

# Anatomical studies on the leaf and stem of *Tinospora formanii* Udayan & Pradeep (Menispermaceae), an endemic species to Southern Western Ghats, Kerala, India

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## ABSTRACT

The anatomical studies on leaf and stem of *T. formanii* Udayan and Pradeep an endemic species to Southern Western Ghats, Kerala, India was carried out focusing on its macroscopic, microscopic, maceration along with organoleptic evaluation. Distinguishing characters of the stem revealed the presence of calcium oxalate crystals, simple and compound starch grains and pitted lignified fibers. Leaf anatomy showed the anomocytic and paracytic stomata, pitted lignified fibers, spiral vessels, non-glandular small trichomes, C or half-moon shaped vascular bundle, surrounded with sclerenchymatous tissues and rosette and prism shaped calcium oxalate crystals. Whereas, maceration studies revealed the presence of spiral and scalar form vessel, fibers, calcium oxalate crystals, simple starch grains. These anatomical studies are vital in the present-day trade scenario not only helpful in the proper identification of the genuine materials in use but also to distinguish different species of *Tinospora*, where the stem and leaf are often admixed with other species of *Tinospora* in the crude drug markets.

**KEYWORDS:** Anatomical studies; Leaf; Stem; Endemic species

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## INTRODUCTION

Medicinal plants play a pivotal role in the traditional systems of medicines (Saleem *et al.*, 2001). All traditional systems of medicine mainly focus their attention on the natural products that help the human race to sustain longer and stronger life. Among the world population, 80% of those belongs to developing countries still rely on traditional medicine because of its efficacy, cultural acceptability and lesser side effects (Shrestha & Dhillon, 2003). Lack of systematic written proof and poor-quality control are major cause in less acceptance of the use of alternative medicine. Therefore, there is an immediate need to have standards for all medicinal plants. For this there are several technique and methodology and pharmacognostic and phytochemical studies is one of such tools. Such efforts will be useful in developing standardization of the identity of the plant. Correct characterization and quality assurance of the plant material is an

essential step to ensure reproducible quality of herbal medicine which will help to justify its safety and efficacy (Ahmed *et al.*, 2006; Odugbemi 2008). For this purpose, anatomical studies of *Tinospora formanii* are carried out in this study.

In the present study, the focus was on one of the endemic plant species in Kerala, *T. formanii*. The genus *Tinospora* belonging to the family *Menispermaceae* has about 32 species distributed in Tropical Africa, Madagascar, Asia to Australia and Pacific Islands (Rohwer & Bittrich 1990; Mabberley 1997). In India, the genus is represented by 4 species. Among them, 2 species namely, *T. cordifolia* (Willd.) Miers. ex Hook. f. & Thomson and *T. sinensis* (Lour.) Merr. are known to occur in southern India. The remaining two species *T. crispa* (L.) Miers. ex Hook. f. and Thomson and *T. glabra* (Burm. f.) Merr. are reported from North-East India and Andaman Islands (Pramanik & Gangopadhyay 1993).

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*T. formanii* Udayan and Pradeep, a new species described recently from the Western Ghats of Thrissur district, Kerala, southern India (Udayan *et al.*, 2009). *T. formanii* is a woody dioecious climber growing at an altitude of 500–650 m and is endemic to Kerala. Leaves are ovate to elliptic-lanceolate, coriaceous and glabrous with reticulation more prominent on the lower surface. Female inflorescence is greenish-yellow, glabrous, stout compound elongated pseudo-racemes with six petals. Drupes are globose and red when mature. It differs from *T. crispa* by the absence of tuberculate stems and the glandular patches in the basal nerve axils of the leaves, and in having six petals as opposed to the three of *T. crispa* (Sheema *et al.*, 2017).

## MATERIALS AND METHODS

### Sample Collection

The fresh plant specimens are used in the present study were collected from Vellanimala forest (Latitude 10° 34' N and Longitude 76°19' E) in Thrissur District, Western Ghats of Kerala in Southern India. Plant specimens were identified and herbarium samples present in herbarium of (Udayan *et al.* 03374 B CAL, CALI and MH, Female Plant).

### Macro and Microscopic Studies

Macroscopic and organoleptic characters such as colour, size, odour and taste were documented. Fresh materials of petiole, leaf and stems are cut into 1-2 cm small pieces, fixed with Formalin acetic alcohol (FAA) and take freehand sections stained with safranin and TBO mounted with glycerin. Stomatal number and index were studied in the leaves. All the photomicrographs were taken using Olympus CX 33 light microscope.

### Histochemical Studies

Fresh materials of mature stems were cut into small pieces and freehand sectioning was done. Detailed identification characters were noted with or without staining procedures (Krishnamurthy, 1988). Histochemical studies on stem were carried out using lignin Potassium iodide-iodine-sulphuric acid method (Chamberlain, 1924), Starch Iodine-Potassium-iodide reaction (Johansen, 1940), total proteins Fast green method (Ruthmann, 1970).

### Macerations Techniques

The fresh material of the mature stems was cut into small slices or slivers about 300 micrometers thick. Then soaked in macerated solution of equal parts of 10% aqueous nitric acid and 10% aqueous chromic acid. This solution kept into the room temperature in 24 hours (Jeffery, 1917).

## RESULT

### Organoleptic Evaluation

The organoleptic characters of leaves showed the light green appearance from both sides having small elongated glands. The

leaf powder was yellowish green in colour, soft in texture, with unpleasant odour and light bitter in taste. The stem was green in nature (soft) having light brown colour from the basal side.

### Macroscopic Characters

The species is restricted to the moist deciduous forest at an altitude of 550 m at Vellanimala forest, Thrissur district of Kerala. It is rare and sparsely distributed only 3 mature plants were noted in the area along with *Tinospora sinensis*. *T. formanii* is a woody dioecious climber, with pale yellowish watery sap when cut; bark smooth, shining, papery, peeling off into scales, with prominent leaf-scars. Stems up to 4 cm thick, with several prominent concentric rings, lenticellate. Leaves (Figure 1, Table 1) alternate, ovate to elliptic-lanceolate, 6–14 x 4–8 cm, coriaceous, glabrous, acuminate at apex, cordate at base, entire; 3-nerved basally, lateral nerves intra-marginal, running to tip of lamina, distinctly looping along the margin; lateral nerves 3–4 pairs, reticulation more prominent on lower surface, glandular patches present in basal nerve axils; petioles slender, slightly swollen and geniculate at base, 5–12 cm long, often drying blackish at least at the swollen base and near the lamina. The sufficient number of female flowers and fruits could be collected only after two years of continuous field observations. This liana reaches the top canopy of host trees such as *Diospyros*



**Figure 1:** Field images of *T. formanii* Udayan & Pradeep. a. Habit, b. Fruits and c. Flowers.

*crumenata* Thw. (Ebenaceae); *Drypetes oblongifolia* (Bedd.) Airy Shaw (Euphorbiaceae) and *Bambusa bambos* (L.) Voss (Bambusaceae). This plant while flowering usually devoid of leaves, (January – May) and soon after flowering and fruiting, leaves emerge in the early monsoon (June) and thus the leaves are produced gregariously. Only 3 female plants could be located in the entire study area without male plants.

## Microscopic Characters

### Petiole

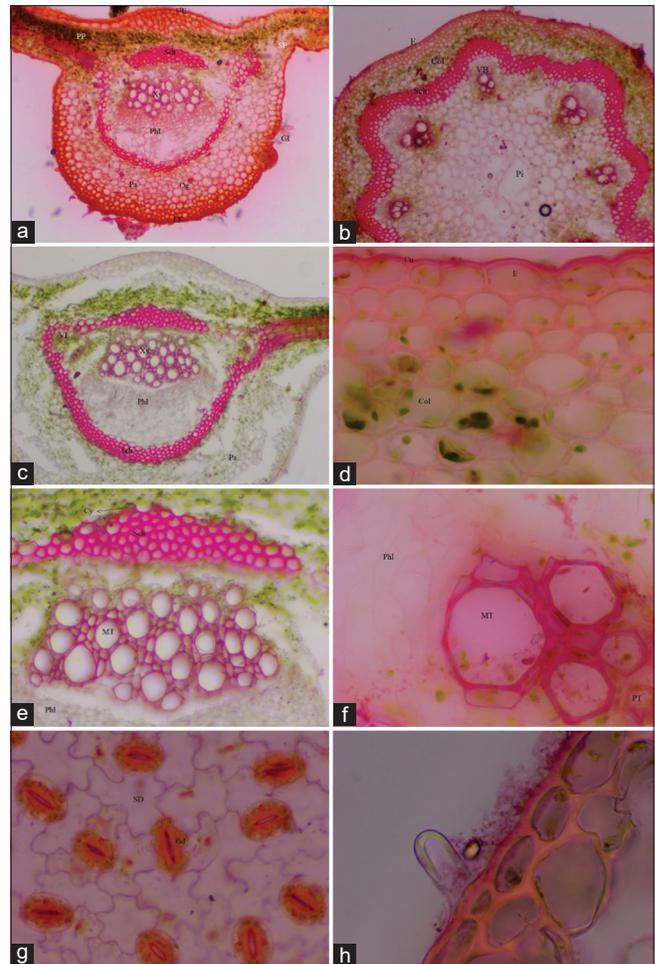
Transverse section of the petiole shows (Figure 2, Table 1) the epidermis, ground tissue, vascular bundles and centrally located parenchymatous tissue. Outermost single layered epidermis consisting of narrow shaped, compactly arranged thick walled cuticle. Beneath the epidermis 4-6 layer of collenchyma tissue consisting of compactly arranged and thin-walled collenchyma cells. Beneath cortex there is 3-4 layered pericycle fibers. These thick wall cells are covered with a cap like structure of the vascular bundles and prism-shaped calcium oxalate crystals. Vascular bundles are 6-8 number of cells, collateral, open and endarch arranged in a ring. Metaxylem towards the sclerenchymatous region and protoxylem towards the parenchymatous region. Medullary ray single layered parenchymatous cells are interrupted to the xylem region.

### Leaf

Transverse Section of *T. formanii* leaf passing through the midrib (Figure 2) shows slightly convex nature, however non-glandular small hairs are present in abaxial side veins only. The adaxial and abaxial side epidermis is single layered rectangular shaped and compactly arranged, covered with thin wall cuticles. The lamina is a dorsiventral structure made up of mesophyll tissues which can be divided into adaxial side palisade and abaxial side spongy tissues. The mesophyll cells mostly appear in the chlorophyll pigments. Palisade parenchymatous cells are single layered, columnar cells. The spongy cells are loosely arranged. Midrib vascular bundles are collateral and open. Xylem is more than 10 numbers of the cells. Xylem rays are present in the fibers and rosette calcium oxalate crystals. The C or half-moon shaped structure of sclerenchyma cells covered with vascular bundles. This thick-wall cell is present in the square and prism-shaped calcium oxalate crystals. The phloem arrangement is towards the midrib abaxial side. Anomocytic and paracytic stomata are present on both sides of the leaves. This stoma surrounded by four subsidiary cells, which are indistinguishable in size and shape.

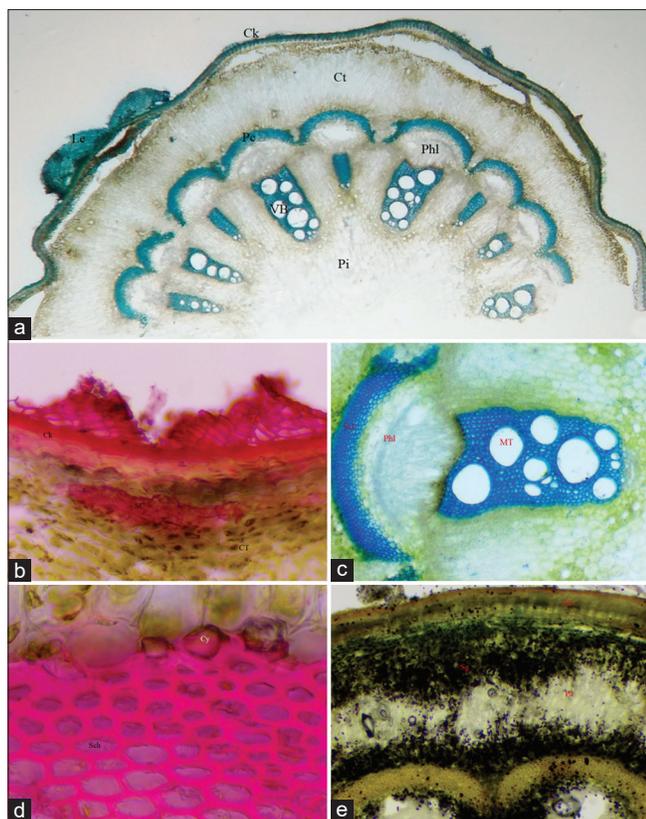
### Stem

Transverse Section of the stem is circular in outline (Figure 3, Table 1) with cork, cortex and stellar region. The cork comprises of an outer zone of thick-walled reddish-brown compressed cells and an inner zone of thin-walled colourless, tangentially arranged cells. The cork tissue is broken at some places due to lenticels. The outer zone of



**Figure 2:** Transverse section of *T. formanii* Petiole and Leaf (a & b) ground plan 4x; c – Leaf midrib vascular bundle 10x; d – Epidermis of petiole 40x; e – Metaxylem and Phloem 10x; f – Petiole vascular bundle 40x; g & h – Anomocytic and Paracytic stomata and non-glandular hair 40x; UE – Upper Epidermis; LE – Lower Epidermis; Col – Collenchyma; MT – Metaxylem; Pi – Pith; PT – Protoxylem; Ph – Phloem; Pa – Parenchyma; PP – Palisade Parenchyma; SD – Subsidiary cells; GD – Guard cells; Scl – Sclerenchyma; Tr – Tracheid; VB – Vascular Bundle.

cortex consists of 20 to 25 layers of tangentially elongated parenchymatous cells, patches on some narrow-shaped stone cells and prism-shaped calcium oxalate crystals and the cells located towards inner side are polygonal in shape and filled with abundant oval and elliptical shape starch grains. Pericyclic cells are lignified fibers associated with a large number of prism-shaped crystals, broad and narrow shaped stone cells containing an outer layer towards the cortex. Stellar region is composed of discrete vascular strands with 8 major and 6 minor wedge-shaped strips of xylem, externally surrounded by circular strips of 10 layers of phloem on alternating, with wide medullary rays. The medullary rays are made up narrow-shaped parenchymatous cells with simple starch grains. Cambium is composed of 2 to 3 layers of loosely arranged thin walled cells. Xylem consists of vessel elements, tracheid, parenchyma and fibers. Multilayered medullary rays filled with starch grains. Pith made up of 30 layers compactly



**Figure 3:** TS of the *T. formanii* stem a – Ground plan of the stem; b – Enlarged view of the lenticels 10x; c – Enlarged view of the single vascular bundles 10x; d – Enlarged view of the prismatic crystals 40x; e – Enlarged view of the abundant starch grains 10x; Le – Lenticels; Ck – Cork; Ct – Cortex; Cy – Crystals; MT – metaxylem; Pa – Parenchyma; Pi – Pith; Pc – Pericycle; Sc – Stone cells; Sg – Starch Grains; Scl – Sclerenchyma; VB – Vascular Bundles.

arranged, large thin-walled parenchymatous cells containing simple, elliptical shaped abundant starch grains.

### Maceration Techniques

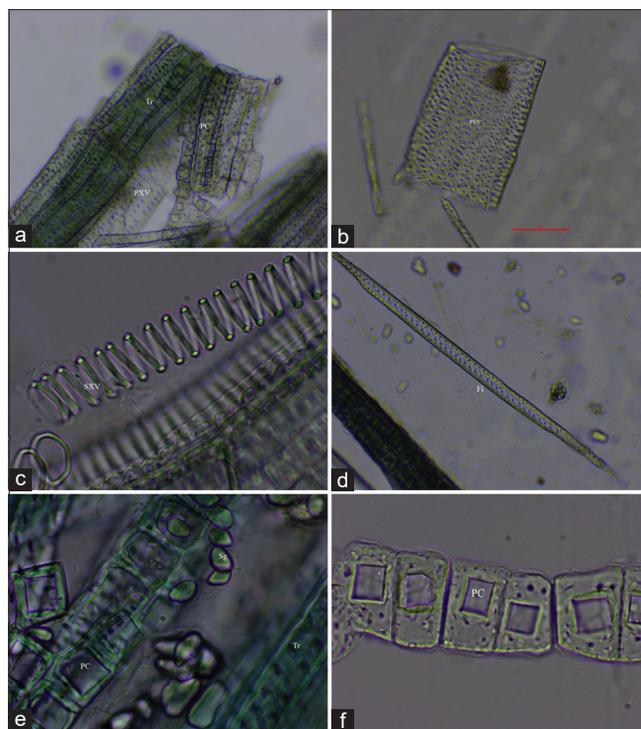
Macerated stem shows scalariform pitted vessels, tracheid, xylem vessels with spiral thickening, fibers, prism-shaped calcium oxalate crystals and simple, oval to elliptical starch grains (Figure 4).

### Stomatal Index

Stomata index was calculated on both leaf surfaces. Stomata index is more on the abaxial epidermis compared to the adaxial epidermis. It is stomatal number on the abaxial and adaxial side was found as 41 and 19, respectively and Stomatal Index of the abaxial and adaxial surface is 34.4 and 21.8 respectively.

## DISCUSSION

*T. cordifolia* has an enormous importance in pharmacology. The species of *Tinospora* are widely distributed in the tropical and subtropical regions of India and China and have multiple



**Figure 4:** Macerated section of the *T. formanii* stem a – Xylem thick-walled cells 10x; b – Pitted xylem vessels 40x; c – Spiral xylem vessels 40x; d – Xylem fiber 40x; e – Starch grains 40x; f – Prism crystals 40x; Tr – Tracheid; PC – Prism Crystals; PXV – Pitted Xylem Vessels; SXV – Spiral Xylem Vessels; Fi – Fiber; Sg – Starch grains.

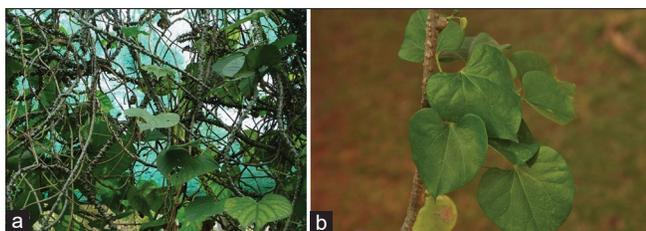
**Table 1:** Anatomical characters of *T. formanii*

Characters	Leaves	Stem
<b>Macroscopic &amp; Organoleptic</b>		
Shape	Ovate to elliptic-lanceolate	Circular
Glands & Lenticels	small elongated glands in both surface	Lenticellate
Powder colour	yellowish green	light brown
Odour	Unpleasant	Unpleasant
Taste	little bitter	No
<b>Microscopic</b>		
Epidermis/Bark	Single layered in both epidermis	Smooth, shining, papery
Cortex	Mesophyll cells in lamina and Midrib parenchyma and collenchyma	20-25 layers of tangentially elongated parenchymatous cells
Vascular bundles	Midrib vascular bundle	8-9 collateral and open collateral and open
Pith	Absent	Large compactly arranged, thin walled parenchyma cells
<b>Histochemical</b>		
Starch Grains	Absent	Present
Calcium oxalate crystals	Rosset	Prism-shaped
Lignin	Absent	Present

applications in different folk medicine, including the Indian Ayurvedic System (Sadik *et al.*, 2001; Ayyanar and Ignacimuthu, 2011; Sutar and Pal, 2015). Various studies including pharmacognostic, quantitative and qualitative analysis are available for these plants. Morphological and anatomical

**Table 2. Comparative stem anatomical characters**

Character	<i>T. cordifolia</i>	<i>T. formanii</i>
Shape in TS	Wedge	Circular
Bark	Warty, glabrous with round lenticels.	Smooth, shining, papery, several prominent lenticellate.
Cortex	Cortex is 15-22 layers parenchyma cells are rounded and elongated cells	Cortex is 20-25 layers of tangentially elongated parenchymatous cells
Pericycle	Cap-like structure of 5-6 layers of narrow pericycle made from sclerenchyma with lignified	Cap-like structure of 6-8 layers of tangentially pericycle made from sclerenchyma with lignified
Secretory sac	Present	Present
Vascular bundles	5-7 collateral and open	8-9 collateral and open
Starch grains	Present in cortical, vascular bundles and pith	present in cortical, vascular bundles and pith
Pith	Large compactly arranged, thin walled parenchymatous cells	Large compactly arranged, thin walled parenchymatous cells
Crystals	Present	Present
Source	Bonde & Upaehye, 1989; Current work Nasreen et al., 2010	



**Figure 5:** Filed vegetative images of a. *T. crispa* (L.) Miers. ex Hook. f. and Thomson & b. *T. cordifolia* (Willd.) Miers. ex Hook. f. and Thomson.

studies have shown the distinctive and differentiating characters between *Tinospora* species. Metcalfe and Chalk (1950) reported Ranunculaceous (Anomocytic) stomata in *Menispermaceae*. Similarly, in the present study our observations on Anomocytic and Paracytic stomata, non-glandular hairs with *T. formanii* leaf. *Tinospora* species stem and leaf macro-microscopic diagnostic key characters as described in earlier studies viz., *Tinospora cordifolia*, *T. sinensis* and *T. crispa* appear similar in external morphological characters by having broadly cordate leaves, cylindrical twining stems, often lenticels and aerial adventitious roots. *T. cordifolia* stem cylindrical, slender, wedge shaped and 5-7 vascular bundles. *T. sinensis* stem, circular outline and most abundant in unicellular trichome, 5-8 vascular bundles and *T. crispa* stem circular in outline, 5-7 vascular bundles (Begum et al., 2019; Parveen et al., 2020). The study has been undertaken to differentiate and standardize *T. formanii* with that of *T. cordifolia*. Stems anatomical characters of both these species have been provided in detail (Table 2) along with morphological comparative characters of *T. crispa* and *T. cordifolia* (Figure 5).

## CONCLUSION

This type of anatomical studies will help to establish identity, purity, quality and safety of the drug for various pharmaceutical

industries in the preparation of AYUSH medicines when a drug is admixed with other species of *Tinospora* and it is helpful to research scholars, students in carrying out further research studies for further establishment of different species of *Tinospora*; wherever is a very important drug in the indigenous system of medicine for further documentation of research studies.

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