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RESEARCH ARTICLE

Macroscopic and Microscopic Studies of *Phyllanthus virgatus* Forst. (Euphorbiaceae)

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Abstract

Phyllanthus virgatus Forst. Euphorbiaceae has adequate ethnomedicinal value and is often confused with other herbaceous members of this genus. This study is aimed at identification of the plant based on macroscopic and microscopic observation. *Phyllanthus virgatus* is an herb with long branches arising from the short and flattened stem, stipules peltate, capsule smooth, long stalked and seeds with minute tubercles. Lamina is dorsiventral, amphistomatic, with paracytic type of stomata. Petiole has thick epidermal cells with papillate outer tangential walls and semicircular vascular strand. The stem in section shows opposite conical wings. The cortical cells of root possess dense accumulation of tannin. The secondary xylem of the root exhibits diffuse porous growth rings. The ovule is tricarpellous, syncarpous with ovules on axile placentation. The fruit has thick conical stalk with persistent perianth and the mesocarp are thick walled with dense tannin content. The endocarp is thick and sclerotic. These above mentioned characters clearly distinguish the plant from the other taxon of this genus.

Keywords: *Phyllanthus virgatus*, amphistomatic, paracytic stomata, vascular strand, conical stalk.

Introduction

Euphorbiaceae is one of the most cosmopolitan plant groups within angiosperm, consisting of about 334 genera (Webster, 1994) and over 8000 species, (Redcliffe-Smith, 2001). The genus *Phyllanthus,* Euphorbiaceae, contains approx. 550 to 750 species and are widely distributed throughout tropical and subtropical countries (Unander *et al*., 1995). The genus *Phyllanthus* have long been used in folk medicine in India and other countries for thousands of years for the treatment of a broad spectrum of diseases, such as disturbances of the kidney and urinary bladder, intestinal infections, diabetes and the hepatitis B virus (Calixto *et al*., 1998).

Phyllanthus virgatus (synonym: *Phyllanthus simplex* Retz.) was used to cure many diseases in indigenous system. Roots in mammary abscesses; seeds and fruit in gonorrhea; fresh leaf extract used as wash for wounds and eye diseases. Leaf extract mixed with buttermilk was used to cure itches of children when bruised. Decoction of the plant was given against cold and fever (Islam, 2009). In China, the extract of this plant was fed to children suffering from malnutrition due to worm infestation. This herb was used as an antiseptic and anti-inflammatory agent by the Gonad tribe of India (Tiwari and Padhye, 1993). Structural similarity among the herbaceous plants of Euphorbiaceae leads to a great confusion in the identification of the species. Hence, isolation of bioactive compound from plants with specific therapeutic value may become impossible. A typical pharmacognostical studies are normally quite adequate for quality control of herbal drugs (Radha, 2008).

According to WHO (1998), the macroscopic and microscopic description of a medicinal plant is the first step toward establishing its identity and purity and should be carried out before any tests were undertaken (Ahmed, Dar, 2008; Shri, 2010). Along with morphological studies, the present investigation is aimed to do the anatomical studies to identify the species.

Materials and methods

Plant collection: The plant was collected from Vellore district, Tamil Nadu, India and identified by Dr. P. Jayaraman. Herbaria of voucher specimens (voucher No. PARC/2015/3094) were prepared and the specimens are lodged in the Plant Anatomy Research Centre, West Tambaram, Chennai. The samples were fixed in FAA (formalin: acetic acid: ethanol in the ratio 5: 5: 90 v/v) for 24 h. The specimens were processed as per the procedure of Sass (1940) and sections were made as per Johansen (1940) and staining was done as per O'Brien *et al*. (1964). For studying the stomatal morphology and venation pattern, paradermal sections as well as clearing of vein were prepared according to Sass (1940). Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit.

Results

Morphological characters: Phyllanthus virgatus is an herb growing up to 50 cm and sometimes up to 75 cm (Fig. 1). Stem is short, thick and flattened with normal distichous leaves. Long branches arise from the base of the stem. The leaves are often reddish, oblong lanceolate, 1-1.5 cm long, 0.3-0.5 cm broad.

Fig. 1. *Phyllanthus virgatus* - Habitat.

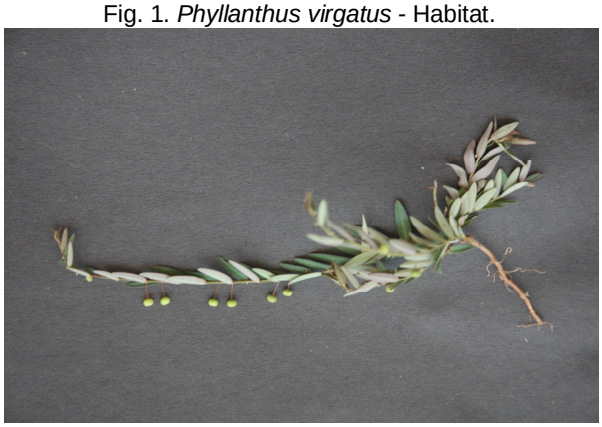
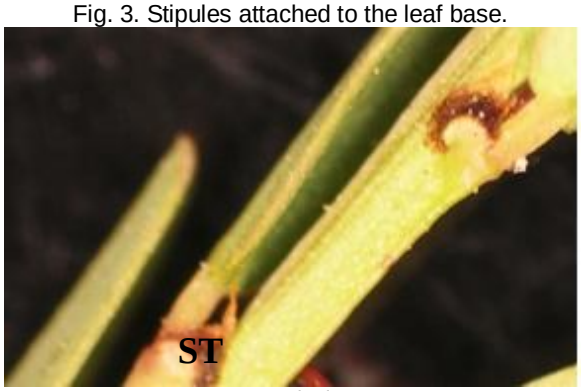


Fig. 2. Dorsal view of an entire leaf.

Fig. 3. Stipules attached to the leaf base.



ST- Stipule

Fig. 4. Male flowers.

Fig. 5. Fruits with large peduncles.

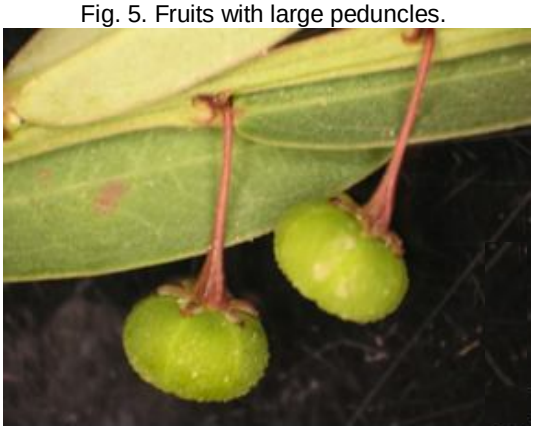


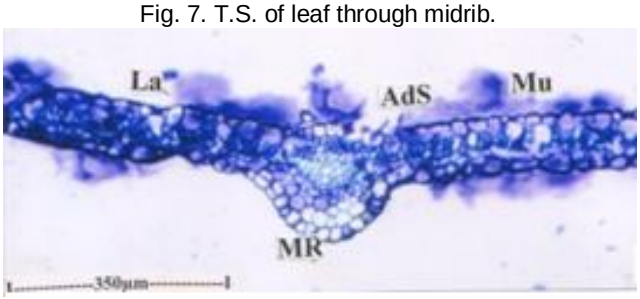
Fig. 6. Outer view of seeds.

The basal part of the leaf is rounded, the apex is obtuse to acute, apiculate (Fig. 2) stipules are subsagittate, peltate and acuminate (Fig. 3). The male flowers are located in the lower axils 2 mm across; female flowers occur above the male flowers measuring 3 mm across, the tepals are 6 and oblong, 1 mm long obtuse; the tepals of female flowers are mucorate, stamens 3, free and anthers subsessile 0.3 mm long dehiscing transversely (Fig. 4). The disc glands are 6 in number ovary 1 mm styles, 3 horizontally spreading 0.7 mm long. The disc is annular, crenulate, capsule is 3 mm across, globular, 3 lobed and the surface is warty (Fig. 5). Seeds are brownish black, triquetrous and minutely tubercled (Fig. 6).

Anatomical characters

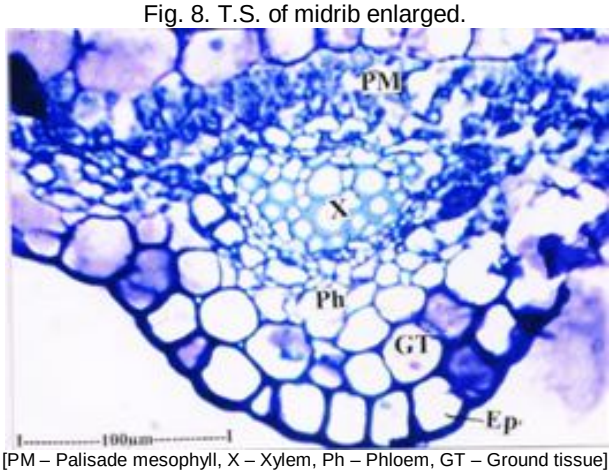
Leaf: In cross sectional view, the leaf exhibits plano convex midrib and fairly thick lamina. Mucilage is secreted in abundance from the cells of the lamina (Fig. 7). The midrib is plano convex measuring 190 µm thick and 158 µm wide. The adaxial and abaxial epidermal layers of the midrib are wide and thick, vertically oblong and thick walled. The epidermal cells are 25 x 20 µm in vertical and horizontal planes. The ground tissue in the abaxial part consists of 3 or 4 layers of wide, circular and thick walled cells. The palisade tissue is horizontally transcurrent along the adaxial part of the midrib. The vascular bundle is single, planoconvex with 5 or 6 short vertical lines of angular, thin walled wide xylem elements. Phloem represented by few cells along the abaxial part of the xylem strand (Fig. 8).

Fig. 7. T.S. of leaf through midrib.



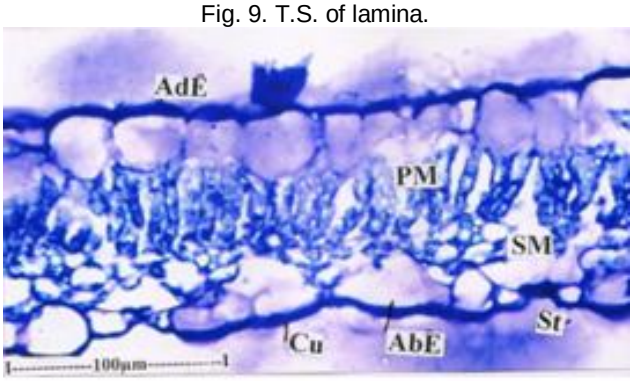
[La- Lamina, MR – Midrib, Ads – Adaxial side, Mu – Mucilage]

Fig. 8. T.S. of midrib enlarged.



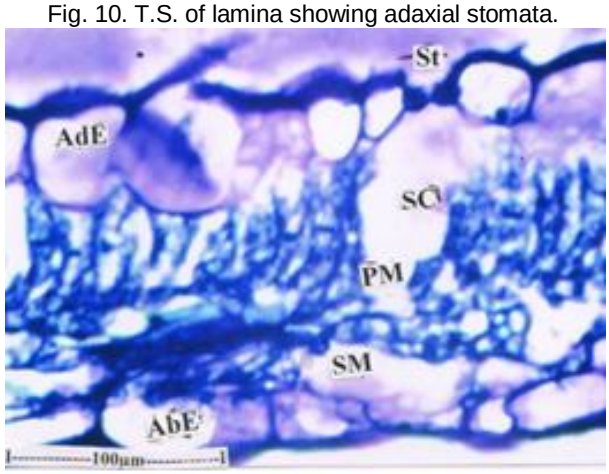
[PM – Palisade mesophyll, X – Xylem, Ph – Phloem, GT – Ground tissue]

Fig. 9. T.S. of lamina.



[AdE – Adaxial epidermis, AbE – Abaxial epidermis, PM – Palisade mesophyll, SM – Spongy mesophyll, St – Stomata, Cu – Cuticle]

Fig. 10. T.S. of lamina showing adaxial stomata.

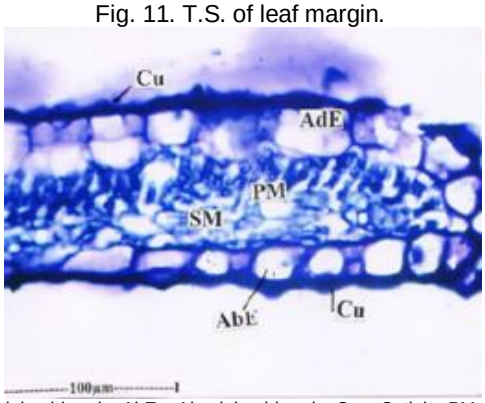


[AdE- Adaxial epidermis, AbE – Abaxial epidermis, PM – Palisade mesophyll, SC – Secretory Canal, SM – Spongy mesophyll, St – Stomata]

Lamina: The lamina is dorsiventral, mesomorphic, 80 µm thick, amphistomatic with thick cuticle. The adaxial epidermis is thick and square shaped measuring 25 µm thick. The abaxial epidermis is comparatively thin; the cells are cylindrical and narrow with thick cuticle (Fig. 9). The mesophyll tissue is differentiated into adaxial layer of single row of cylindrical palisade cells which are loosely arranged. The spongy mesophyll tissue consists of small, lobed less compact parenchyma cells. Stomata occur on both the adaxial and abaxial epidermis. The guard cells are just below the level of the subsidiary cells (Fig. 10).

Leaf margin: The marginal part of the lamina is slightly conical and measures 52 µm in thickness. The epidermal cells along the leaf margin are smaller in size but possess thick walls and thick cuticle. The mesophyll tissue is normal as in the middle part of the lamina (Fig. 11).

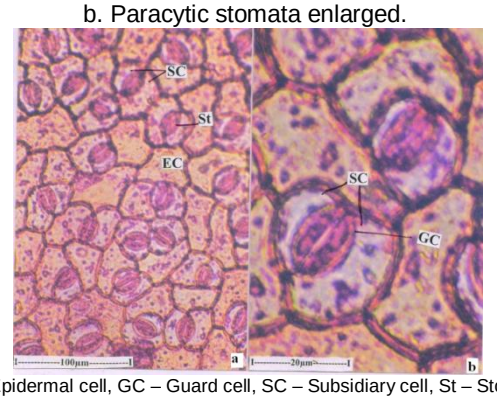
Fig. 11. T.S. of leaf margin.



[AdE- Adaxial epidermis, AbE – Abaxial epidermis, Cu – Cuticle, PM – Palisade mesophyll, SC – Secretory Canal, SM – Spongy mesophyll]

Epidermal cells and stomata: The adaxial epidermis consists of thick walled wavy anticlinal walls and paracytic type of stomata. The stomata are abundant and diffuse in distribution on the abaxial epidermis (Fig. 12a). The abaxial stomata are also paracytic with 2 subsidiary cells lying parallel to long axis of the guard cells (Fig. 12b). The guard cells are broadly elliptical with narrow slit like stomatal aperture. The guard cells are 15 x 20 µm in size. The epidermal cells possess dense cytoplasm with prominent solid particles inside the cells.

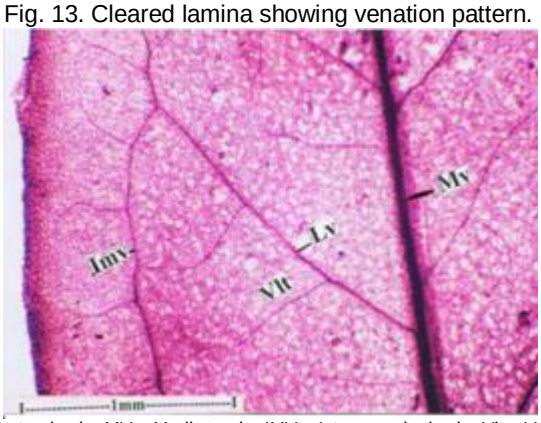
Fig. 12a. Epidermal cells and stomata enlarged;
b. Paracytic stomata enlarged.



[EC – Epidermal cell, GC – Guard cell, SC – Subsidiary cell, St – Stomata]

Venation pattern: The leaf consists of a thick straight mid vein and thin straight lateral veins. The lateral veins are inter-connected by vertically running intra marginal veins from which arise several thin, unbranched, wavy horizontal veinlets running towards the margin. The vein islets are formed by the lateral veins and vein terminations are less common when present the vein terminations arise from the mid veins extending into the vein islets (Fig. 13).

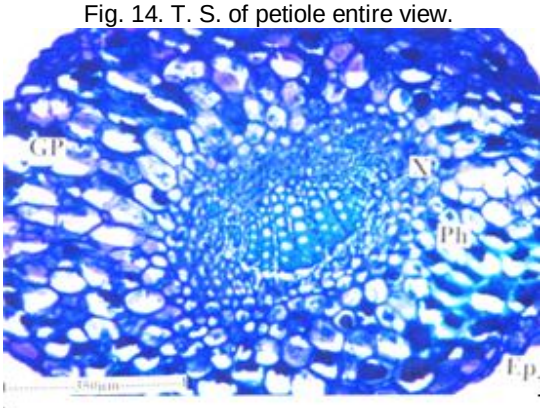
Fig. 13. Cleared lamina showing venation pattern.



[LV – Lateral vein, MV – Median vein, IMV – Intra marginal vein, Vlt – Veinlet]

Petiole: The petiole is short and less conspicuous measuring 1.1 mm in dia. It consists of thick epidermal cells with papillate outer tangential walls. The ground parenchyma includes wide, angular, compact thin walled parenchyma cells with amorphous cell inclusions (Fig. 14). The vascular strand is semicircular, small and centrally placed. It consists of thick mass of xylem elements which are in compact radial lines of about 6 elements in each row. On the lower end of the xylem strand occur phloem elements which consist of a continuous row of small, compact sieve elements mixed with parenchyma cells. The xylem elements are up to 25 µm in dia.

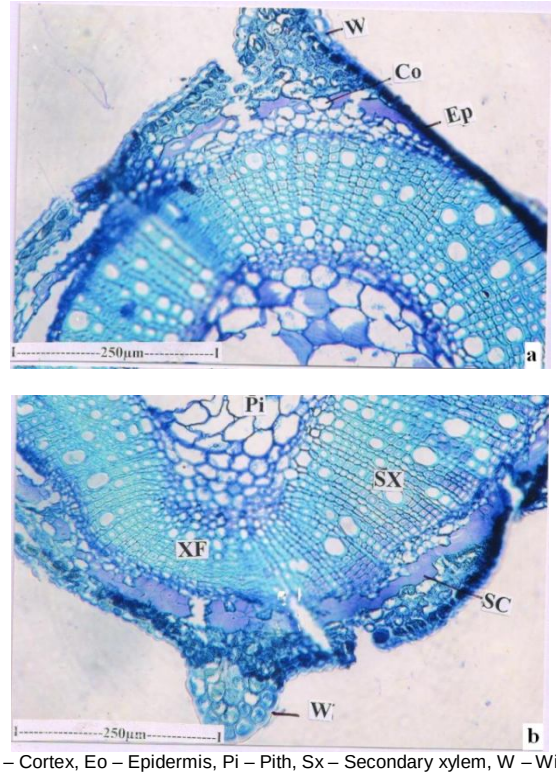
Fig. 14. T. S. of petiole entire view.



[X-Xylem, Ph-Phloem, GP-Ground Parenchyma]

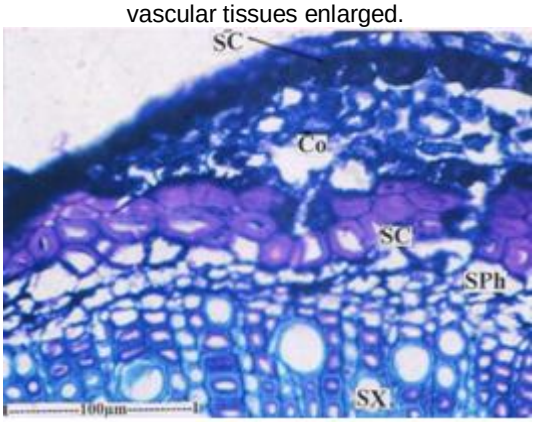
Stem: The stem is circular with two opposite, thick, short, conical wings (Fig. 15a,b). The stem consists of a thin layer of epidermis comprising small, rectangular thin walled cells with thick cuticle along the outer tangential walls.

Fig. 15a and b. T. S. of stem showing 2 opposite conical wings.



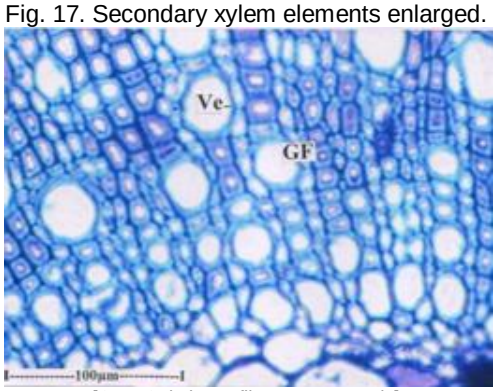
[Co – Cortex, Eo – Epidermis, Pi – Pith, Sx – Secondary xylem, W – Wing, XF – Xylem fibres, Sc – Sclerenchyma]

Fig. 16. T. S. of stem cortical zone and secondary vascular tissues enlarged.



[Co – Cortex, Sc – Sclerenchyma, SPh – Secondary phloem, SX – Secondary xylem]

Fig. 17. Secondary xylem elements enlarged.

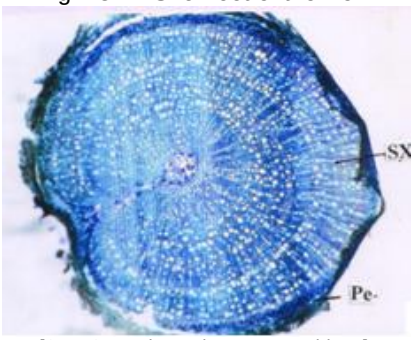


[GF – Gelatinous fibre, Ve – Vessels]

The cortical zone is narrow with circular loosely arranged parenchyma cells. Inner to the cortex is a wide cylinder of mucilaginous sclerenchyma cells which are 1 to 3 cells in thickness. Inner to the sclereid zone, occurs the secondary phloem. The secondary phloem includes outer crushed sieve elements and narrow inner zone of non-collapsed phloem. The secondary xylem cylinder is hollow. It comprises of primary xylem elements around the pith and secondary xylem cylinder towards outside. Secondary xylem includes narrow thick walled fibres and solitary radial discontinuous lines of vessels. The vessels are angular and measure up to 30 µm in dia (Fig. 16 and 17). The ground tissue of the secondary xylem includes partially mucilaginous, squarish thick walled fibres which show gelatinous inner walls. The pith region consists of large, less compact, thin walled parenchyma cells. The two ridges of the stem include thick walled parenchyma cells (Fig. 15a).

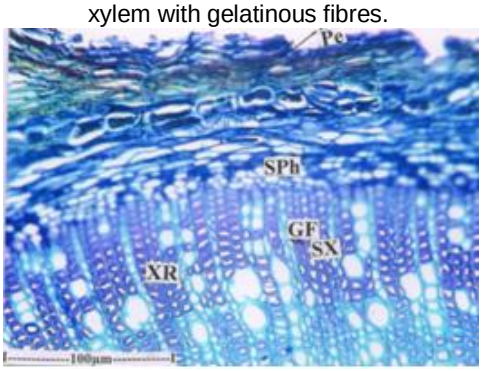
Root: The root is thick, circular and measures about 1.8 mm in dia. It consists of well-developed periderm which shows deep irregular fissures (Fig. 18). The secondary xylem exhibits 2 or 3 less distinct growth rings. The periderm includes a few layers of superficial, tubular, thin walled suberized cells (Fig. 19). Inner to the periderm is narrow cortical zone where the cells are radially stretched and rectangular, thin walled and compact. Some of these cortical cells possess dense accumulation of tannin (Fig. 20). Secondary phloem occurs on the outer peripheral part of the secondary xylem cylinder. It includes small, thin walled, angular sieve elements, companion cells and parenchyma cells (Fig. 20). The secondary xylem is about 710 µm in radial plane. It consists of either solitary or short radial multiples of 2 or 4 vessels which are circular and thin walled. The vessels vary in dia. Both narrow and wide vessels are inter-mixed with each other. Two or more growth rings are seen with fairly distinct growth ring border. The growth ring border consists of a narrow layer of small thick walled fibres. The vessels located in the growth ring border are wider and they become gradually narrow towards the periphery. Thus, the growth ring seen to be diffuse porous. The vessels are up to 40 µm in dia. The xylem fibres are thick walled, rectangular with narrow lumen. The cell walls are thick and lignified (Fig. 19).

Fig. 18. T. S. of root entire view



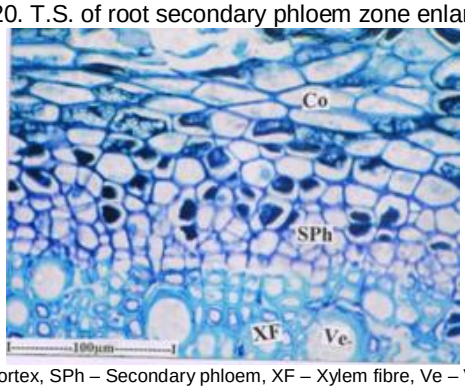
[SX – Secondary xylem, Pe – Periderm]

Fig. 19. T.S. of root showing periderm and secondary xylem with gelatinous fibres.



[Pe – Periderm, SPh – Secondary phloem, GF – Gelatinous fibre, SX – Secondary xylem, XR – Xylem ray]

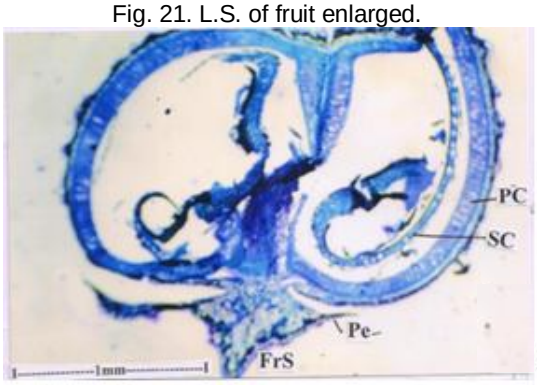
Fig. 20. T.S. of root secondary phloem zone enlarged.



[Co – Cortex, SPh – Secondary phloem, XF – Xylem fibre, Ve – Vessel]

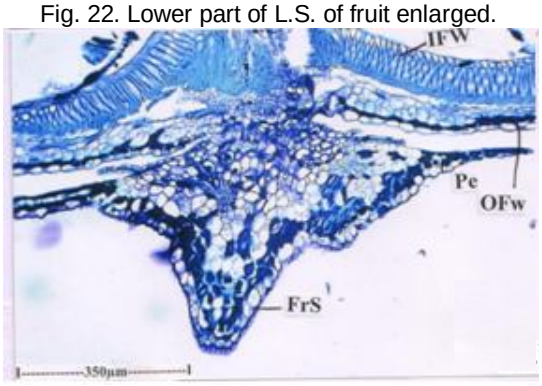
Fruit: The fruit is a capsule with 3 carpels, syncarpous with ovules on axile placentation (Fig. 21). The mature fruit has a thick conical stalk, with persistent perianth at the top of the stalk (Fig. 22). The pericarp measures 140 µm consists of a thin layer of epidermal cells which are elliptical and narrow. Inner to the epidermal layer is 2 or 3 layers of mesocarp, which are thick walled with dense tannin content. Inner to these layers the mesocarp consists of elliptical, thin walled, compact, parenchymatous layer (Fig. 23 and 24), vascular bundle located in mesocarp region (Fig. 24). The endocarp is thick and sclerotic with vertically oblong, thick walled columnar sclereids or macro sclereids. This zone is 100 µm thick (Fig. 24). The inner border of the sclerotic endocarp has a thin layer of squarish thick walled lignified epidermal cells.

Fig. 21. L.S. of fruit enlarged.



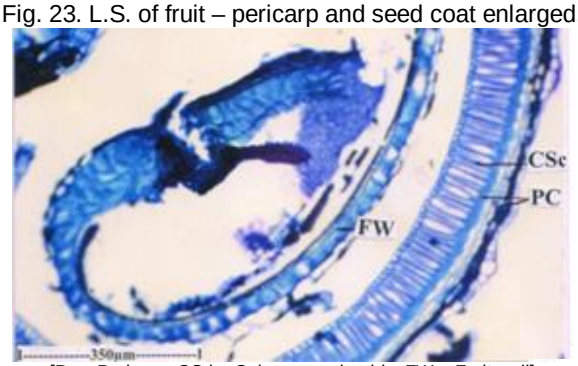
[Pc – Pericarp, Sc – Seed coat, Pe – Perianth, FrS – Fruit stalk]

Fig. 22. Lower part of L.S. of fruit enlarged.



[IFw – Inner fruit wall, OFw – Outer fruit wall, Pe – Perianth, FrS – Fruit stalk]

Fig. 23. L.S. of fruit – pericarp and seed coat enlarged.

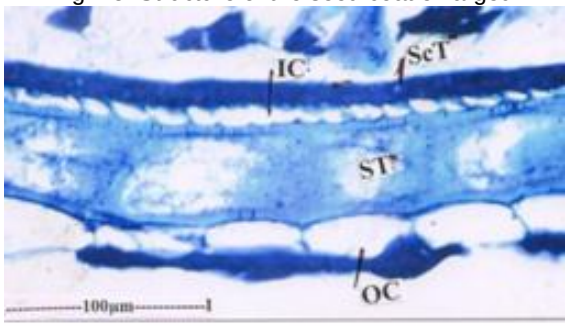


[Pc – Pericarp, CSd – Columnar sclereids, FW – Fruit wall]

Fig. 24. A portion of the pericarp showing epicarp, mesocarp and sclerotic endocarp.

[VB- Vascular bundle, Mc – Mesocarp, Ep – Epidermis, CScl – Columnar sclereids]

Fig. 25. Structure of the seed coat enlarged



[ScT – Sclerotesta, ST – Sarcotesta, OC – Outer seed coat, IC – Inner seed coat]

Seed coat: The seed coat is 70 µm thick. It consists of outer layer of wide, rectangular thin walled cells. The outer tangential walls of the cells have uneven thickness of cuticle. The middle zone is wide, comprising of large, rectangular thin walled cells. Inner to this layer occurs a single layer of small squarish fairly thick walled cells. The inner most part of the seed coat consists of thick darkly stained layer of cells (Fig. 25).

Discussion

Gamble (1921) described *P. virgatus* as a stiff almost woody herb with long flattened branches. Ugemuge (1985) described the plant as an erect herb, woody below, branches long, straight, flattened; stipules sagittate, reddish brown, linear-oblong, mucronate, capsules smooth, long stalked, seeds red, tubercled. Singh (1988) explored the habit and habitat of the plants and reported that the plant grows up to 30 cm height, herbs amongst grasses, under the shade of rocks, on bouldery slopes in open forests up to 1050 m alt. and along the roadsides and also in cultivated fields. In the present study, macroscopic features of *P. virgatus* like leaf shape oblong–elliptic, absence of branchlets, branches long arising from the short and flattened stem, stipules sagittate, reddish brown, peltate, capsule smooth, long stalked and seeds with minute tubercles are observed. Kandavel *et al*. (2011) observed the morphological and anatomical characters of herbaceous *Phyllanthus* spp. seen in Thiruchirappalli district and gave distinct characters of *P. virgatus* by virtue of its unique characters like leaf shape, absence of cataphylls, absence of branchlets etc. Webster (1956-1957) laid much stress on the branching pattern; he described the species *P. virgatus*, the phyllotaxy was distichous; the flowers may occur on any node, and so there is still no differentiation of the axes. Correlated with the distichous phyllotaxy is trend towards more or less flattened stem with two lateral wings or angles.

In the present study, it is observed that the leaf shows mucilaginous cells in the lamina. The lamina is found to be dorsiventral, mesomorphic, 80 µm thick and amphistomatic, paracytic type of stomata on both the surfaces. A clear venation pattern with thick straight midvein, thin straight lateral veins inter-connected by vertically running intra-marginal veins which ends in vein islets is noted. This reticulate venation marks the character of the species as a dicotyledon. Metcalfe and Chalk (1950) recorded mucilaginous epidermal cells of leaf in certain species of *Phyllanthus*. A brief account given by Sharma and Sheela (2011) describes that in *P. simplex*, the epidermal cell walls of leaves are straight and they showed anisocytic and paracytic type of stomata. Dentate type cells are found all along the margins of leaf lamina. A cluster of calcium oxalate crystals is observed in the ground tissue. The palisade tissue is discontinuous and restricted to only lamina. In our study, the sectional view of petiole shows thick epidermal cells with papillate outer tangential walls.

The vascular strand is semicircular, small and centrally placed. The ground parenchyma cells appear darkly stained due to accumulation of amorphous cell inclusions. Sarala *et al*. (2014) studied the petiole of *P. virgatus* and found that the individual vascular bundle is arc shaped with phloic sclerenchymatous patches. There is no hypodermal mechanical tissue reported in petiole. The observations made in the sectional view of vegetative and reproductive organs reveals a clear view of internal structure of the plant. The paradermal section of the leaf showing the epidermis and stomatal pattern is an added value to the taxonomic significance.

Conclusion

The above characters observed in the study clearly specify the plant as *Phyllanthus virgatus*. This study forms a base for the further pharmacognostic and pharmacological investigation of this plant.

References

1. Ahmed, Z. and Dar, A. 2008. Advances in Natural Products; Delhi: Divya Publishing House, p.132.
2. Calixto, J.B., Santos, A.R., Filho, V. and Yunes, R.A. 1998. A review of the plants of the genus Phyllanthus: Their chemistry, pharmacology and therapeutic potential. *Med. Res. Rev*. 18: 225-258.
3. Gamble, J.S. 1921. Flora of the Presidency of Madras part IV Rubiaceae to Ebenaceae, published under the authority of the Secretary of State for India in Council, London. (Volume II).
4. Islam, M. 2009. *Medicinal plants of North East India*, Aavishkar Publishers Distributors, Jaipur 302003 (Raj) India, p.92.
5. Johansen, D.A. 1940. Plant Microtechnique. McGraw Hill Book Co; New York, p.523.
6. Kandavel, D., Kiruthika Rani, S., Vinithra, M.G. and Sekar, S. 2011. Systematic studies in herbaceous Phyllanthus spp. (Region: Tiruchirappalli district in India) and A simple key to authenticate 'Bhumyamalaki' complex members. *J. Phytol.* 3(2): 37-48.
7. Metcalfe, C.R. and Chalk, L. 1950. Anatomy of the Dicotyledons. Vol.I & II. Clarendon Press, Oxford, p.1212.
8. O'Brien, T.P., Feder, N. and McCull, M.E. 1964. Polychromatic Staining of Plant Cell walls by toluidine blue-O. *Protoplasm.* 59: 364-373.
9. Radha, R., Sivakumar, T. and Arokiyaraj, S. 2008. Pharmacognostical evaluation of Pulumeriaalba Linn. *Res. J. Pharm. Technol*. 1: 496-501.
10. Redcliffe-Smith, A. 2001.*Genera Euphorbiacearum*. Royal Botanical Gardens. Kew, p.464.
11. Sarala, C., Tadavi. and Bhadane, V.V. 2014. Taxonomic significance of the Rachis, Petiole and Petiolule anatomy in some Euphorbiaceae. *Biolife.* 2(3): 850-857.
12. Sass, J.E. 1940. Elements of Botanical Microtechnique. McGraw Hill Book Co; New York, p.222.
13. Sharma, S.K. and Sheela, M.A. 2011. Pharmacognostic evaluation of leaves of certain Phyllanthus species used as a botanical source of Bhumyamalaki in Ayurveda. *J. Res. Ayurveda*. 32(2): 250-253.
14. Singh, N.P. 1988, Flora of Eastern Karnataka, Vol. II, p.576.
15. Tiwari, V.J. and Padhye, M.D. 1993. Ethnobotanical study of Gond tribe of Chandrapur and Gadchiroli districts of Maharashhtra State, India. *Flitoterap.* 64(1): 58-61.
16. Ugemuge, N.R. 1985. Flora of Nagpur district, p.334, 335.
17. Unander, D.W., Webster, G.L. and Blumberg, B.S. 1995. Usage and bioassays in *Phyllanthus* (Euphorbiaceae)-IV: Clustering of antiviral uses and other effects. *J. Ethnopharmacol.* 45(1): 1-18.
18. Webster, G.L. 1956-1957. A monographic study of the West Indian sps of Phyllanthus. *J. Arnold Arboreteus.* 37(2): 297.
19. Webster, G.L. 1994. Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Ann. Missouri Bot. Garden*. 81: 33-144.