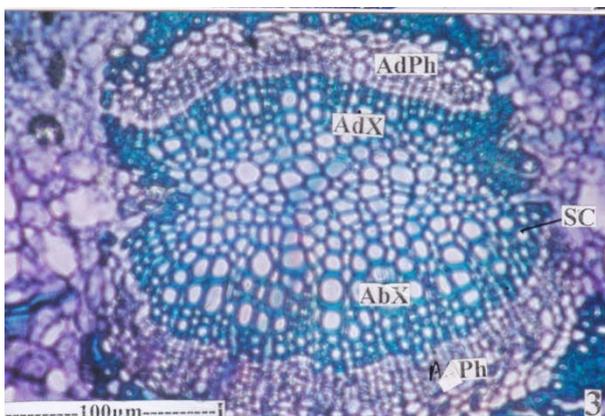


Fig.1.3. Vascular bundle of the midrib

(Ab ph-Abaxial phloem; Abs- Abaxial side; Abx- Abaxial phloem; Adph-Adaxial phloem; Ads-Adaxial side; Adx-Adaxial xylem; Cu-cuticle; Ep-Epidermis; La-Lamina; Mr-Midrib; Sc-Secretory cavity; VB-Vascular bundle)

Epidermal Tissues: The epidermal cells and the stomata were studied from paradermal sections. The cells appear in surface view. The adaxial epidermis consists of polygonal cells with highly thick walls. The anticlinal walls are straight. The cells have abundant simple circular pits. (Fig 3.1, 2,3). The pits are diffuse in distribution. Due to the presence of pits, the anticlinal walls appear beaded (Fig 3.2). The adaxial epidermis is apostomatic (without stomata). The abaxial epidermal cells are also polygonal in outside. The cells are small; they have thick and straight anticlinal walls. The cells are densely pitted; the pits are simple and circular (Fig 4.1, 2, 3). The abaxial epidermis is stomatiferous. The stoma are diffuse in distribution (Fig 4.1,2). The guard cells circular in surface view with semicircular guard cells.

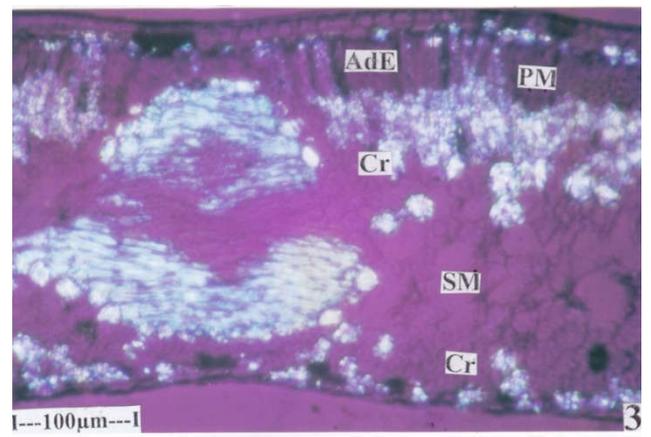


Fig.2.3. Crystal distribution in the mesophyll (AbE-Abaxial Epidermis; AdE-Adaxial Epidermis; Cr-Crytals; Ep-Epidermis; LM-Leaf Margin; PM-Palisade Mesophyll; SM-Spongy Mesophyll; VB-Vascular Bundle; Sc-Sclerenchyma)

The stomata are cyclocytic type. Each stomata is surrounded by a circle of four or five subsidiary cells. The subsidiary cells are rectangular and curved (Fig 4.1,3; 5.2). The stomata are 30 X 30 µm in size.

Venation pattern (Fig 6.1,2) The venation of the lamina is densely reticulate. The primary lateral veins are very thick; the secondary vein and the vein-lets are also fairly thick. The vein-lets are elongated and narrow. They are bordered by thick vein boundaries (Fig 6.1). The vein terminations are many in each islet. The terminations are either unbranched or branched once or twice (Fig 6.2). The terminations are slightly curved.

Starch grains (Fig 7.1,2) Starch grains are abundantly in the mesophyll cells of the leaf. The starch grain simple type with central hilum and + shaped polarimark.

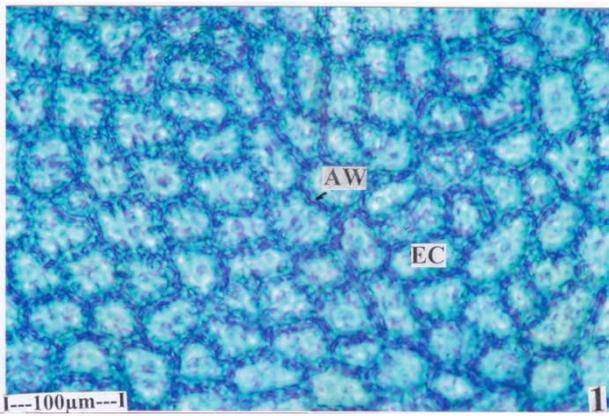


Fig.3.1. Paradermal sections of the adaxial epidermis

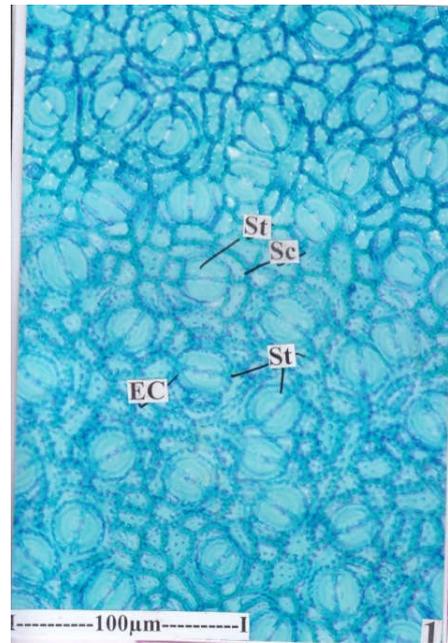


Fig. 4.1. Abaxial epidermis in surface view

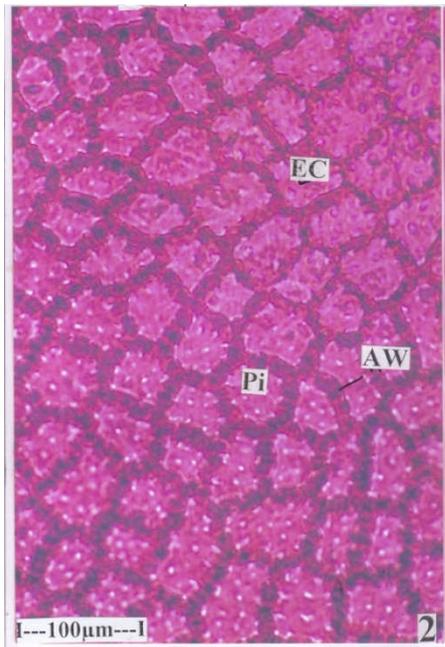


Fig. 3.2. Epidermal cells in surface view

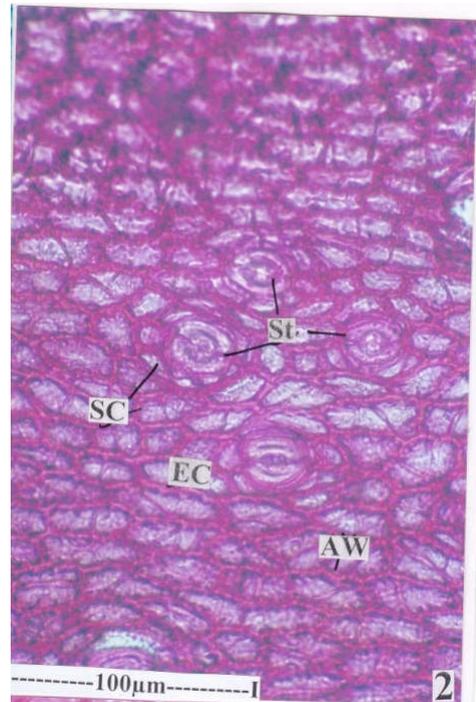


Fig. 4.2. Leaf paradermal section

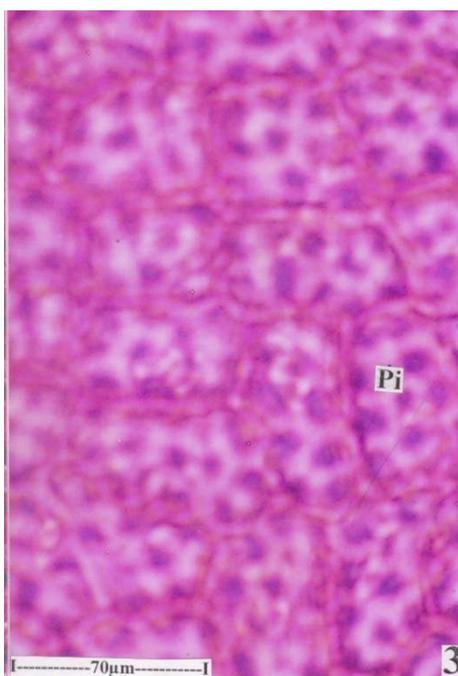


Fig. 3.3. Showing thick beaded anticlinal walls and simple pits. (AW-Anticlinal walls; EC-Epidermal Cells; Pi-Pits)



Fig. 4.3. Leaf paradermal section (AW-Anticlinal walls; EC-Epidermal cells; GC-Guard cells; SC-subsiary cells; St-Stomata)

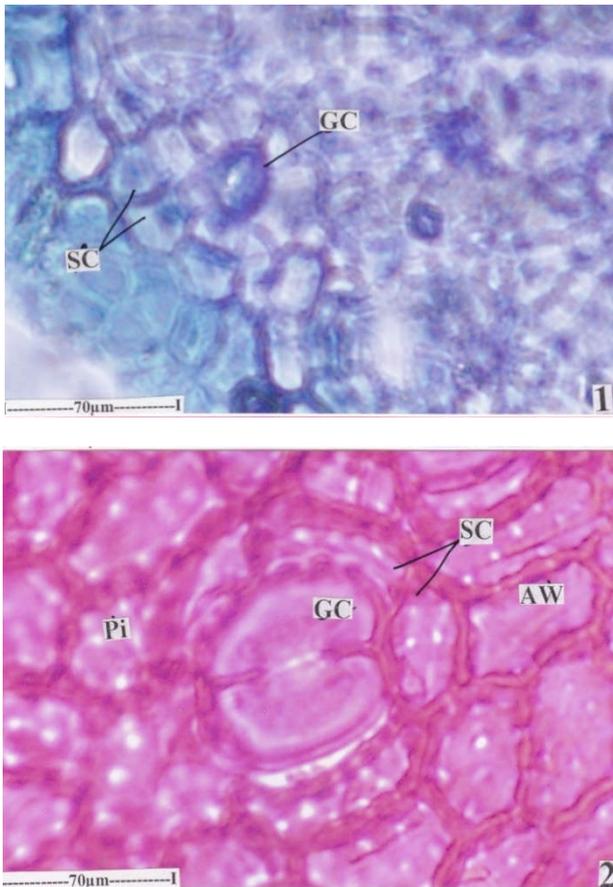


Fig. 5.1& 2. Stomata enlarged showing cyclocytic subsidiary cells (AW-Anticlinal walls;; Gc-Guard cells; Pi-Pits; Sc-Subsidiary cells)

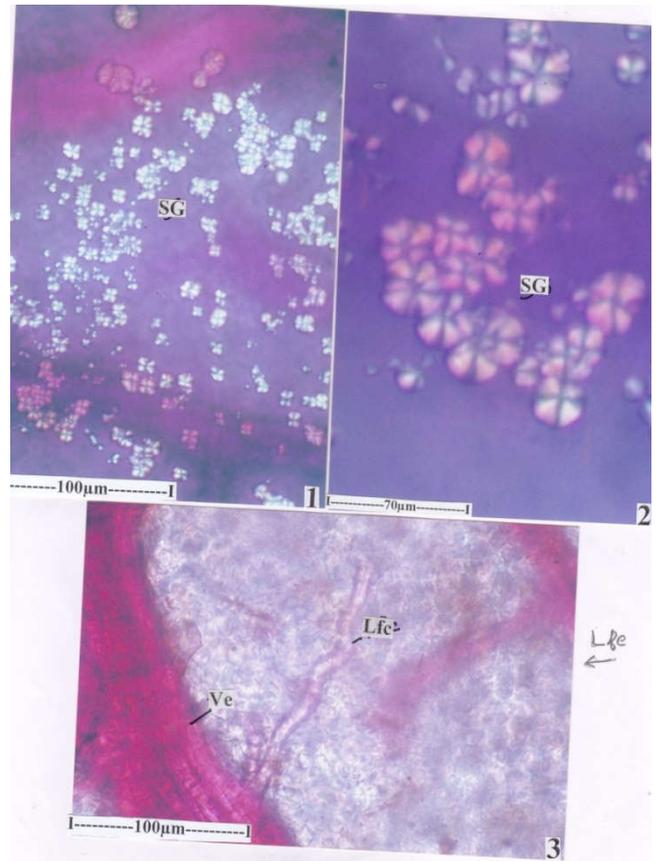


Fig.7.1. Starch grains in the seaf mesophyll (as seen under polarized light) 3Foliar scleroid in the leaf.

The starch grains are 10µm in diameter. Foliar scleroids (Fig. 7.3) Sclerides are often seen in the leaf. They are commonly seen associated with the veins. The sclerids are long, thin, unbranched curved thread like and lignified.

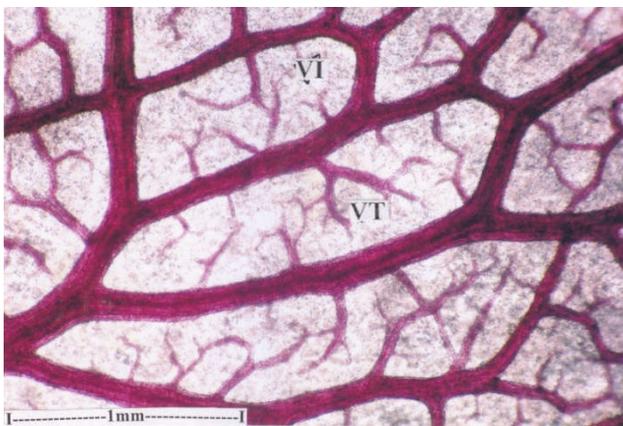


Fig.6.1 Venation pattern of the lamina

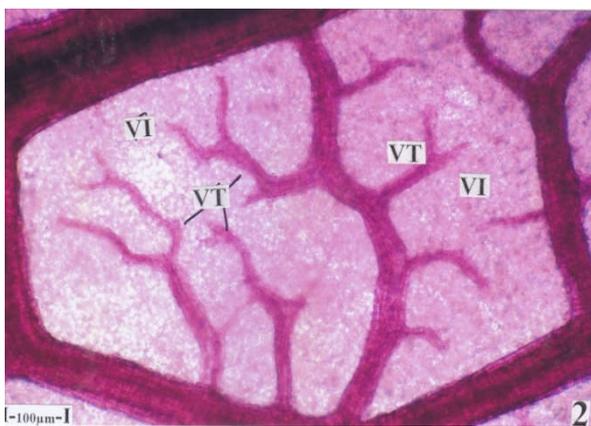


Fig 6.2. Vein-islet and vein terminations enlarged (VI-Venation Islet; VT- Vein Terminations)

Petiole (Fig 8.1, 2,3). The petiole is semicircular in sectional view. The adaxial side is flat and the abaxial side is convex (Fig8.1). The petiole is 1.3mm in horizontal plane and 1.1mm in vertical plane. The epidermal small epidermal cells with smooth the thick cuticle. The ground tissue of the petiole is homogenous, parenchymatous and thin walled. These are wide, circular secretory cavities distributed in the ground tissue. The cavities 40µm wide (Fig 8.2). There is a single vascular bundle comprising two segments (Fig 8.3). On the adaxial part is a flat thick vascular segment which consists of short radial rows of xylem and xylem fibres and adaxially located phloem unit.

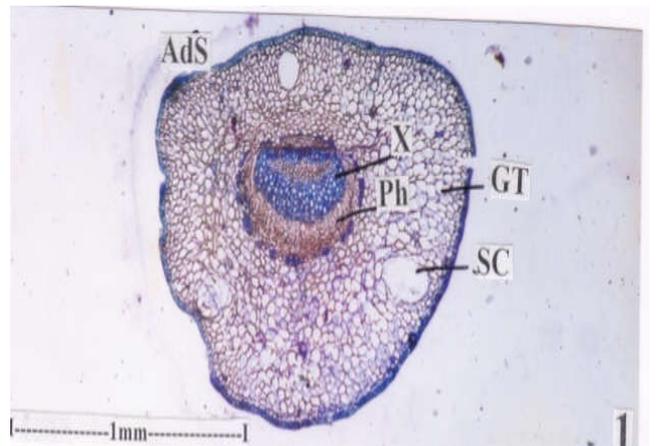


Fig. 8.1. T.S. of petiole – entire view

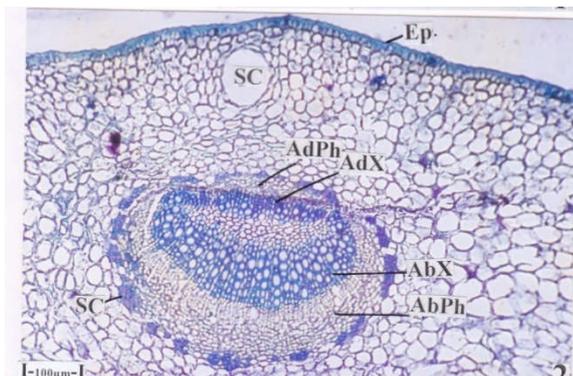


Fig. 8.2. T.S. of petiole – A sector

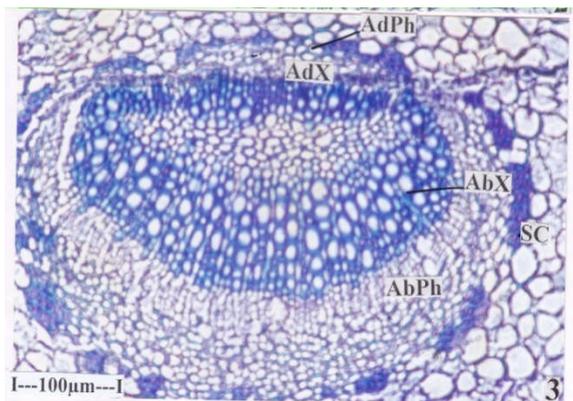


Fig. 8.3. T.S. of petiole – vascular strand enlarged (Ab Ph- Abaxial Phloem; Abx- Abaxial xylem; AdPh-Adaxial phloem; Adx-Adaxial xylem; Ep-Epidermis, Ph-Phloem; SC- Secretary Cavity; X-Xylem)

The abaxial segment includes a thick are shaped xylem strand and wide and thick are of phloem formed on the lower part of the xylem (Fig 8.3) The entire vascular completed in enclosed within a thin discontinuous layer of sclerenchyma cells. The vascular structures measure 480µm in horizontal plane and 350µm in vertical plane.

Summary and Conclusion

Atalantia racemosa a rare species in Tailnadu is known for its ethnomedicobotanical relevance. Few studies have been carried out by other researchers which have fragmentary information on specific parts. No Morphological and antatomical studies have been done till date. Our investigation highlights the botanical standardization of whole plant of *Atalantia racemosa*, which is claimed as potent ethnodemical plant in the form of drug. This work presents a descriptive study of the anatomical characters of leaf.

We conclude that, the anatomical findings viz., xylem and phloem fibers, vessel elements, epidermal tissues, calcium oxalate crystals, trichomes, and unique foliar architecture with micrographic information which has provided taxonomic value for identifying and classifying the desired plant taxon to the other against adulteration.

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