

Morpho-Anatomical studies of *Atalantia racemosa* Wight ex Hook., an important medicinal plant of Chittoor Dt. of Andhra Pradesh, India.

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Abstract: *Atalantia racemosa* Wight ex Hook., belongs to the family Rutaceae and is widely used as ethnomedicine in and around chittoor district of Andhra Pradesh for various ailments. In this paper we attempted for authenticity in morphological identification of *Atalantia racemosa* with the Morpho-anatomical studies of root, stem and leaf in order to provide a scientific basis for this important medicinal plant. The observations on taxonomical, macroscopical and microscopical characters confirmed that *A. racemosa* has apparent anatomical distinctiveness and peculiarities which differentiate with any adulterant taxa.

Key words: *Atalantia racemosa*, Morpho-anatomy, Adulteration.

Introduction

Botanical identity is important prerequisite for understanding the analysis of medicinal properties of any plant. Many of the plant species are also sold in herbal drug market by the same vernacular name. If the plant identity of the drug is incorrect, the entire work on the plant becomes invalid (Tulasi Rao *et al.*, 2012). Sivaji *et al.*, (2012) and Ramesh *et al.*, (2013) worked on the taxonomic ambiguity and adulteration of phytodrugs.

In this botanical investigation, *Atalantia racemosa* was selected due to the interesting ethnobotanical claim and morphological and microscopical studies of Root, Stem and Leaf were done as an attempt to establish genuine morpho-anatomical characterization and to differentiate with other adulterated speculated species.

The observed Adulterants of *Atalantia racemosa* are *Glycosmis pentaphylla* (Retz.) DC., *Pamburus missionis* (Wight) Swingle. and *Toddalia asiatica* (L.) Lam. of Rutaceae family.

Taxonomic Description of *Atalantia racemosa*

Atalantia racemosa Wight & Arn. Prodr. 91.1834; Hook. F. Fl. Brit. India 1:512, 1875; Gamble, Fl. Pres. Madras 1:114. 1957.

Family : Rutaceae (Citrus family), Common names are Bombay *Atalantia*, Wild lime.

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Vernacular name (Telugu) reported are Adavinimma, Kondanimma, Karunimma, Murikinimma.

Atalantia racemosa is a shrub/ small thorny tree with a compact crown, upto 4 m tall. Branches are many with long, strong, straight, sharp axillary spines with brachlets terete, glabrous. Bark is brownish, smooth; blaze yellowish. Leaves are simple (unifoliolate), alternate, spiral; petiole 0.5-1.0 cm long, articulate; lamina 4.5-9.0 cm, elliptic to elliptic-ovate, base acute to rounded, margin entire, apex emarginate or obtusely acute, coriaceous, glabrous, gland dotted, drying olive green, midrib raised above; secondary nerves 10-18 pairs; tertiary nerves admedially ramified. Flowers are white coloured, arranged in axillary elongated racemes, faintly fragrant; pedicels 0.4 cm long. Sepals 4, regularly lobed. Petals 4, free, white. Stamens 8, filaments fused into a staminal tube; anthers are large, heart shaped. Ovary superior, 2 locular, ovules 2 per locule, axile. Fruit berry, globose, 2 cm across. Seeds-4. Flowering and fruiting occurs in December- May (Fig.1A-1D). Ethnobotanical documentation in Chittoor district revealed that *Atalantia racemosa* is a potent medicinal plant which brought the interest to investigate (Fig. 1.E)



Materials and Methods

Collection of Plant material

Plant material (Root, Stem and Leaves) of *Atalantia racemosa* were collected from Papavinasana theertham and Chakra theertham in Tirumala (Fig.1.E& 1.F).

Identification of the plant was done by the second Author. Perusal of standard literature (Gamble, 1915; Madhavachetty *et al.*, 2018), collections from the herbarium of Botany Department, S.V University, Tirupati and e-herbarium (K, JSTOR) confirmed the taxonomic identity of the selected taxa. Voucher specimen deposition (SVUTY/RT-NPD/3908) was done in the Herbarium, Department of Botany, Sri Venkateswara University, Tirupati.

Transverse sections (T.S) of fresh root, stem and leaves of *A.racemosa* were made and were immobilized in FAA solution (formalin: glacial acetic acid: 70% ethyl alcohol; 5:5:90) for macro- and microscopic observations (Johansen, 1940). Microtomy was done according to Sass (1940) and modified protocol of Ramesh *et al.*, (2013). Powder microscopic studies were carried out by examining the powder of the plant samples as well as by macerating the plant samples using Jeffery's maceration fluid (Johansen, 1940; Sass, 1940). Photomicrographs were taken using Olympus BX51 light microscopy coupled with Olympus DP70 digital camera. The micromorphological and anatomical observations were made at different magnifications (Fig.2.A-2.P).



Figure 1: A: Habit

B: Twig

C: Flowering stage D: Fruiting stage



E: Ethnobotanical documentation



F: Plant raw material (Root, Stem and Leaf)

Results and Discussion

The studies reveal the macroscopic, microscopic features (anatomy of root, stem and leaf), powder microscopy, cell inclusions, measurements of different tissue systems and cells of *Atalantia racemosa*.

Macroscopic (Organoleptic) Studies

Macroscopic studies reported are as follows: The root is thick, woody, brown, pungent, aromatic odour and lite bitter taste. The stem is thick, very hard, woody, rough, blackish,

aromatic and bitter taste. The leaf is unifoliate, glossy, glabrous, gland dotted, thick green, olive green when dry, pungent, aromatic and bitter taste.

Morpho-Anatomical studies

Anatomy of Root (2.A- 2.D)

Thick old root was studied measure about 2.8 mm in diameter. The root consists of outer highly fissured and wavy periderm, narrow cortical zone, thick secondary phloem and dense cylinder of secondary xylem. There are

three layers of successive periderm which are highly fissured with reflexed broken periderm segments. In between the successive periderm cylinders are seen narrow, compressed regions of cortical parenchyma cells. The cells of the periderm are suberised and curved. The outer most part of periderm consists of intact layer of epidermis and hypodermal cells. The epidermal cells are highly thick walled, with narrow lumen. The hypodermal layer of cells are vertically oblong and thin walled. The cortical zone consists of angular, thin walled compact parenchyma cells which possess dense starch grains. There are wide, slightly

wavy walled secretory cavities in the cortical zone (Fig.2.C).

Secondary phloem: Secondary phloem zone is 230 mm thick. It consists of about six successive thick cylinders of discrete segments of fibres. The segments are rectangular with narrow space in between segments. In secondary phloem tissue occurs in between the cylinders of fibres (Fig.2.B).

The phloem elements are almost crushed, collapsed and compressed. The phloem just outside the xylem cylinder is intact and noncollapsed consisting of functioning sieve elements.

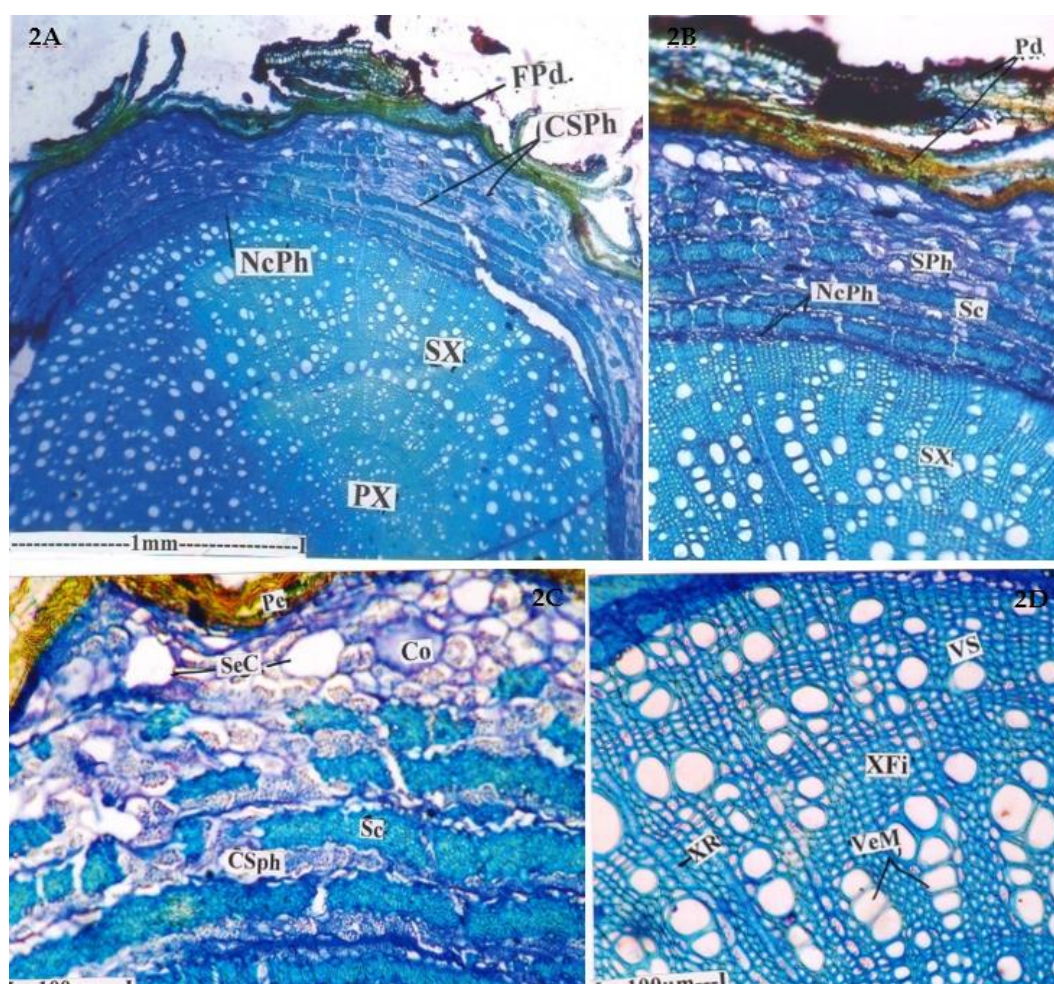


Figure 2.A: Transverse section. of Root-enlarged; **2.B:** T.S. of root periderm; **2.C:** T.S. of root collapsed secondary phloem; **2.D:** T.S. of root secondary xylem.

FPd: Fissured periderm; PX: Primary Xylem; SX: Secondary xylem; CSPh: Collapsed phloem; NcPh: Non-collapsed phloem; SPh: Secondary phloem; Sc: Sclerenchyma; Co: Cortex; Pe: Periderm; XFi: Xylem fibres; VeM: Vessels in multiples; XR: Xylem ray; VS: Vessel solitary;

Secondary xylem: The secondary xylem is a thick, dense cylinder with central core of primary xylem (Fig. 2.A). The xylem comprises distinct circles of vessels in the

inner zone. In the outer zone the vessels are the solitary or short and long multiples. The vessels are narrow or wide and thick walled. The vessels are 10-40 mm in diameter.

Xylem fibres (Fig.2.D): These are angular in transactional view, thick walled, lignified and in regular radial lines. The xylem rays thin, straight or slightly curved and the ray cells are radially elongated. Some of the fibres and all ray cells possess dense accumulation of starch grains.

Anatomy of Stem (Fig. 2E & 2F)

Thick, old stem was studied. The stem measuring 5mm thick was studied. The stem consists of a thin superficial periderm, narrow cortex, thick secondary phloem and dense and thick secondary xylem. The periderm is superficial, and it consists of two thin layers of phellem cells; the phellem is broken frequently and raised the phellem layers occurs a thin space of three to five radial files of cortical cells. Beneath the periderm zone is cortical tissue which consists of several brick shaped rectangular cells which are in horizontal, compact layers. Wide circular secretory cavities and thick fragments of fibres are sparsely seen in the cortical zone.

Secondary phloem: Secondary phloem is thick comprising several tangential discontinuous blocks of sclerenchyma cells. The phloem elements located in between the sclerenchyma segments are collapsed and the cells are crushed. The parenchyma cells of the collapsed phloem are intact and dilated. The innermost part of the secondary phloem is the non-collapsed, intact tissue. The cells of the non-collapsed phloem are in regular radial compact layers.

Secondary xylem: The secondary xylem is thick and dense cylinder and exhibits numerous narrow, distinct growth rings. The growth rings are abrupt, and the growth ring boundaries are marked by thick bands of parenchyma. The vessels diffuse in distribution; they are circular in sectional view and the diameter of the vessel vary from 20-50 mm in diameter.

The axial xylem parenchyma exhibits to various distribution in relation to the association to vessels. The parenchyma cells are paratracheal banded and paratracheal scanty. Paratracheal banded parenchyma cells are in the tangential bands and pass through touching the vessels. The

paratracheal scanty parenchyma cells are seen in thin, incomplete sheath around the vessels. Sometimes the parenchyma may be apotracheal band, not touching the vessels. Xylem rays are thin comprising single row of elongated cells. The rays are weak, and they curve as they pass across the vessels. The xylem fibres are small, angular in outline, thick walled and lignified.

Crystal distribution (Fig.2.H): Calcium oxalate crystals of prismatic and pyramidal types are abundant in pith cells and in the xylem ray cells. The crystals are random in distribution and vary in shape and size. The cells possessing the crystals are not modified in shape and size.

Anatomy of Leaf (Fig. 2G)

The leaf consists of a thick midrib and thin smooth lamina (Fig.2G). The midrib is biconvex with semicircular parts both on the adaxial and abaxial sides. The adaxial part of the midrib is broad and thick and top of the midrib is flat. The abaxial part is wider and thicker and semicircular. The midrib is 610 mm in vertical plane and the adaxial part is 450 mm wide and 50mm thickness. The abaxial part of the midrib is 550 mm wide.

The epidermal layers of the midrib are thin and the epidermal cells are smaller with thick cuticle. Inner to the epidermis are two layers of small, thick walled elliptical cells followed internally by 3 to 5 layers of larger darkly stained cells. The vascular system is wide and occupies a major part of the midvein. The vascular system consisting of plano-convex cylindrical; the adaxial part is flat and the abaxial part is a wide deep bowl shaped. The adaxial flat part includes several vertical compact thick-walled xylem elements and a thick horizontal layer of darkly stained phloem elements. The bowl-shaped part has several vertical layers of wide, circular thick-walled xylem elements and thick layer of phloem elements present in the xylem along lower side. The central core has compact, angular thick-walled lignified ground cells. There is a flat thick segment of fibres on the adaxial part of the phloem. The bowl shaped abaxial vascular cylinder of xylem and phloem is ensheathed by thick densely stained fibres which are narrow and thick walled and lignified.

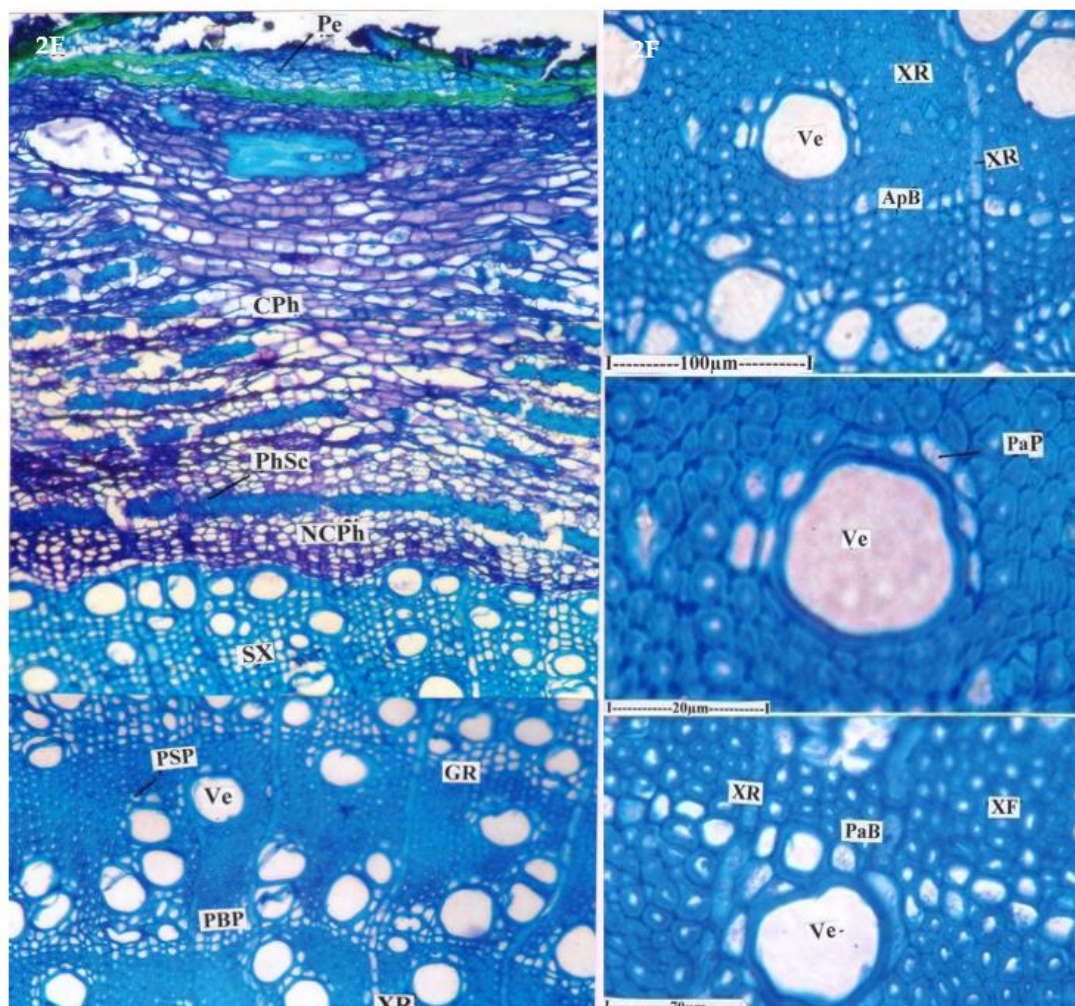


Figure. 2E & 2F: T.S. of stem showing periderm, cortex, parenchyma, banded parenchyma secondary phloem and secondary xylem and vessels

Pe: Periderm; GR: Growth ring; PBP : Para tracheal banded parenchyma; SX : Secondary xylem;XF : Xylem fibre; XR : Xylem ray; PSP : Paratracheal scanty parenchyma; CPh : Collapsed phloem; PhSc : Phloem sclerenchyma; NCPPh : Non-collapsed phloem; ApB: Apotracheal banded parenchyma;PaP : Paratracheal parenchyma; PaB : Paratracheal band; Ve : Vessels.

Lamina: The lamina is dorsiventral and bifacial. The surface of the lamina is smooth and even. The lamina is 180 mm thick. The adaxial epidermal layer consists of small spindle cells with thick cuticle. The abaxial epidermal cells are slightly larger and have thick cuticle. There are two layers of oblong thin walled hypodermal cells. There is a single layer of short compact cylindrical palisade cells along the adaxial part. The palisade cells are 50 mm thick. The spongy mesophyll tissue consists of 8 or 9 layers of tangentially elongate thin walled more or less

compact parenchyma cells. There are prominent and less prominent lateral veins and veinlets distributed along the median part of the mesophyll tissue. Petiole anatomy was reported in Fig. (2.1)

Crystal distribution: Calcium oxalate crystals of minute prismatic bodies are seen in the subepidermal (hypodermal) cells and in the lower epidermal cells. The crystals are in single layer and they appear bright white due to birefringent property when viewed under polarised light. (Fig. 2.O & 2.P)

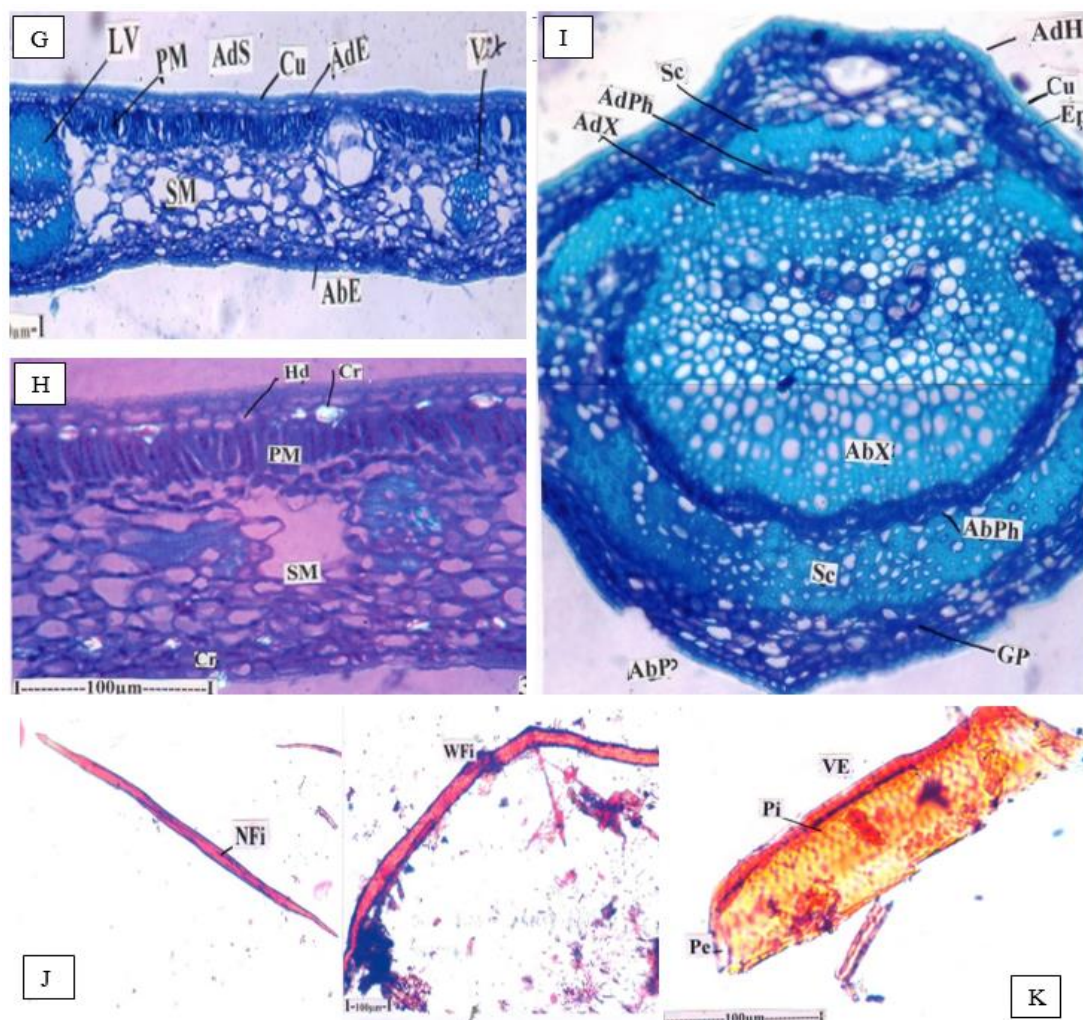


Figure.2.G.: Transverse section of leaf through midrib; **Figure.2.H:** T.S. of lamina through lateral vein; **Figure.2.I:** Petiole anatomy.

Powder Microscopy: Figure.2.J: Normal & Wide fibres **Figure.2.K:** Vessel element;

AbP : Abaxial part; AbPh : Abaxial phloem; AbX : Abaxial xylem; AbS : Abaxial side; Ep : Epidermis; AdE : Adaxial epidermis; AdPh : Adaxial phloem; AdS : Adaxial side; AdX : Adaxial xylem; AdH : Adaxial hump; Cu : Cuticle; La : Lamina; MR : Midrib; Sc : Sclerenchyma; VS : Vascular strand; Cr : Crystals; Hd : Hypodermis; Lv : Lateral vein; PM : Palisade mesophyll; SM: Spongy mesophyll; Vit : Veinlet; Cr: Crystal; PhR : Phloem ray; Pi : Pith; NFi : Narrow fibre; WFi : Wide fibre; Pe : Perforation; Pi : Pits; VE : Vessel element

Powder Microscopy (Fig.2J,2K)

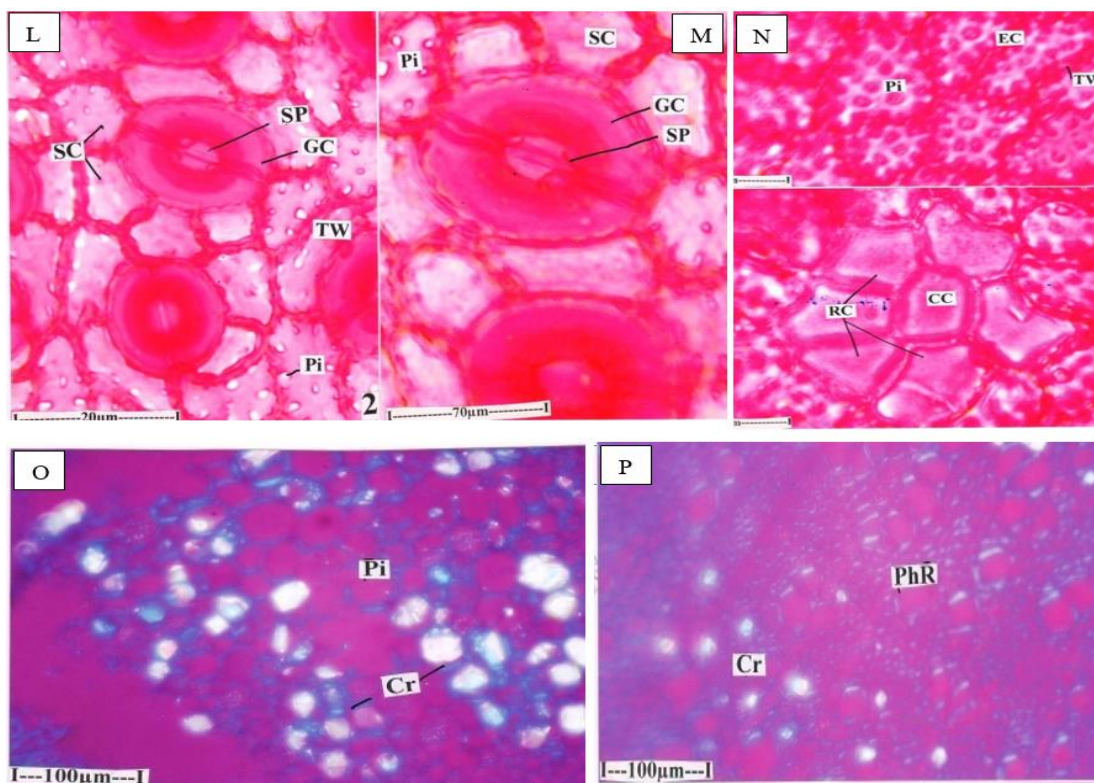
Dried Powder preparation of the whole plant was examined under the microscope and the following elements were observed.

Fibres: Libriform fibres are abundant in the powder of the stem. There are two types of fibres; some fibres are thin, narrow and pointed at the ends. These narrow fibres are 750 mm long and 20 mm thick. The other of fibre is longer, wide and wide lumened. The wide fibres are more than 1 mm long and 30 mm thick.

Vessel elements (Fig. 2F): The vessel elements of different types are frequently seen in the

stem powder. Some of the vessel elements are short, wide and cylindrical with elliptical, oblique, simple end wall perforations. The vessel elements have multiseriate circular lateral wall circular bordered pits. The vessel elements are 250 mm long and 50 mm wide.

The other type of vessel elements are long, narrow, cylindrical with long or short end wall tails (Fig. 2F). These longer and narrow vessel elements have circular end wall perforations and are slightly oblique. The lateral wall pits are circular, bordered and multiseriate. The vessel elements are 180-230 mm long and 20-25 mm thick.



Powder Microscopy: **Figure 2.L, 2.M & 2.N:** Adaxial & Abaxial epidermis with pits.

Figure 2.O & 2.P: Crystals in pith and ray cells

EC: Epidermal cell; CC: Central cell; SC: Subsidiary cell; St: Stomata; TW: Tangential wall; Pi: Pits; XR: Xylem ray GC: Guard cell; SP: Stomatal pore. Cr: Crystal.

Epidermal peelings of the leaf are common in the leaf powder. The adaxial epidermal cells are polyhydral and thick walled. Dense circular simple pits are densely occur in the tangential as well as periclinal walls, so that the walls appear beaded (Fig. 2L,2M,2N).

The abaxial epidermal peeling is seen in the powder. The abaxial epidermis is densely stomatiferous. While the adaxial epidermis is apostomatic. The abaxial epidermal cells have dense circular pits.

The stomata are circular or slightly elliptical. The stomatal pores are narrow, slit-like. The circular stomata are 20 mm horizontally (diameter); the elliptical stomata are 20 mm in horizontal plane and 30 mm in vertical plane. The stomata are cyclocytic type; each stomata is encircled by a ring of 5-8 subsidiary cells; the subsidiary cells are rectangular to squarish and they are horizontally aligned with guard cells (Fig. 2L,2M,2N).

Traditional plant morphology has apparently become marginalized. However, morphology

as an integrative discipline has an important role to play and the broader phylogenetic context need to be considered. (Endress, 2005). New and exciting techniques for structural research such as micro computer tomography and modern morphometric analyses are opening possibilities for a further integration of comparative morphological studies (Schonenberger and Balthazar, 2012).

Summary and Conclusion

Atalantia racemosa a rare species in Chittoor district 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 tilldate. Our investigation highlights the botanical standardization of whole plant of *Atalantia racemosa*, which is claimed as potent ethnodemicinal plant in the form of drug. This work presents a descriptive study of the morphoanatomical characters of vegetative parts viz., Root, Stem and Leaf. photomicrographs of Tranverse sections

(2.A–2.P) show distinct characters which authenticate the identification of *Atalantia racemosa*. We conclude that, the Morpho-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.

Acknowledgements

Authors are thankful to the Head, Department of Botany, Rayalaseema University, Kurnool, Andhra Pradesh (India) for providing necessary facilities. Authors also express gratitude towards the University Authorities for Ph.D. registration of the First author and DCM Committee for constructive suggestions that improved the research work.

Conflict of Interest

We declare that no conflict of interest.

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Cite this article as:

Naga Padmavathi V. and K. Madhava Chetty. Morphoanatomical studies of *Atalantia racemosa* Wight ex Hook., an important medicinal plant of Chittoor Dt. of Andhra Pradesh, India. *International Journal of Bio-Pharma Research*, Volume 8, Issue 2 (2019) pp. 2491-2498.



<http://dx.doi.org/10.21746/ijbpr.2019.8.2.3>

Source of support: Nil; Conflict of interest: Nil.

