

## Foliar Anatomical Study of *Cordia* Species (Boraginaceae) from the Northern Western Ghats, India

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### ABSTRACT

The plant family Boraginaceae worldwide distributed and its contained 145 genera. Carolus Linnaeus described the *Cordia* genus in the format of *Cordia* L., Sp. Pl. 1: 190 (1753). The genus *Cordia* L. (Boraginaceae) is a medicinally important taxon. The first species of *Cordia dichotoma* G. Forst. has been used for the treatment against fever, ulcers, dyspepsia, ringworm, etc. It has also been reported for its activities such as analgesic, antidiabetic and anti-inflammatory actions. Another second species, *C. sinensis* Lam., which is endemic to the northern Western Ghats, is a potential medicinal plant and is presumed to have similar biochemical activities. Since the species is consumed mainly in the crude form, it is difficult to establish the identity based on only leaf or stem characters. A comparative study has been carried out to authenticate the identity of these two species based on foliar anatomical characters. The anatomical sections of a leaf (TS), and petiole (TS) of both the species were observed under the bright-field microscope, and characters were documented. Though the diagrammatic outline of the leaf sections is similar, detailed anatomical characters are significantly different. The structure and number of the trichomes are observed abaxial and adaxial sides of the leaves. The trichomes were also found to be useful characters for identification. In the case of leaf, the number and arrangement of vascular bundles, arrangement of collenchyma at the lower epidermis, palisade cells, and distribution of storage cells are the major characters to differentiate both species. During the study, we also observed the detailed characters of the cuticular layer, trichomes, and stomatal characters. Ranunculious type stomata and the presence of cystoliths in the basal cell of the trichome are the common characteristics observed in both species. On the basis of the primary study, the species can be differentiated based only on foliar anatomical characters.

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### Introduction

The plant family Boraginaceae worldwide distributed and its contained 145 genera. Carolus Linnaeus described the *Cordia* genus in the format of *Cordia* L., Sp. Pl. 1: 190 (1753). In the *Cordia* genera *C. dichotoma*, *C. domestica*, *C. macleodii*, *C. sinensis*, *C. wallichii*, this five specie distributed in the Northern Western Ghats Maharashtra state (Singh & al., 2000). *Cordia* species are perennial tree species. The *C. sinensis* medicinal uses are diarrhea & dysentery, anthelmintic, antidiabetic activity, throat pain treatment, this plant bark making a decoction and these decoction use as a gargle for throat treatment and economic use- *Cordia* use as most timber yielding plant (Kirtikar & Basu, 1918; Metcalfe & Chalk, 1965; Modhvadia, 2009; Lohidas & al., 2015; Patil, 2018). Fresh leaves use as treat to jaundice and fruit jounce use as fevers treatment. Human consume a plant parts as food considering a primary requirements but sometime human use a plant part as fodder and medicines. In Ayurvedic medicinal system both plant considered as source of Laghushleshmataka (Maundu, 1999; Khare, 2007).

Anatomy is the scientific study of the internal structure of organisms including organs and different kinds of tissues

(Bozman, 1967; Gray, 2003). The present work completely depend on a detailed anatomy study leaf, and petiole of *Cordia sinensis* Lam., Tabl. Encycl. n. (1914) and *Cordia dichotoma* G.Forst., Fl. Ins. Austr. 18, n. 110 (1786) species from Northern Western Ghats of Maharashtra. Understanding and evaluation of *Cordia* species anatomy is important to the study of emergence branches are plant taxonomy, plant systematic, plant physiology, ecology, paleobotany and evolutionary biology. During the study we cover many of comparative aspects of physical and anatomical structure of leaf and petiole. Describe a internal structure of *Cordia* spp. using a hand lens, light compound microscope and Stereo Zoom Microscope (MSZ Bi). During the literature study of *cordia* species we observed many of anatomical variation TS leaf (Epidermis, Palisade layer, Collenchyma, Number vascular bundles, and Parenchyma cell ) and TS of petiole (Epidermis, Palisade layer, Collenchyma, Number vascular bundles, and Parenchyma cell) of both the species were observed under the bright-field light compound microscope and their characters were documented (Metcalf & Chalk, 1965; Khandelwal & Sethi, 2019). Both the species produces a annual ring and observed a secondary growth. These plant

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shows annual rings because of well developed secondary growth. In stem woody anatomy observed a broad range arrangement of vessels and fibers produces a porous wood (Metcalf & Chalk, 1965). Xylem is a complex tissue system composed of different cell types. The term tracheary element is derived from "trachea," a name originally resembling insect tracheae (Katherine, 1960). Two basic types of tracheary element: tracheids and vessel elements. The vessel elements adjoining to each other, at their end possess perforations, whereas tracheids elements lack these perforations. Tracheids elements are elongated, lignified, thick walled and pointed at both end. The perforations several openings which are scalariform perforation plate. Xylem vessels showing a simple pitted in their lateral walls. To observe xylem structure, mature stem are sectioned transversely (transverse section: TS) and longitudinal planes: along the rays (radial longitudinal section: RLS and tangential longitudinal section: TLS). Rays are termed one cell wide tangentially called uniseriate and more than one cell wide called multiseriate observed in both species, viewed in TS and TLS. The vascular cambium produces from secondary xylem and phloem of stem. Prismatic calcium oxalate crystals observed in *C. sinensis* in the pith region of stem (Evert, 2006; Rudall, 2007; Khandelwal & Sethi, 2019). Starch grains is one of the non-nitrogenous stored form observed in studied plant species. Tapioca starch grain type observed in both plant species (Stevens, 1907; Wallis, 1939). The tannins red or brown stained in the cells. Tannins are a polyphenolic important secondary metabolite compound. It is observed in mature leaves and petiole of *C. sinensis*. The primary important function of tannins is protective mechanism repellent to predators ( Rao & Das, 1979; Zobel,

1985; Evert, 2006). Plants that produce and show a allelopathy phenomenon. Some plants leaf synthesized released in response to environmental stress (Bussotti et al., 1998). The stone cells are observed in both species. Stone cells important function is thickening and lignification of the walls of thin-walled parenchyma cells. Stone cell providing a strengthening and toughness of parenchyma cells. The presence of stone cells are frequent and important landmarks in the study of powdered drugs. In powdered drug stone cells are use apparently. Sclerenchyma cells are classified into two categories fibers and sclereids ( Rao, 1957; Evert, 2006).

In leaf observed outermost protodermal single cell layer i.e epidermis tissue that covers the entire the epidermis is a arise from radial patterning of L1 layer of tunica. The cortex and endodermis arises from L2 layer and combinely L1 and L2 is a part of tunica. The pericycle and vasculature arises radial patterning from L3 layer of carpous. Meristemoid tissues get superficial change of cells that gives rise epidermal outgrowths trichome and stomata. In both species observed a ranunculaceous type stomata and simple uniseriate trichome (Taiz & Zeiger, 2002). The present study revealed the cystoliths unique character in *Cordia* species that are important for identify the taxa at their vegetative stage (Evert, 2006). Ranunculious type stomata are observed on the abaxial and adaxial surface of the leaf (Van Cotthem, 1970). Trichome is specialized epidermal cell are used as a trait in taxonomy. Collenchyma and sclerenchyma cell are supporting tissues in plants. In both species, vascular bundles are observed in parts of leaf and petiole but in both species of petiole arc type vascular bundles observed. Abundance of tapioca starch grains type found in stem of both *Cordia* species (Stevens, 1907; Wallis, 1939).

**Table 1. Classification of *Cordia sinensis* and *Cordia dichotoma***

Kingdom	Plantae	Plantae
Class	Dicotyledons	Dicotyledons
Sub-class	Gamopetalae	Gamopetalae
Series	Bicarpellatae	Bicarpellatae
Order	Polemoniales	Polemoniales
Family	Boraginaceae	Boraginaceae
Genus	<i>Cordia</i>	<i>Cordia</i>
Species	<i>C. sinensis</i> Lam.	<i>C. dichotoma</i> G. Forst.
Common names	Gondani, Gondi, Gundi, Naruvili	Lasura, Bhokar, Lasore and Gunda

(Bentham & Hooker, 1862)

**Table 2. Morphology of *Cordia sinensis* and *Cordia dichotoma***

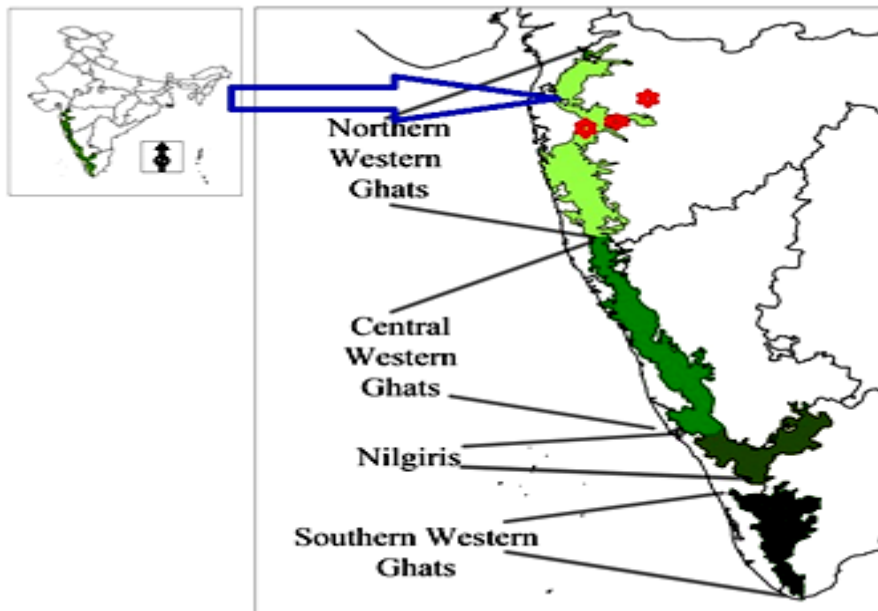
Characteristics	<i>Cordia sinensis</i>	<i>Cordia dichotoma</i>
Height	5.8 m high	5 to 10 m high
Bark	Brown in colour	White in colour
Leaves	simple	simple
Flower	white in colour	white in colour
Inflorance	axillary cymes	cymose
fruit	reddish.brown, pulpy, edible, single seeded.	fruit yellow or pink, edible, single seeded.

(Singh et al., 2000)

### Materials and Methods

**Study area and collection:** Leaf and stem bark of *Cordia sinensis* were collected from Pune, Nashik and Aurangabad district during 2019-2020 of Maharashtra, India. The plant was identified and authenticated by. Plant parts were collected from different localities of Maharashtra state (Table 1)

**Anatomical study of leaf and petiole:** Make a thin sliced from fresh mature stem samples for anatomical study (8mm to 10mm), leaf (main vein and lamina) and petiole (centre of the petiole) from both plants species. Prepare hand sections stained were stain using 0.1% Safranin and mount in glycerin and examined under bright-field compound microscope (Chamberlain, 1920; Khandelwal & Sethi, 2019). Measure a morphometric observations of length, diameter, breadth and number of layers ( $\mu\text{m}$ ). Calculate stomatal index for the abaxial leaf surface using. Calculate trichome density and identify a type of trichomes. (Salisbury's, 1927; Evert, 2006)



## Results

### 1. Anatomical characteristics of leaf

Leaf anatomical studies using Bright-field light microscope, the transverse section (t.s.) of the leaf at the midrib region, Ovate shape (*C. sinensis*.), elongated lanceolate (*C. dichotoma*) (Figs.- 2, 4 and 5 Table- 2, 4 and 5)

**Epidermis:** Single cuticular layer observed on adaxial and abaxial surface of the leaf. Structures such as cystoliths are present on adaxial and abaxial surface of the leaf. Epidermal cell structure are straight, isobilateral, unicellular and papillose lower surface of leaf. There were significance variation between taxa i.e papillose projection lower surface epidermis observed in *C. dichotoma* and absent in *C. sinensis*. Cystoliths were present in both species of adaxial and abaxial surface of leaf. The *Cordia* species studied showed “Ranunculious” type of stomata, The stomatal index was calculated abaxial surface of leaf. Trichomes were present in both species of adaxial and abaxial surface of the leaf. Calculate trichome density. Simple uniseriate trichomes observed in both species.

**Collenchyma cell:** The terms collenchyma derived from the Greek word *colla* i.e glue. Thick glistening cell wall is a unique characteristic of Collenchyma cell. Collenchyma is a living tissue with cell wall is thickened (Fig.). In studied species 3 - 4 layered and 3 - 5 layered of collenchymas cell in *C. sinensis* and *C. dichotoma* respectively. Generally observed a chloroplast in collenchyma cell, but in studied species chloroplast are not observed. collenchyma cell layer is observed beneath the epidermis. The wall thickening in collenchyma cell aobserved several patterns, on the basis of that patterns collenchyma cells are classified (1) lamellar, or plate, collenchyma (2) angular collenchymas (3) the lacunar, or lacunate, collenchymas (4) annular collenchym. During the study lamellar, or plate, collenchyma type of collenchymas are observed (Katherine, 1960; Evert, 2006; Rudall, 2007).

**Parenchyma cell:** The word parenchyma is derived from the Greek *para*, beside, and *en-chein*, to pour. intercellular spaces are observed in both species. Parenchyma is a living tissue with cell wall is thin. In studied species 3 - 4 layered and 2 - 3 layered of parenchyma cell in *C. sinensis* and *C. dichotoma* respectively.

**Palisade layer:** A transverse section of *Cordia* leaf the palisade layer composed a single layered parenchyma cells that were closely packed. Palisade parenchyma cells are arranged vertically elongated. bundle sheath extension observed in between palisade layer of leaf lamina *Cordia* leaf.

**Vascular Tissue:** Vascular bundle composed a xylem and phloem, in which smaller vein vertically transcurrent by sclerenchyma. The water conducting tissue, xylem on the adaxial side and the food conducting tissue, phloem on the abaxial side of leaf. Xylem tissue are thick walled and phloem tissue are thin walled. *Cordia* species can be categorised into two types i.e. broad Ushaped and narrow U-shaped. The species *C. sinensis* and *C. dichotoma* show broad Ushaped, show narrow U-shaped.

### 2. Characteristics of petiole

Transverse section (T.S.) of the petiole, petiole shape are ovate (Figs. 3, Table- 3). The petiole shape of *C. sinensis* and *C. dichotoma* abaxial surface concave and adaxial surface convex. The epidermis is a single layer of cells. Trichomes were found in *C. dichotoma* and absent in *C. sinensis*. A well-developed collenchyma cells are beneath to the epidermal cells. In collenchyma cells observed a tanniniferous secondary compounds. Collenchyma 3 - 4 and 5 - 7 layered *C. sinensis* and *C. dichotoma* respectively. The pitted parenchymatous cells are irregular in outline in *C. dichotoma*. The parenchymatous cells are parenchymatous cells are hexagonal or irregular. Parenchyma 3 - 4 and 4 - 5 layered *C. sinensis* and *C. dichotoma* respectively. t.s of petiole generally shows an 10 - 13 and 22- 24 vascular bundle with arc type of separate vascular bundle *C. sinensis* and *C. dichotoma* respectively. vascular bundle were broad U-shaped in both species. Both cell types contain chloroplasts

### Discussion

The inclusive current study was to find out documentation of anatomical characterisation and its intimation for the identification of *Cordia* species in vegetative stage.

**Leaf anatomy:** At the base of epidermal hairs cystoliths are observed. Cystoliths are a Greek word (*kustis*, bag; *lithos*, stone). In cystoliths calcium carbonate crystals are accumulated. (Katherine, 1960; Evert, 2006; Rudall, 2007). Cystoliths are confined to a *Cordia* species (Boraginaceae). Large amount of silica accumulated in cystoliths and reported in species of Boraginaceae (Metcalf & Chalk, 1965; Evert, 2006).

The vascular bundles organization in both species is collateral open. The region of vascular cambium and pith region of *C. sinensis* calcium carbonate observed. Variations in the arrangement of the shape of vascular bundles were observed in the present study. During the study, two types of vascular bundles are arranged i.e. broad U-shaped and narrow U-shaped.

During the studies showed that the trichomes were of single types in the present study: Non-glandular trichomes. Ranunculious type stomata are observed on the abaxial and adaxial surface of the leaf. The stomatal index of leaves differs in studied *Cordia* species. Stomatal Index (SI) was highest observed in *C. dichotoma* and the lowest in *C. sinensis*. Highest trichomes density in *C. dichotoma* and the lowest in *C. sinensis* of abaxial and adaxial leaf surface.

**Petiole anatomy:** During the study, two types of vascular bundles are identified i.e. broad U-shaped and narrow U-shaped. The vascular bundles organization in both species is collateral open. In both species of petiole arc type vascular bundles observed. The region of vascular cambium and pith region of *C. sinensis* calcium carbonate observed. Epidermal outgrowths i.e trichomes are observed in *C. dichotoma*. During the study we observed broad pith parenchymatous cells in *C. dichotoma* and narrow pith parenchymatous cells in *C. sinensis*. During the present study, the parenchymatous cells, collenchymas cell of ground tissue showed structures such as cystoliths, calcium carbonate crystals and tannin cells seen in various region. The region pith of petiole pited parenchyma cell are observed in *C. dichotoma* and not observed in *C. sinensis*, there are a unique characteristics important to taxonomical identification of *Cordia* species. The significance of collateral open types of vascular bundles was observed of species of *Cordia* species. New anatomical documentation of petiole anatomy are taxonomically informative characters (Metcalf & Chalk, 1965; Evert, 2006).

The culmination, this study of the anatomical documentation of leaf and petiole of the *Cordia* species of Northern Western Ghats of Maharashtra. The revealed unique anatomical characters which could be of enormous taxonomic importance. Documentation of anatomical characterization is helpful to taxonomical identification of *Cordia* species. Using foliar anatomical characters followed distinguishing vital role for plant taxonomy, plant systematic, plant physiology, ecology, paleobotany, evolutionary biology and histology, were useful for diagnostic purposes

#### Table Caption

**Table 3. *Cordia* species studied from Northern Western Ghats of Maharashtra.**

Taxon	Place of Collection	Geographic coordinates	Elevation (m)
<i>Cordia sinensis</i> Lam.	N 19.265168, E 73.978847	Otur, Maharashtra	673
	N 19.701139, E 75.082472	Aurangabad, Maharashtra	431
<i>Cordia dichotoma</i> G.Forst	N 19.960905, E 73.668170	Nashik, Maharashtra	689
	N 19.263584, E 73.976226	Otur, Maharashtra	664

**Table 4. Comparative Leaf anatomical characteristics of *Cordia* spp.**

Characters	<i>C. sinensis</i>	<i>C. dichotoma</i>
Upper Epidermis	1 layered	1 layered
Epidermal outgrowths	Present	Present
Palisade layer	1 layered	1 layered
Collenchyma	3 - 4 layered	3 - 5 layered
Parenchyma	3 - 4 layered	2 - 3 layered
Number of vascular bundle	13 - 15 layered	10 - 13 layered
Shape of vascular bundle	Broad U-shaped	narrow U-shaped
Lower epidermis	1 layered	1 layered
Pith- pited Parenchyma cell	Absent	Present

**Table 5. Comparative Petiole anatomical characteristics of *Cordia* spp.**

Characters	<i>C. sinensis</i>	<i>C. dichotoma</i>
Upper Epidermis	1 layered	1 layered
Epidermal outgrowths	Absent	Present
Collenchyma	3 - 4	5 - 7
Parenchyma	3 - 4	4 - 5
Number of vascular bundle	10 - 13	22- 24
Arc vascular bundle	2	2
Shape of vascular bundle	Narrow U-shaped	Broad U-shaped
Lower epidermis	1 layered	1 layered
Pith- pited Parenchyma cell	Absent	Present

**Table 6. Stomatal characteristics of abaxial leaf surface of *Cordia* spp.**

<i>Cordia</i> spp.	Leaf Surface	No. of stomata/ mm- 2	No. of cells/ mm-2	Stomatal Index
<i>C. sinensis</i>	Abaxial	24.2±3.03	103.2±35.66	0.20
<i>C. dichotoma</i>	Abaxial	20.8±0.83	55.4±6.06	0.27

\*Each value expressed as mean± S.D of 05 replicates from 5 different leaves.

**Table 7. Trichome characteristics of abaxial and adaxial leaf surface of *Cordia* spp.**

<i>Cordia</i> spp.	Leaf Surface	No. of Trichome/ mm- 2	Total area/ mm-2	Trichome Density
<i>C. sinensis</i>	Abaxial	67.1±26.51	4±0.00	16.77
	Adaxial	83.5±17.39	4±0.00	20.87
<i>C. dichotoma</i>	Abaxial	95.4±31.92	4±0.00	23.85
	Adaxial	117±34.57	4±0.00	29.25

\*Each value expressed as mean± S.D of 05 replicates from 5 different leaves.





Figure 1. Morphological appearance *Cordia* species from Northern Western Ghats of Maharashtra. (a) *Cordia sinensis* and (a) *Cordia dichotoma*

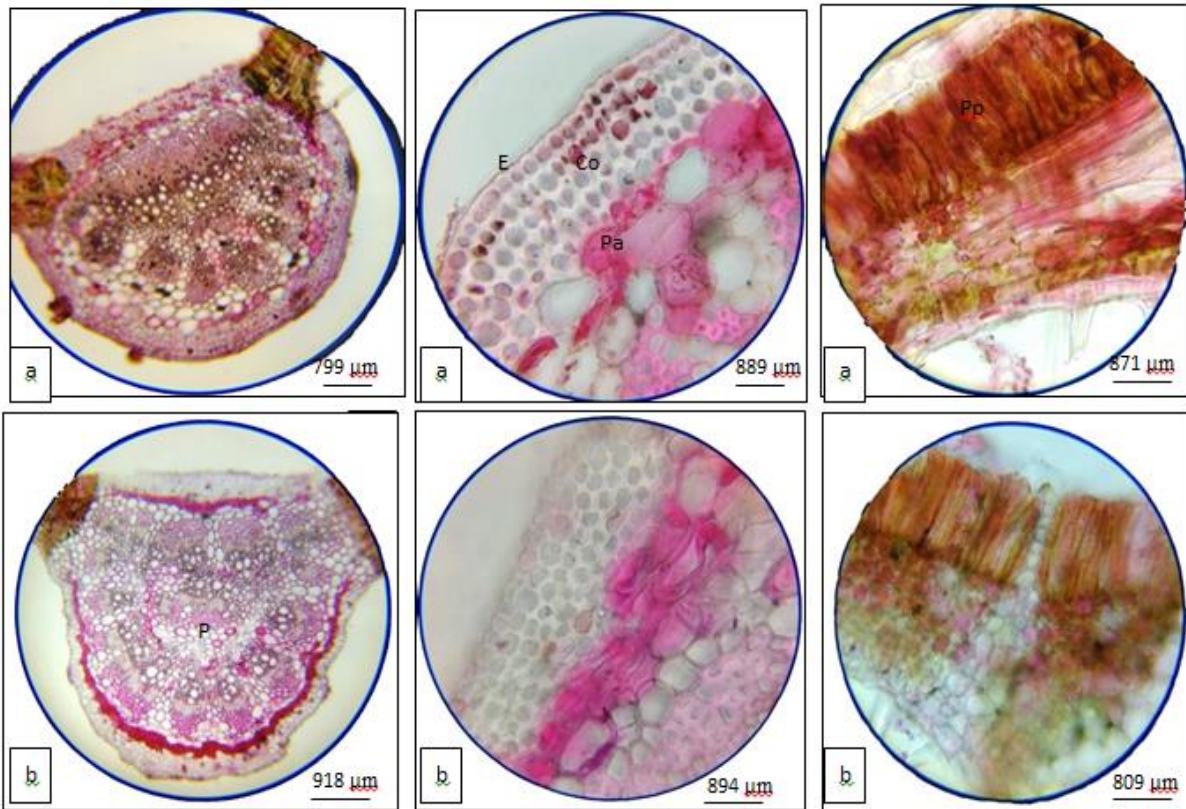
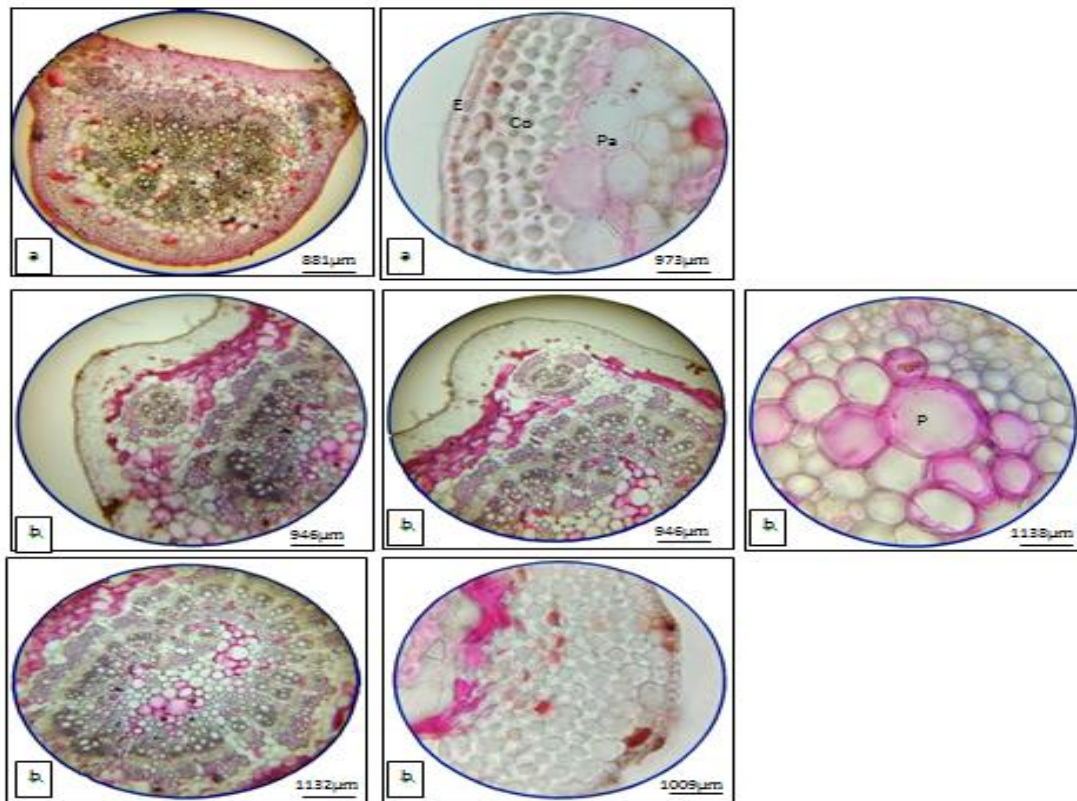


Figure 2. Leaf anatomy of *Cordia* spp. (a) *C. sinensis* (b) *C. dichotoma*

(a) *C. sinensis* (broad U-shaped ), (b) *C. dichotoma* leaf (narrow U-shaped)

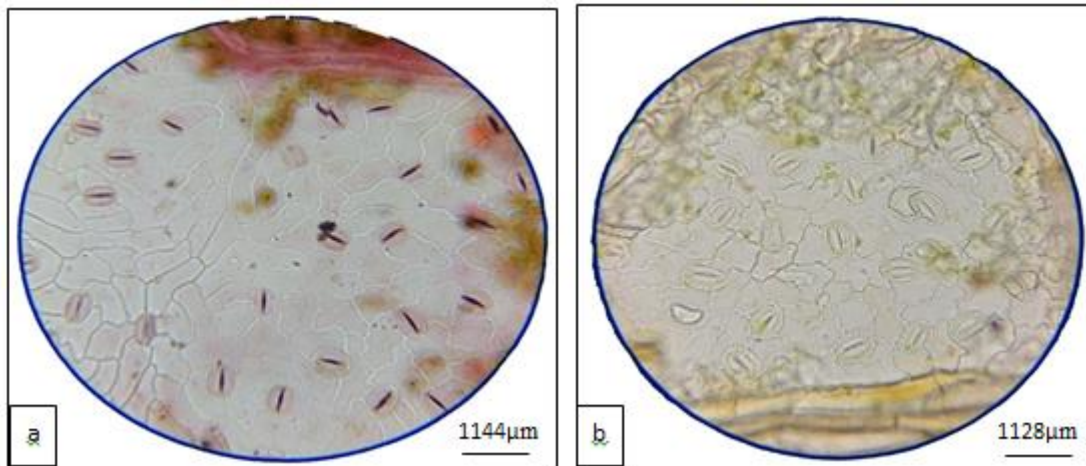
E= Epidermis, Pp=Palisade parenchyma, Co= Collenchyma, Sp= Spongy parenchyma, Pa= Parenchyma P= Pith, Cyt = cystoliths  
 (a) *C. sinensis* (b) *C. dichotoma* (narrow U-shaped vascular bundle), (b) *C. dichotoma* Pith- pited Parenchyma cell (10x)



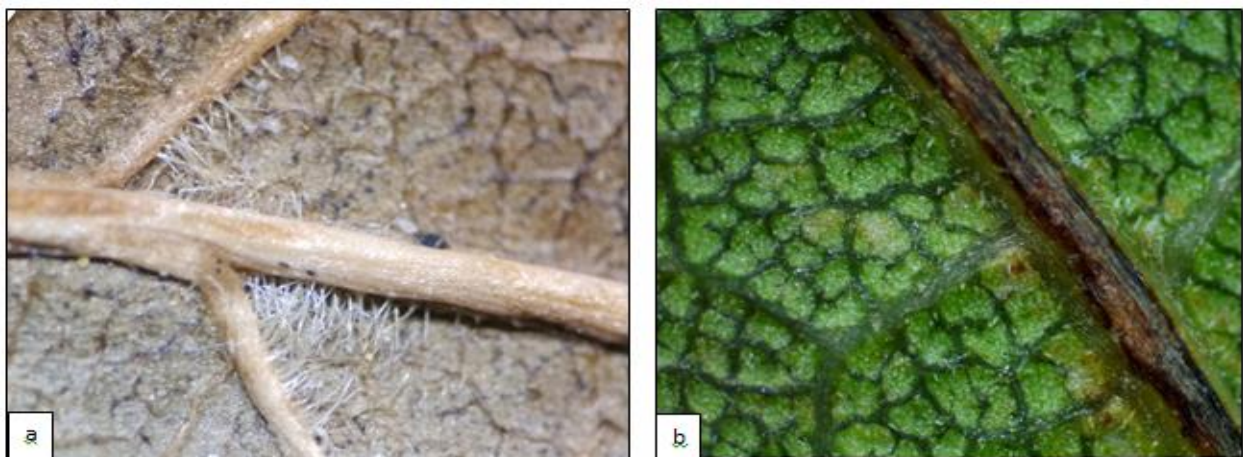


**Figure 3. Petiole anatomy of *Cordia* spp. (a) *C. sinensis* (b) *C. dichotoma***

Showing structure of main vascular bundle (a) *C. sinensis* (b) *C. dichotoma* (U-shaped), Arc vascular bundle  
 E= Epidermis (40x), Pp=Palisade parenchyma, Co= Collenchyma, Sp= Spongy parenchyma, Pa= Parenchyma, P= Pith (40x)  
 (a) *C. sinensis* (b) *C. dichotoma* (narrow U-shaped vascular bundle), (b) *C. dichotoma* Pith- pited Parenchyma cell (10x)



**Figure 4. Stomata type of *Cordia* spp. (a) *C. sinensis* (b) *C. dichotom* (anomocytic type of stomata ) (10x).**



**Figure 5. Trichome characteristics of abaxial and adaxial leaf surface of *Cordia* spp. (a) *C. sinensis* (b) *C. dichotom* (anomocytic type of stomata ) (10x). Non- glandular type trichome observed on the surface of both species leaf.**

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