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## Vegetative anatomy of *Tabernaemontana alternifolia* L. (Apocynaceae) endemic to southern Western Ghats, India

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**ABSTRACT** The anatomical description of vegetative parts of *Tabernaemontana alternifolia* L. belonging to the family Apocynaceae was investigated in the present study. The leaves of *T. alternifolia* is hypostomatic with paracytic stomata, uniseriate epidermis made up of thin-walled parenchymatous cells covered by thin cuticle on both adaxial and abaxial surfaces. The hypodermis comprises of angular collenchyma cells. Mesophyll is dorsiventral containing silica bodies and vascular bundles are bicollateral. The petiole is flattened adaxially and arch-shaped abaxially with a uniseriate epidermis covered by a thin cuticle. The hypodermis is 7-8 layered angular collenchyma cells consisting of laticifers and parenchymatic, cortical layers consisting of silica bodies and thick-walled fibers and U-shaped bicollateral vascular bundles. Secondary growth in stems is characterized by the formation of periderm and thick-walled fibers in the vascular tissues. Bicollateral vascular bundles are covered by sclerenchymatous patches, parenchymatous cortex and pith consist of fibers, laticifers and silica bodies. The root possess unicellular root hairs, compactly arranged thin-walled uniseriate epidermis, 16-18 layered cortex containing silica bodies and fibers, indistinct endodermis, radially arranged vascular bundles and 14-16 arched xylem. Pitted water-storage cells are present in the conjunctive tissue. Lignin deposition was observed in the root stelar region and pith is absent.

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### Introduction

The genus *Tabernaemontana* belonging to the family Apocynaceae comprises of around 102 species occurring throughout the tropical and also in some subtropical regions of the world (Van Beek et al. 1984). *Tabernaemontana* includes shrubs or small deciduous trees bearing white latex, dichotomously branched stem and opposite leaves. *Tabernaemontana alternifolia* L. (= *Tabernaemontana heyneana* Wall) is a small tree growing up to 8 m tall and produce milky white latex (Ignacimuthu et al. 2006). It is commonly found in open forests of Western Ghats from Maharashtra to Kerala at an elevation of 900 m (Sathishkumar et al. 2012) and it is endemic to southern Western Ghats (Manasa and Chandrashekar 2015). Typically, this species is considered to be a potential medicinal plant as it possesses antimicrobial activity, antioxidant and is used in the treatment of the nervous disorder, diabetics, chronic bronchitis, lymphocytic leukaemia, snake bite; respiratory and skin problems (Sukumaran and Raj 2008; Sathishkumar and Baskar 2012). Besides these medicinal uses, *T. alternifolia* also consists of several

secondary metabolites including alkaloids, flavonoids, steroids, tannins, glycosides and resins (Srivastava et al. 2001; Roy et al. 2002; Sathishkumar et al. 2008).

Anatomical investigations help in understanding the phylogenetic interactions as well as the physiological progression (Yeung 1998; Liu and Zhu 2011) and support in the identification of plants when floral characteristics are unavailable (Dengler 2002). The anatomy of vegetative parts of certain species of Apocynaceae has been examined. However, most of these studies have examined only the foliar anatomy and the stem and root anatomy of Apocynaceae is not well studied. Duarte and Larrosa (2011) examined the anatomical features of leaf and stem in *Mandevilla coccinea* (Hook & Arn.) Woodson and reported uniseriate epidermis with thick and striate cuticle, paracytic stomata on both the leaf surfaces, dorsiventral mesophyll and collateral vascular bundles in leaves; and cambium formation, sclerenchymatous sheath with non-lignified fibers, bicollateral vascular bundles, large parenchymatous pith with amyloplasts in the stem; and occurrence of laticifers and phenolic compounds in both stem and leaf. A comparative study on the anatomical characters of *Nerium oleander* L. and *Catharanthus roseus*

(L.) G. Don leaf by Abdalla et al. (2016) revealed that the dorsiventral leaf consisted of a single-layered epidermis with sparse epidermal hairs in *C. roseus* and isobilateral leaves, four-layered epidermis bearing numerous epidermal hairs, presence of sunken stomata and calcium oxalate crystals in *N. oleander*.

Although literature is available on the morpho-anatomical features of leaf and stems of various genus of Apocynaceae (Rio et al. 2005; Larrosa and Duarte 2006; Maciel et al. 2010; Abdalla et al. 2016), anatomical description of the genus *Tabernaemontana* is very limited. Omino (1996) investigated the leaf anatomy 37 species in 26 genera of Apocynaceae including four species of *Tabernaemontana* (*Tabernaemontana pachysiphon* Stapf, *Tabernaemontana elegans* Stapf, *Tabernaemontana stapfiana* Britten, *Tabernaemontana ventricosa* Hochst. ex A.DC) occurring in Africa and observed paracytic stomata in all the examined species, single-layered epidermal cells on both the foliar surfaces except in *T. pachysiphon* (1-2 layered), bifacial mesophyll with numerous intercellular spaces, collenchymatous cells in the ground tissue of midrib, clustered crystals in both the epidermal layer, mesophyll and in the ground tissue of midrib region in *T. ventricosa*; numerous solitary crystals in lower epidermis and star-shaped crystals in palisade layer of *T. pachysiphon* whereas small star-like crystals were reported in phloem parenchyma of *T. stapfiana*. The vascular bundles were deep V-shaped in all four species of *Tabernaemontana* (Omino 1996). The wood anatomy of *Tabernaemontana eglandulosa* Stapf, and *Tabernaemontana siphilitica* (L.f) Leeuwenb., revealed distinct growth rings, vessel grouping in radial multiples, vessels with sporadically double simple perforations, septate fibers with simple to minutely bordered pits and axial parenchyma were either scarce or absent (Lens et al. 2008).

Guidoti et al. (2015) studied the morpho-anatomical characters of *Tabernaemontana catharinesis* A.DC leaves and observed uniseriate epidermis devoid of trichomes, six to seven layers of angular collenchyma, thin-walled parenchymatous cortex, and bicollateral vascular bundle. *Tabernaemontana catharinesis* stem possessed uni-stratified epidermis, angular collenchyma cells and fibers in the cortical region and the secondary growth revealed the presence of periderm and fibers in external phloem patches. The petiole in *T. catharinesis* had uni-stratified epidermis, thick cuticle, and angular collenchyma adjacent to the epidermis and bicollateral vascular arrangement (Guidoti et al. 2015). As *T. alternifolia* is an endemic plant with high medicinal value, it is important to conserve this species through various means including vegetative propagation. Therefore, anatomical investigation of vegetative parts like leaf, stem and root could be helpful in understanding the regeneration of the plant dur-

ing vegetative propagation. In addition, information on vegetative anatomy of *Tabernaemontana* species is scanty. Therefore, the present study was carried out to investigate the vegetative characters including the leaf, petiole, stem and root in *T. alternifolia*.

## Material and methods

Samples of leaf, stem, and roots were collected from mature plants of *T. alternifolia* during the month of February 2019 from Nadugani, Gudalur taluk of Nilgiri district, Tamilnadu, India. The latitude and longitude of the study site are 11.4718° N to 76.4107° E at an elevation of ~ 1000 m a.s.l., and the average rainfall is 2020 mm. The plant specimens were authenticated by Botanical Survey of India, Southern circle and a voucher was deposited in the Bharati Herbarium, Department of Botany, Bharathiar University, Coimbatore, India (accession number: 007744). The collected plant samples were transferred to the laboratory by placing the samples in an ice-box. The plant materials were washed with distilled water and preserved in formalin-acetic acid-alcohol (FAA) solution until processing. The leaf and petiole sections were taken from the fully developed 5<sup>th</sup> leaf from the top. Stem sections were taken at the 5<sup>th</sup> internode from the shoot tip and root sections were taken from 5cm from the root tip. The preserved material (leaf, stem, petiole and root) of *T. alternifolia* were freehand sectioned using a sharp razor blade for the histological observations using different types of stains like safranin, phloroglucinol-HCl, and toluidine blue O to identify the cell inclusions such as cutin, suberin and lignin (Gurav et al. 2014). Around 1 cm square leaf pieces were placed in Jeffrey's maceration solution for 72 hours at 35 °C for the observation of the epidermal layer (Kigkr 1971). The specimens in Jeffrey's fluid were later washed, stained with safranin and examined under an Olympus BX51 light microscope attached with a fluorescence setup (Olympus, U- RFL-T, U-25 ND 25 neutral density filter).

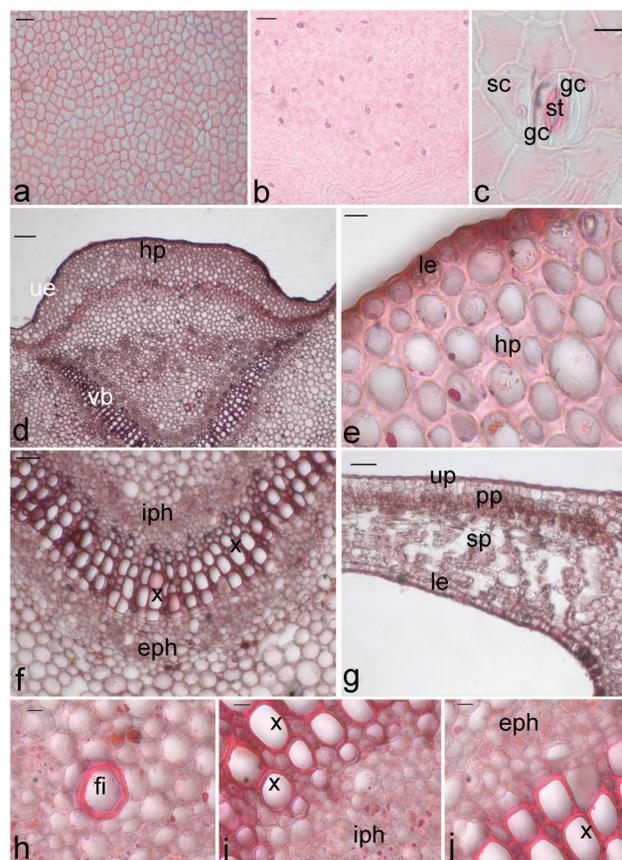
A calibrated ocular scale was used to measure the dimensions of the cells and the size of the different regions in the sections. The variables measured include the thickness of cuticle, the pore size of stomata, the length and width of the epidermis, collenchyma cells, palisade and spongy parenchyma cells, cortex, sclerenchymatous fibers and vascular bundles and pith cells. Images and autofluorescence of the observed specimens were captured with a ProgRes3 camera fitted to the Olympus BX 51 light microscope. The stomatal index (SI %) was calculated according to Salisbury (1927) using the formula  $(S/S+E) \times 100$  where S and E denotes the number of stomata and epidermal cells respectively. All the observations are

presented either as an average value or range (minimum value and maximum values) or average  $\pm$  standard error. Two dimensional values are presented as length  $\times$  breadth.

## Results

### Leaf

Leaves are hypostomatic containing stomata only on abaxial surface and the adaxial surface is devoid of stomata (Fig. 1a,b). The stomata are of the paracytic type with two subsidiary cells parallel to the guard cells (Fig. 1c). The cell dimensions of subsidiary cells and guard cells are respectively  $26.75 \pm 0.91 \times 10.5 \pm 0.89 \mu\text{m}$  and  $22.75 \pm 1.17 \times 7.5 \pm 0.64 \mu\text{m}$ . The stomatal pore measures  $13.5 \pm 0.55 \times 2.75 \pm 0.25 \mu\text{m}$ . The leaf consists of both adaxial and abaxial epidermis covered by a thin cuticle. The cuticle on the adaxial surface ( $5 \mu\text{m}$ ) is 25.85% thicker than the cuticle on the abaxial surface ( $3 \mu\text{m}$ ). The average stomatal index is 8.76%. The upper and lower epidermis is uniseriate, consisting of a compactly arranged square to rectangular thin-walled parenchymatous cells (Fig. 1g). The length and width of epidermal cells of the upper and lower surface of the leaves are respectively  $16.20 \pm 0.43 \times 25.62 \pm 0.74 \mu\text{m}$  and  $18.08 \pm 0.45 \times 24 \pm 0.62 \mu\text{m}$ . Hypodermis on the abaxial surface is 5-6 layered and is composed of angular collenchyma cells whereas on the adaxial side, hypodermal cells are 7-8 layered (Fig. 1e). The adaxial and abaxial collenchyma cells measures  $16.20 \pm 0.43 \times 25.62 \pm 0.73 \mu\text{m}$  and  $20.6 \pm 1.17 \times 15.66 \pm 0.82 \mu\text{m}$ , respectively. The mesophyll is dorsiventral differentiated into palisade and spongy parenchyma cells. The palisade parenchyma is 2-3 layered and composed of thin-walled parenchymatous cells measuring  $16.5 \pm 1.18 \times 11.75 \pm 0.65 \mu\text{m}$ . The palisade parenchyma is followed by 8-10 layered spongy parenchyma cells bearing large intercellular spaces. The length and width of spongy parenchyma cells are  $23.5 \pm 1.79 \times 20.75 \pm 1.13 \mu\text{m}$ . Silica bodies are present in both palisade and spongy parenchyma cells. In cross-section, the leaf midrib is biconvex. A sub-epidermal layer comprising of 14-18 layered thin-walled parenchyma cells are observed on both adaxial and abaxial sides (Fig. 1d). This region is characterized by the presence of thick-walled fibers (Fig. 1h), lactiferous cells and silica bodies. A V-shaped bicollateral vascular bundle is present in the midrib region. Xylem consists of metaxylem and protoxylem vessels. The metaxylem and protoxylem cells measures  $30.5 \pm 0.78 \times 25.25 \pm 0.53 \mu\text{m}$  and  $14.12 \pm 0.32 \times 14.37 \pm 0.39 \mu\text{m}$ , respectively. The protoxylem is oriented towards the upper epidermis and metaxylem is located towards the lower epidermis. Xylem is surrounded by internal and external phloem (Fig. 1f). The internal phloem forms a continuous strand (Fig. 1i) whereas, the



**Figure 1.** Vegetative anatomy of *Tabernaemontana alternifolia* leaf (a-j). **a.** Epidermal peeling of the adaxial surface of the leaf; **b.** Abaxial surface showing the distribution of stomata; **c.** Stomata with guard cells arranged parallel to the subsidiary cells; **d.** Transverse section (T.S.) of leaf along the midrib region showing upper epidermis, hypodermis and vascular bundle; **e.** Lower epidermis covered by a thin cuticle, and collenchymatous hypodermis; **f.** Bicollateral vascular bundles, xylem surrounded by internal phloem and external phloem; **g.** Leaf lamina uniseriate upper epidermis, lower epidermis, palisade parenchyma and spongy parenchyma; **h.** Thick-walled fibers; **i.** Internal phloem adjacent to xylem cells; **j.** External phloem and xylem.

st = stomata, gc = guard cell, ue = upper epidermis, hp = hypodermis, vb = vascular bundle, le = lower epidermis, x = xylem, iph = internal phloem, eph = external phloem, pp = palisade parenchyma, sp = spongy parenchyma, fi = sclerenchymatous fiber. Scale bars = 50  $\mu\text{m}$ .

external phloem appears as patches or small groups (Fig. 1j). The xylem arches range between 40 and 43.

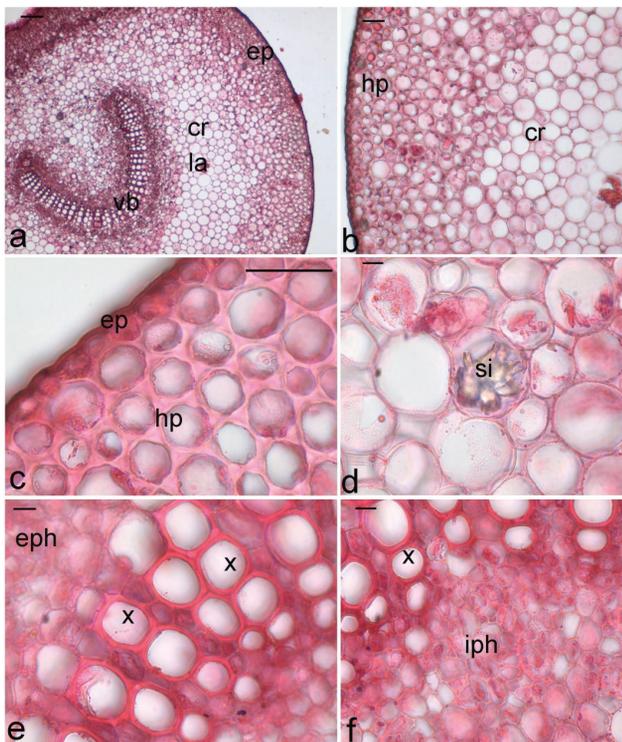
### Petiole

The petiole is a flattened arch and lightly winged at the edges on the adaxial side in transverse section. The epidermis is single-layered with a compactly arranged round to oval thin-walled parenchymatous cells measuring  $15.3 \pm 0.56 \times 11.2 \pm 0.25 \mu\text{m}$  and covered by 2-3  $\mu\text{m}$  thick smooth cuticle (Fig. 2a). The hypodermis is a continuous band of 7-8 layered angular collenchyma cells measuring

$35.75 \pm 2.29 \times 28 \pm 2.49 \mu\text{m}$  (Fig. 2c). Laticifers occur in the hypodermis. Around 12-16 cell layers of thin-walled parenchyma cells enclosing triangular or rectangular intercellular spaces subtends the hypodermis (Fig. 2b). Silica bodies and thick-walled fibers are present in the parenchymatic layer (Fig. 2d). The length and width of the parenchyma cells are  $42.25 \pm 3.90 \times 43.5 \pm 2.83 \mu\text{m}$ . Vascular bundles are bicollateral and U-shaped. Xylem consists of protoxylem and metaxylem that measures  $24.5 \pm 1.29 \times 22.5 \pm 0.55 \mu\text{m}$  and  $11.25 \pm 0.55 \times 11.5 \pm 0.85 \mu\text{m}$ , respectively. The protoxylem is oriented towards the epidermis. Xylem is surrounded by small patches of external and internal phloem (Fig. 2e,f). The external phloem patches measures  $44.25 \pm 2.07 \times 47.25 \pm 4.17 \mu\text{m}$  and the internal phloem patches measure  $37.75 \pm 2.09 \times 39 \pm 2.77 \mu\text{m}$ .

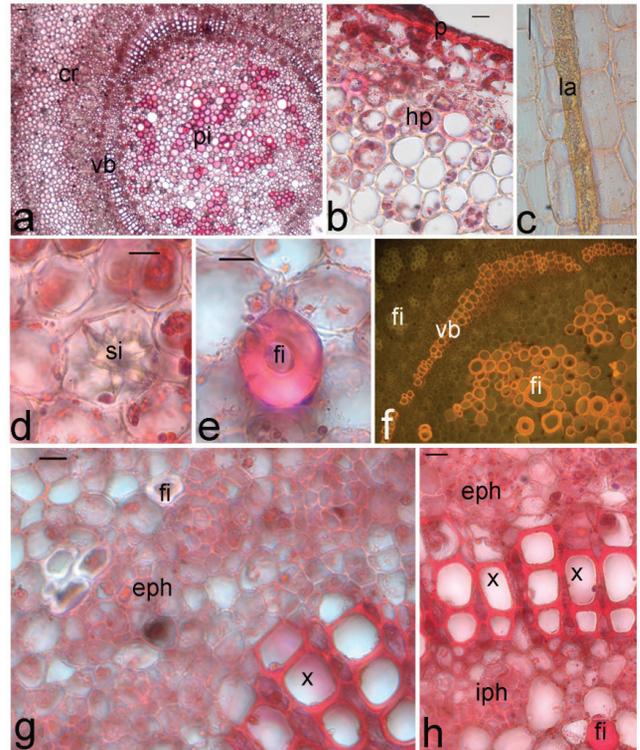
### Stem

The stem is circular in outline (Fig. 3a). The cuticle is



**Figure 2.** Vegetative anatomy of *Tabernaemontana alternifolia* petiole (a-f). **a.** Transverse section (T.S.) of petiole showing epidermis, cortex, laticifers and U-shaped vascular bundle; **b.** hypodermis and cortex; **c.** Uniseriate epidermis and hypodermis; **d.** Silica bodies in the cortical region, **e,f.** Vascular bundle showing xylem bordered by external phloem and internal phloem.

ep = epidermis, cr = cortex, la = laticifers, vb = vascular bundles, hp = hypodermis, si = silica bodies, eph = external phloem, iph = internal phloem, x = xylem. Scale bars = 50  $\mu\text{m}$ .



**Figure 3.** Vegetative anatomy of *Tabernaemontana alternifolia* stem (a-h). **a.** Transverse section (T.S.) of the stem showing periderm, bicollateral vascular bundle and pith; **b.** Periderm and hypodermis made up of angular collenchyma cells; **c.** Longitudinal section (L.S.) of the stem showing non-articulated laticifers; **d.** Silica bodies in cortex; **e.** Thick-walled sclerenchymatous patches and fibers; **f.** Fluorescent image showing deposition of lignin in vascular bundles, sclerenchymatous fibers and sclerenchymatous fibers patches; **g.** Vascular bundles with xylem enclosed by external phloem and sclerenchymatous fibers; **h.** Xylem covered by external phloem and internal phloem and fibers in the pith region.

p = periderm, vb = vascular bundle, hp = hypodermis, la = laticifers, si = silica bodies, fi = sclerenchymatous patches and fibers, x = xylem, eph = external phloem, iph = internal phloem, pi = pith. Scale bars = 50  $\mu\text{m}$ .

smooth and 4-5  $\mu\text{m}$  thick. The periderm is three-layered forming an outer layer and consists of loosely arranged thin-walled parenchymatous cells. The hypodermis is composed of 5-6 layers of angular collenchyma cells measuring  $16.3 \pm 0.50 \times 14.15 \pm 0.37 \mu\text{m}$ . Idioblasts comprising of phenolic compounds are observed in the collenchymatic hypodermis (Fig. 3b). The parenchymatous cortex is 11-13 layered with cells measuring  $30.25 \pm 0.72 \times 30.2 \pm 0.84 \mu\text{m}$ . Non-articulated laticifers, silica bodies and thick-walled fibers occur in the parenchymatous cortical cells which enclose triangular or squarish intercellular spaces (Fig. 3c-e). The sclerenchymatous fibrous patches surrounding the vascular bundles are irregular and measures  $67.7 \pm 2.30 \times 64.1 \pm 2.33 \mu\text{m}$  and cells with lignin thickened walls (Fig. 3f).

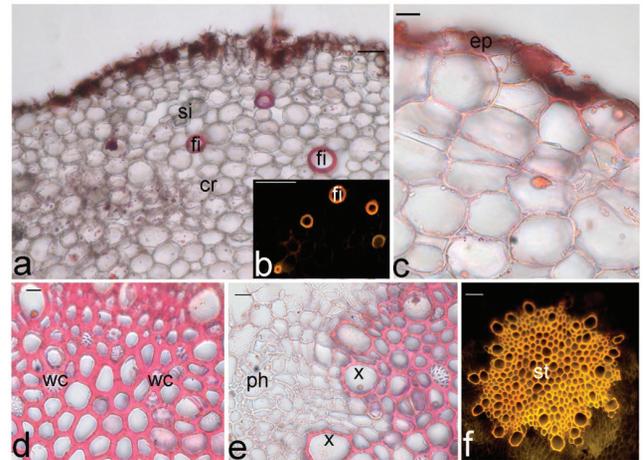
The vascular bundles are bicollateral and oval-shaped. The cambial zone consists of 2-3 rows of thin-walled small rectangular and meristematic cells arranged radially. The external and internal phloem are arranged in small groups or patches adjoining the xylem (Fig. 3g,h). The internal and external phloem patches measure  $34.5 \pm 1.95 \times 38.5 \pm 1.95 \mu\text{m}$  and  $38.33 \pm 1.51 \times 48.33 \times 2.01 \mu\text{m}$ , respectively. The vessels of the metaxylem measure  $41.95 \pm 0.86 \times 32.2 \pm 0.65 \mu\text{m}$  and the protoxylem cells measure  $20.1 \pm 0.68 \times 14.9 \pm 0.47 \mu\text{m}$ . Pith is composed of thin-walled parenchyma cells with intercellular spaces. Fibers, laticifers and silica bodies occur in the pith region (Fig. 3a). The pith cells measure  $50.5 \pm 5.51 \times 53.83 \pm 6.11 \mu\text{m}$ . The anatomical features of the mature stem are almost similar to that of the young stem except with few differences. The epidermal layer forms the outer layer in young stems and sclerenchymatous sheath consisting of fibers around the stellar region in mature stems is absent in the young stems. Moreover, the epidermis in young stem is replaced by the periderm in the older stems.

### Root

The root appears circular in transverse section (Fig. 4a). Unicellular root hairs are present. The root hair measures  $45.5 \pm 3.6 \times 12.6 \pm 0.45 \mu\text{m}$ . The epidermis is uniseriate, compactly arranged, and composed of oval to round shaped thin-walled parenchymatous cells (Fig. 4b). The cell dimensions of epidermal cells are  $33.66 \pm 2.24 \times 39.23 \pm 2.38 \mu\text{m}$ . Cortex is 16-18 layered, made up of thin-walled larger to smaller circular to oval-shaped parenchymatous cells enclosing triangular or squarish intercellular space. The cells in the cortical region measure  $36.45 \pm 1.26 \times 44.83 \pm 2.03 \mu\text{m}$ . Silica bodies and thick-walled fibers occur in the cortex (Fig. 4a). The length and width of fibers are  $36.66 \pm 2.24 \times 39.23 \pm 2.38 \mu\text{m}$ . Endodermis and pericycle are indistinct. Vascular bundles are arranged radially. Xylem is exarch. Xylem and phloem cells are differentiated by conjunctive tissue made up of parenchymatous cells (Fig. 4e). Xylem arches are 14-16 and the stele bears pitted water storage cells, and cells with lignin thickened walls (Fig. 4d,f). The water-storage cells measure  $17.00 \pm 1.27 \times 11.05 \pm 0.87 \mu\text{m}$ . Pith is absent.

### Discussion

The vegetative anatomy of *T. alternifolia* revealed certain variations in their anatomical traits when compared to other members of the Apocynaceae family. Trichomes are absent in leaves of *T. alternifolia* as reported in other species of *Tabernaemontana* (Omino 1996; Guidoti et al. 2015). However, trichomes have been reported in members of the Apocynaceae like *Mandevilla velutina* (A.DC.) Woodson and *N. oleander* (Santos et al. 2009; Maciel et



**Figure 4.** Vegetative anatomy of *Tabernaemontana alternifolia* root (a-f) **a.** Transverse section (T.S.) of root showing epidermis, sclerenchymatous fibers, silica bodies in the cortex **b.** Fluorescent image (insert) showing deposition of lignin in sclerenchymatous fibers, **c.** Epidermis, **d.** Pitted water-storage cells in the stellar region, **e.** vascular bundles with xylem and phloem, **f.** Fluorescent image of stele indicating the presence of lignin deposition. Scale bars = 50  $\mu\text{m}$ .

ep = epidermis, fi = sclerenchymatous fiber, si = silica bodies, cr = cortex, wc = water-storage cells, x = xylem, ph = phloem, st = stele. Scale bars = 50  $\mu\text{m}$ .

al. 2010). The hypostomatic leaves and paracytic stomata in *T. alternifolia* are similar to those reported by Omino (1996) in *T. stapfiana*, *T. pachysiphon*, *T. ventricosa* and *T. elagans*. However, in contrast to the observation of the present study, amphistomatic leaves were reported in *T. catharinensis* (Guidoti et al. 2015) and *M. coccinea* (Durate and Larrosa 2011). The presence of stomata on the abaxial surface of the leaf in *T. alternifolia* helps to minimize the water loss (Mbagwu et al. 2008). Moreover, paracytic type of stomata is the characteristic feature of the genus *Tabernaemontana* (Metcalf and Chalk 1950, 1988; Omino 1996). The stomatal index of 73.42% in *T. alternifolia* is less than that of the stomatal index reported in *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult. However special type of stomata called giant stomata is reported in *T. divaricata* (Gowramma and Sultan 2018) is absent in leaves of *T. alternifolia*. The differences in the stomatal index could be due to the physiological responses to various environmental factors (Adegbite 2008; Aworinde et al. 2012). Cuticle acts as an apoplastic barrier against water loss and also prevents the entry of other solutes from external sources into the plant tissue (Yeats and Rose 2013). Leaves of *T. alternifolia* possessed thin-cuticle over the leaf that contradicts studies where a thick cuticle has been reported to cover the leaves in *Tabernaemontana* species (Omino 1996; Guidoti et al. 2015). However, Mayberry (1937) observed thin cuticle covering the leaves of *Amsonia tabernaemontana* Walter. In contrast to the observations

of the present study, a bilayered epidermis covering both the leaf surfaces has been reported in *T. pachysiphon* and *T. stapfiana* (Omino 1996).

The mesophyll is dorsiventral in *T. alternifolia* which is similar to the other members of the Apocynaceae family (Metcalf and Chalk 1950). The ecological anatomy of the tree species suggested that the degree of mesophyll differentiation mostly depends on the degree of exposure to the sun (Hanson 1917; Ryder 1954). The mesophyll in *T. alternifolia* resembles that of *T. catharinensis* consisting of 2-3 layers of palisade parenchyma cells (Guidoti et al. 2015). On the contrary, palisade parenchyma was 3-4 layered in *T. stapfiana* (Omino 1996). The spongy parenchyma cells in *T. alternifolia* are similar to those of other *Tabernaemontana* species (Omino 1996; Guidoti et al. 2015). Laticifers and crystals present in mesophyll, multilayered hypodermis and bicollateral vascular bundles in *T. alternifolia* are the common characteristic features of Apocynaceae (Metcalf and Chalk 1950; Omino 1996).

The petiole is flattened arch-shaped with small projections in *T. alternifolia* is similar to those observed in *T. catharinensis*. Nevertheless, concave-convex structured petiole was reported in *Forsteronia glabrescens* Müll.Arg. and *M. coccinea* of Apocynaceae family (Larrosa and Duarte 2006; Durate and Larrosa 2011). The presence of non-lignified fibers in *F. glabrescens* adjacent to the internal phloem is in accordance with the observations of the present study (Larrosa and Duarte 2006). Angular collenchyma cells present in the hypodermal region of *T. alternifolia* is similar to those in *T. catharinensis* (Guidoti et al. 2015) and contrastingly, annular collenchyma was noted in *M. coccinea* (Duarte and Larrosa, 2011). Silica bodies and laticifers observed in *T. alternifolia* are similar to those of *T. catharinensis*. The presence of silica bodies in *T. alternifolia* could prevent from collapsing of the plant during drought (Metcalf and Chalk 1979). In the present study, vascular bundles are U-shaped and bicollateral. Similar observations were reported in *T. elegans*, *T. stapfiana* and *T. catharinensis* (Omino 1996; Guidoti et al. 2015). In contrast to the results of the present study, V-shaped, shallow and curved arc-shaped bicollateral vascular bundles were reported in *Himatanthus sucuuba* (Spruce ex Müll.Arg.) Woodson, *Voacanga thouarsii* Roem. & Schult., *Rauvolfia mombasiana* Stapf and *Strophanthus barteri* Franch (Larrosa and Duarte 2005; Omino 1996).

The secondary growth in *T. alternifolia* stem reveals the formation of the periderm in the outermost layer that is in line with the observations of Larrosa and Duarte (2006) and Guidoti et al. (2015) in *H. sucuuba* and *T. catharinensis*. Nevertheless, an incipient secondary growth in *M. coccinea* stem possessed single-layered epidermis covered with thick striated cuticle (Duarte and Larrosa, 2011). Periderm forms a protective tissue during the secondary

growth replacing the epidermis (Evert 2006). The angular collenchyma cells, sclerenchymatous sheath consisting of lignified thick-walled fibers enclosing the vascular bundles in *T. alternifolia* is as same as *T. catharinensis* (Guidoti et al. 2015). The thick-walled fibers commonly found in patches, provides rigidity and flexibility to the plants (Esau 1974; Costa et al. 2006; Scatena and Scremin-Dias 2006). Similar to *T. alternifolia*, the stem of *T. catharinensis* in transverse section showed the presence of fibers and laticifers in the cortical region (Guidoti et al. 2015). Moreover, the distribution of laticifers differs among the species, for example, laticifers occur only in the cortex in *Himatanthus lancifolius* (Müll.Arg.) Woodson (Baratto et al. 2010a) whereas, in *Rauvolfia sellowii* Stapf, it was reported in the phloem cells and marrow (Baratto et al. 2010b). The laticifers produce latex due to physical damage and consist of varied latex compounds that protect the plants against herbivores or pathogens (Ramos et al. 2019). Occurrence of laticifers is very common in Apocynaceae family (Metcalf and Chalk 1950) and may be branched or unbranched or non-articulated (Esau 1977; Mahlberg 1993). Laticifers in the *T. alternifolia* are non-articulated as observed in *Tabernaemontana coronaria* Willd (Rao and Malaviya 1966). However, articulated laticifers have been reported in the other member of Apocynaceae like *T. catharinensis* (Canaveze and Machado 2016), *Mandevilla atrovioleacea* (Stadelm.) Woodson (Lopes et al. 2009) and *Allamanda blanchetii* A. DC. (Gama et al. 2017)

Silica bodies were present in cortex and pith regions of *T. alternifolia*. However, the idioblasts containing silica bodies were not observed in *T. catharinensis* (Guidoti et al. 2015). Contrastingly to the observations of *T. alternifolia* in the present study, starch grains were reported in some species of Apocynaceae (El-Kashef et al. 2015; Duarte and Larrosa 2011). The intraxylary or internal phloem in *T. alternifolia* is a common feature of Apocynaceae family. In the present study, internal phloem existed as an isolated strand forming a group of patches similar in other species of the family, like *A. tabernaemontana* (Mayberry 1937) and *M. coccinea* (Larrosa and Duarte 2011). In contrast to the results of the present study, internal phloem formed a continuous ring in *N. oleander* (Maciel et al. 2010). According to Metcalf and Chalk (1950), internal phloem commonly occurs in a continuous ring or isolated groups in the Apocynaceae. The function of internal phloem in stems of climbers includes prevention of breakage when stems are coiled or twisted and enhance the mechanical flexibility of stems (Schenk 1893). However, in trees Premakumari et al. (1985) reported that internal phloem help in the translocation of photosynthates. Therefore, the presence of internal phloem in *T. alternifolia* stem may also perform certain functions like translocation of growth substances.

The anatomy of the root in Apocynaceae is relatively less explored when compared leaves and petiole. *Tabernaemontana alternifolia* had uniseriate epidermis. The cortex in *T. alternifolia* is 16-18 layered in contrast to *Carissa macrocarpa* (Eckl.) A.DC. where the cortex is only 12-15 layered (Allam et al. 2016). However, the indistinct endodermis of *T. alternifolia* resembles with endodermis reported in *C. macrocarpa*. Starch grains were absent in the roots of *T. alternifolia* as reported in other species of Apocynaceae (Appezato-da-Glória and Estelita 1997; Boutebtoub et al. 2009; Allam et al. 2016). Laticifers are one of the important characteristic features of Apocynaceae. Nevertheless, in the present study, although, laticifers were observed in leaves, petiole and stem of *T. alternifolia*, it was absent in the roots. Pitted water-storage cells could help *T. alternifolia* during the xerophytic or drought conditions. Vascular bundles are arranged radially in *T. alternifolia*. The pith was absent in *T. alternifolia* root similar to that reported in *C. macrocarpa* (Allam et al. 2016). The lignin deposition in stelar region as supported by the auto fluorescence could also provide additional structural support to the plant (Willemse 1989).

## Conclusion

The present study revealed that the presence of paracytic stomata restricted to abaxial surface of the leaf, thin cuticle on adaxial and abaxial side in leaves, thick-walled sclerenchymatous patches, isolated internal phloem strands in stems; water storage cells, and deposition of lignin in root stelar region could contribute to the identification of this endemic plant.

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