

# Anatomical Studies and Determination of Leaf Constants of *Cosmostigma cordatum* (Poir.) M. R. Almeida

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## ABSTRACT

**Aim:** *Cosmostigma cordatum* is a species of twining shrub in the family *Apocynaceae*, which is widely distributed throughout the tropical regions of Asia. The plant contains several chemical compounds, including alkaloids, flavonoids and terpenoids, which are responsible for its medicinal properties. Overall, *C. cordatum* is an interesting species of plant that has been used in traditional medicine for centuries. This study aimed to examine the anatomical characteristics of the stem, petiole, leaf and root of *C. cordatum* for evaluation. **Materials and Methods:** Hand sections of various parts of the plant materials are taken and stained with safranin and mounted in glycerin under microscope as per standard protocol. Microscopic evaluation of leaf constants such as stomatal number, stomatal index, epidermal cell number, trichome number, palisade ratio, vein islet and vein termination number were examined. **Results and Discussion:** This study provides anatomical information for authentic identification of this plant. The anatomical investigation of stem, petiole, leaf and root of *C. cordatum*, the green milkweed creeper is significant in these perspectives. The leaf lamina of *C. cordatum* contains few rosette calcium oxalate crystals. Both paracytic and anisocytic stomata, multicellular falcate trichomes on the epidermal region of the leaf are the major distinguishing features of *C. cordatum*. **Conclusion:** The present work, identifying the anatomical features of the plant, underlines the need for taking serious measures to protect its existence at any cost. The anatomical characters and structures are key characteristics for verification and authentication. Anatomical data of stem, petiole, leaf and root of *C. cordatum* can serve as a supplement to evolutionary analyses of *Apocynaceae*.

**Keywords:** *Cosmostigma cordatum*, Rosette crystal, Trichome, Sclerenchyma, Vascular bundles.

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## INTRODUCTION

*Cosmostigma cordatum* is also an important plant used in Indian traditional medicine. It is commonly known as green milkweed creeper. It belongs to *Apocynaceae* family.<sup>[1]</sup> *C. cordatum* is distributed in the south Indian states of Kerala, Karnataka and Tamil Nadu and also in southern Maharashtra. In Kerala it is present in all districts. In Malayalam it is called 'Vattuvalli' or 'Vattolam'. *C. cordatum* is believed to possess the properties of hypoglycemic, inflammation reducing and

inhibiting cancer growth and widely recognised for its potential health benefits. The plant was traditionally applied in Ayurvedic medicines for various purposes, including the treatment of fever, inflammation and gastrointestinal disorders. The plant contains several chemical compounds, including alkaloids, flavonoids and terpenoids, which are responsible for its medicinal properties.<sup>[2]</sup>

*Cosmostigma cordatum* is a climber characterized by small greenish flowers with brown dots. It is a twining, glabrous shrub with opposite, membranous, ovate, or cordate leaves. The flowers are small and are found in axillary or lateral racemose cymes with relatively long peduncles. The calyx is divided into five parts, with ciliate lobes and paired glands between them. The corolla is rotate and deeply five-lobed, with the lobes slightly overlapping to the right. The corona processes

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are erect, membranous and adnate to the base of the staminal column, but shorter than the anthers. They are either truncate, bifid, trifid, or slightly toothed. The staminal column is very short and the anthers have a round inflexed membranous appendicle. Pollen masses are erect, clavate and are attached to hard pollen carriers by long, slender, twisted caudicles. The ovary consists of two carpels, with a thick style and a broad, flat, five-angled style apex with a wavy rim. The fruit is composed of one or two large, lanceolate, blunt, smooth, follicular mericarps. Seeds are ovate, margined and end in a silky coma, with flat, elliptical cotyledons.<sup>[3]</sup> Flowers are small, flat and wheel-like with five petals. Petals are long, ovate and yellowish-green with reddish brown speckles. Having erect pollinia and also flowers are axillary or sub-lateral. Fruits are oblong and bluntly pointed. Flowering and fruiting season is April to June.<sup>[4]</sup>

Anatomical characteristics are stable so it can be used as an important tool for taxonomic characterization of plants. For over 150 years, the characteristics of a plant's internal structure have played a crucial role in advancing plant systematic.<sup>[5]</sup> Different types of stomata are also seen in different plant species. Stomata, located in the epidermis and cuticle layer of leaves, serve as the main pathway for water vapour transfer when open.<sup>[6]</sup> The guard cells regulate water loss and uptake and they can initiate stomatal closure in response to water stress.<sup>[7]</sup>

Closely related plant species can be very similar, making it difficult to distinguish them. *C. cordatum* and *Wattakaka volubilis* both plants belonging to the same family *Apocynaceae*. They are morphologically very similar plants in the absence of flowers. But their anatomical characters are different. The present study was conducted to examine the anatomical characteristics of the stem, petiole, leaf and root for evaluating *C. cordatum* and also for the authentic identification of this plant. Anatomical data of *C. cordatum* help to augment the evolutionary analyses of *Apocynaceae*. In this context the present work identifying the anatomical features and determination



**Figure 1:** Image of *Cosmostigma cordatum* (Poir.) M. R. Almeida.

of leaf constants of this plant, underlines the need for taking serious measures to protect its existence.

## MATERIALS AND METHODS

### Plant Material (Figure 1)

The whole plant of *Cosmostigma cordatum* was collected from Pathanamthitta. The samples were authenticated for its botanical identity at Department of Botany, University of Kerala, Kariavattom campus, Thiruvananthapuram (BOT/KUBH/167/24).

### Plant Anatomy

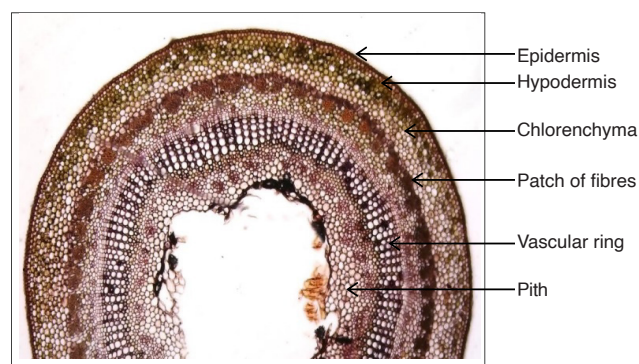
Hand sections of various parts of the plant material were taken and stained with safranin and mounted in glycerin under 10X as well as 40X objective of microscope as per standard protocol.

### Determination of Leaf Constants

The matured fresh leaves of *C. cordatum* were taken and cleaned. The section of the leaf between the midrib and margin from the central region was cut into small pieces. Leaf samples were treated with 10% NaOH solution until it become clear. The leaf sample was rinsed with water, then mounted with glycerin and observed under a light microscope equipped with a digital camera to determine leaf constants. Leaf constants, including stomatal number, epidermal cell number, stomatal index, palisade ratio, trichome number, vein islet and vein termination number etc. were assessed through microscopic evaluation.

## RESULTS

### Transverse section of stem of *Cosmostigma cordatum* (Figure 2)



**Figure 2:** T.S. of stem of *Cosmostigma cordatum*.

T.S. of stem is circular in outline with wavy like appearance at some areas. Epidermis is uniseriate composed of rectangular cells covered with a thick cuticle. It is followed by hypodermis of 2-3 layers of

collenchyma tissues. Next to it, chlorenchyma zone composed of 4-5 layers of chlorophyll containing parenchyma tissues followed by a cortex of thin walled parenchyma tissues. The sclerenchymatic sheath is incomplete, with multiple fibre groups surrounding vascular system. The vascular system's architecture is characterized by a bi-collateral design, where the xylem takes the form of a continuous, unbroken cylinder. Encircling the xylem is the external phloem, comprising a seamless cylinder of sieve elements and parenchymatic cells. In contrast, the internal phloem is organized into discrete clusters, positioned side by side, comprising sieve elements and parenchymatic cells. Central pith is composed of polygonal parenchyma cells which are compactly arranged and contains few rosette Calcium oxalate crystals (Figure 2a).

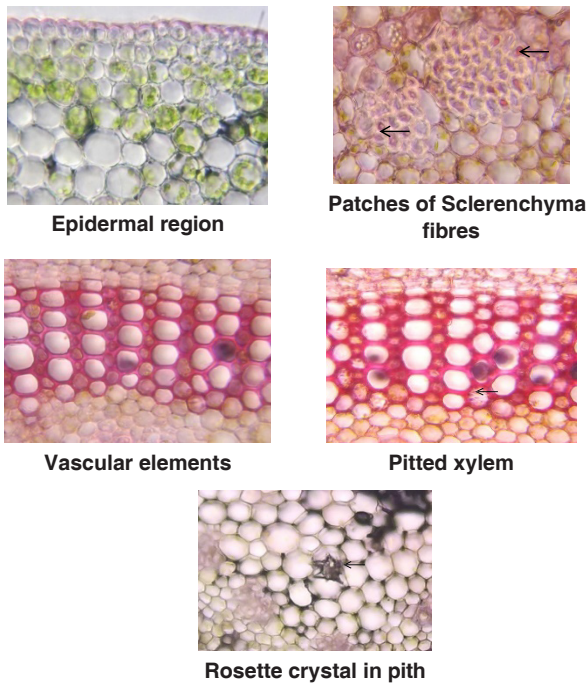


Figure 2a: Enlarged view T.S. of stem of *Cosmostigma cordatum*.

**Transverse section of Petiole of *Cosmostigma cordatum* (Figure 3)**

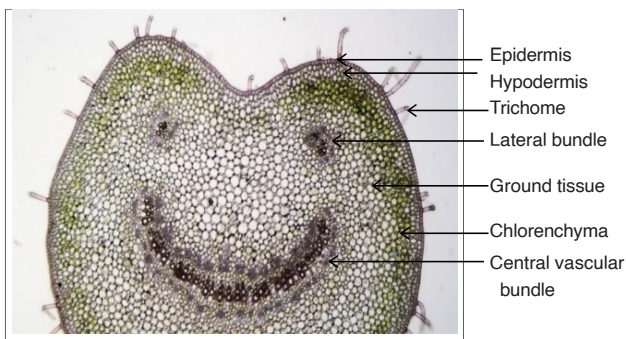


Figure 3: T.S. of petiole of *Cosmostigma cordatum*.

T.S of petiole is circular in outline with two notches. A single layered epidermis consists of thickly cuticularised and squarish cells. Many multicellular trichomes are seen on the epidermal cells. Hypodermis is 2-3 layered composed of collenchyma cells. Chlorenchyma tissues are 5-6 layered. Ground tissues are composed of polygonal parenchyma cells arranged without any intercellular spaces and constitute important portion of the petiole and contains rosette Calcium oxalate crystals. A bicollateral vascular bundle with arc-shaped, is centrally embedded in ground tissue. Two small vascular bundles are located at two lateral sides. Bundle sheath cells form a protective layer around the vascular bundles (Figure 3a).

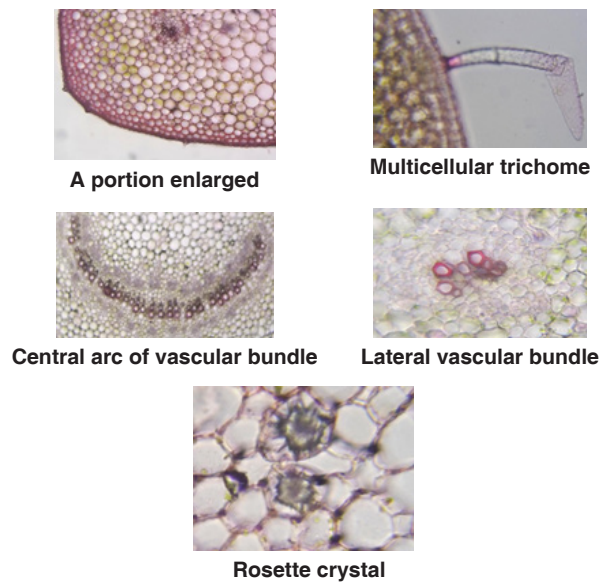


Figure 3a: Enlarged view of T. S of petiole of *C. cordatum*.

**Transverse section of Leaf of *Cosmostigma cordatum* (Figure 4)**

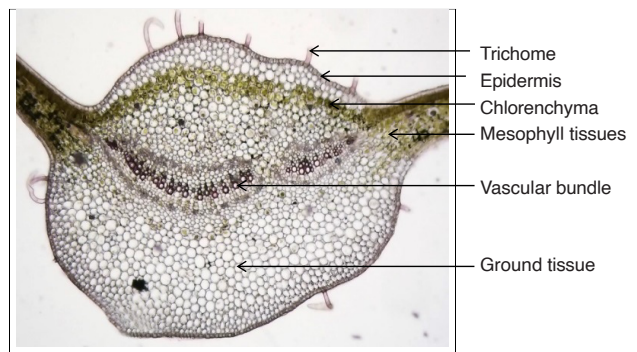
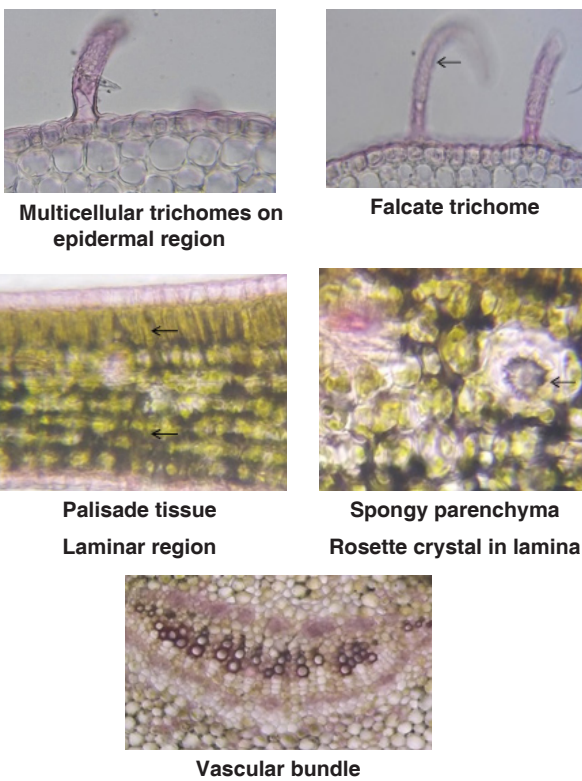


Figure 4: Transverse section of leaf of *Cosmostigma cordatum*.

**Midrib**

The epidermis, which is uniseriate, is composed of cells that are more or less square-shaped covered with

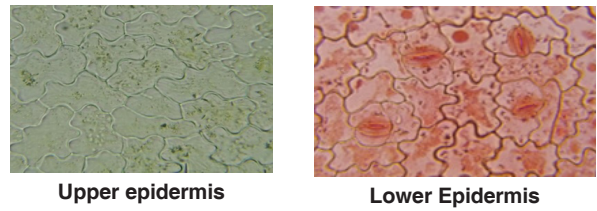
a cuticle and the epidermal cells bear a few trichomes. Below the epidermis, a hypodermis is present; consist of 2-3 layers of collenchyma cells, followed by 4-5 rows of chlorenchyma tissues. The ground tissue consists of thin-walled, compactly arranged parenchyma cells containing rosette-shaped calcium oxalate crystals. A well-developed crescent-shaped bicollateral vascular bundle is located in the midrib region. The outer and inner phloem, composed of sieve tube cells, companion cells and phloem parenchyma, surround the lignified xylem, which is formed of radial vessels with metaxylem in the lower region and protoxylem in the upper region, separated by xylem parenchyma. (Figure 4a).



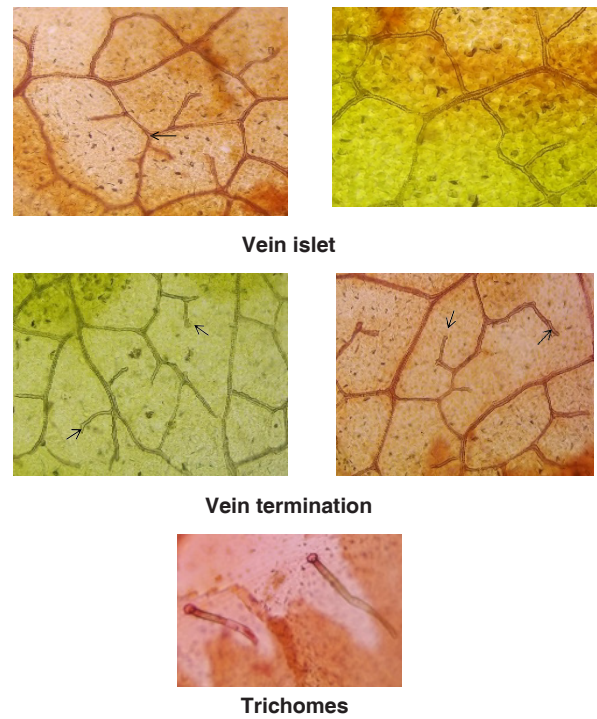
**Figure 4a:** Enlarged view of T. S of leaf of *C. cordatum*.

**Lamina**

The lamina is made up of mesophyll, which consists of a single layer of elongated palisade cells and a broad region of small spongy parenchyma cells. Palisade tissue consist of single layer of columnar cells that are compactly arranged. The spongy cells are arranged without any spaces in 4 layers. Rest of space is occupied by loosely packed spongy parenchyma cells. Between the palisade and spongy parenchyma, the lateral veins appear (Figure 4a).



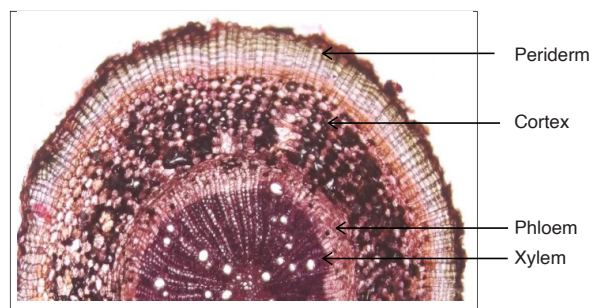
**Figure 5:** Paracytic and anisocytic stomata present in *C. cordatum*.



**Figure 6:** Quantitative microscopic images of leaves of *C. cordatum*.

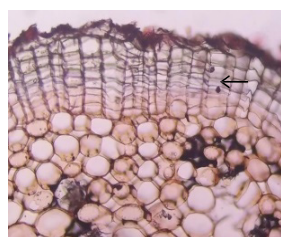
Parameter	Leaf of <i>C. cordatum</i>	
	Upper epidermis	Lower epidermis
Stomatal number (per mm <sup>2</sup> area)	Nil	154-161
Epidermal number (per mm <sup>2</sup> area)	382-418	315-339
Stomatal Index (per mm <sup>2</sup> area)	Nil	32.20-32.83
Palisade ratio (per cell)		4
Vein islet number (per mm <sup>2</sup> area)		5-11
Vein termination number (per mm <sup>2</sup> area)		18-23
Trichome number	9	Nil

## Transverse section of root of *Cosmostigma cordatum* (Figure 7)

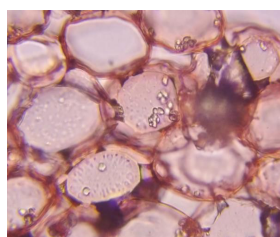


**Figure 7:** Transverse section of root of *Cosmostigma cordatum*.

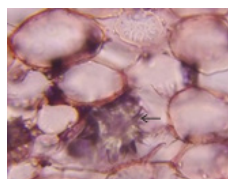
The root appears circular in transverse section. Stratified cork is present, composed of 8-10 layers of rectangular cells. Round-shaped parenchyma cells comprise the cortex and it is arranged with some intercellular spaces. The cortical region is rich in starch grains and rosette calcium oxalate crystals. Vascular bundles are centrally occupied in which Xylem forms the major portion, surrounded by phloem elements, in turn encircled by pericycle and endodermis. Vessels were thin walled, diffused, solitary, circular, or oval in cross-section. Pith is indistinct or absent (Figure 7a).



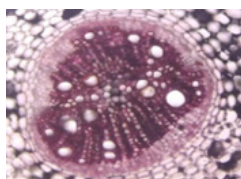
**Periderm region**



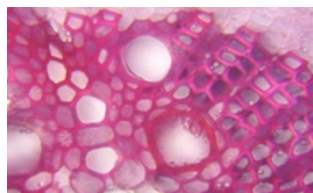
**Cortex containing starch grains**



**Rosette crystal in cortex**



**Vascular bundle**



**Xylem elements**

**Figure 7a:** Enlarged view of T. S of root of *C. cordatum*.

## DISCUSSION

Plant morphology or phytomorphology is the study of the physical form and external structure of plants.<sup>[8]</sup> It is useful in the visual identification of the plant. According to World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.<sup>[9]</sup> Most of the plants are classified according to their macro-morphological features. But an accurate classification results when the information is taken from anatomical studies. Trichome, stomata, vein etc. are important anatomical features which are often good indicators of the family, genera and sometimes species.

*Cosmostigma cordatum* and *Wattakaka volubilis* are morphologically very similar plants in same family *Apocynaceae*. In a transverse section of the stem of *C. cordatum*, the central pith consisted of tightly packed polygonal parenchyma cells, which showed a few rosette-shaped calcium oxalate crystals (Figure 2a). But in T. S of stem of *Wattakaka volubilis* several stellate crystals were present in the parenchyma cells.<sup>[10]</sup> And also, multicellular glandular trichomes were present in *W. volubilis*<sup>[11]</sup> and it was absent in the stem of *C. cordatum*. In T.S of petiole of *C. cordatum* showed many multicellular trichomes on the epidermal cells. Trichomes are the collective term of hairs and papillae. Trichomes are essential for protecting plants from insects.<sup>[12]</sup> Some of them secrete metabolites for the plant. A particular type of trichome is constant in a species. And in the ground tissues of *C. cordatum* rosette calcium oxalate crystals were present (Figure 3a).

In transverse section of leaf of *C. cordatum*, epidermal cell showed few trichomes in the midrib region and in the ground tissue rosette calcium oxalate crystals were present (Figure 4a). In T. S of leaf of *W. volubilis* showed druses type of calcium oxalate crystals, glandular trichomes and oil globules.<sup>[11-13]</sup> These calcium oxalate crystals protect plants from herbivores by being linked to irritating chemicals or proteolytic toxins.<sup>[14]</sup>

Stomata are pores located in the leaf epidermis that regulate gas exchange. These pores are surrounded by a pair of specialized parenchyma cells known as guard cells. The kidney shaped guard cells in dicotyledons but dumb bell shaped in the members of monocotyledons. Subsidiary cells also known as accessory cells, are specialized epidermal cells that surround guard cells and provide support for their movement.<sup>[15]</sup> Here all the leaves were hypostomatic in nature, paracytic and anisocytic type of stomata were present in the lower

epidermis (Figure 5). Stomatal number of lower epidermis ranging from 154-161 and it was absent in the upper epidermis of the leaves. The percentage of stomata to the total number of stomata and epidermal cells in a leaf is the stomatal index. It was ranging from 32.20 to 32.83%. The palisade ratio is the average number of palisade cells below a single upper epidermal cell of a leaf. Here the palisade ratio was found to be 4. It is an important parameter for characterizing and determining leafy drugs. The vein islet is the minute area of photosynthetic tissue encircled by the ultimate division of conducting strands. The vein islet number is the average number of vein islets per square millimeter of the leaf surface. The vein termination is the ultimate free termination of veinlet. The vein termination number means the number of veinlet terminations per square millimeter of the leaf surface. In *C. cordatum* the vein islet and vein termination were calculated as 5-11 and 18-23 (Table 1). According to a study in *W. volubilis* the vein islet and vein termination numbers were calculated as 20 and 11.<sup>[16]</sup> These characters are species specific. The veins and their innumerable variations in leaf venation pattern provided various characters of taxonomic importance (Figure 6). In a study quantitative microscopical results showed that *W. volubilis* leaf have 11-14 stomata/sq.mm and 18.41 stomatal number and stomatal index respectively and only paracytic type of stomata was found in leaf of *W. volubilis*.<sup>[11]</sup>

In T.S of root of *C. cordatum*, rosette calcium oxalate crystals along with starch grains were abundantly present in cortical region (Figure 7a). From a study in T. S of root of *W. volubilis* druses type of calcium oxalate crystals were present.<sup>[13]</sup> Anatomical features such as tissue types, stomatal types, structure of mesophyll in the leaf, trichomes, calcium oxalate crystals etc were discovered through anatomical studies. These features are crucial for identifying the plant *Cosmostigma cordatum*.

## SUMMARY

This study investigated the anatomical characteristics of *Cosmostigma cordatum*, a twining shrub belonging to the *Apocynaceae* family. Using standard protocols, hand sections of the plant's stem, petiole, leaf and root were stained with safranin and mounted in glycerin for microscopic examination. The results revealed that few rosette calcium oxalate crystals in the leaf lamina. Both paracytic and anisocytic stomata, multicellular falcate trichomes etc. were present on the leaf's epidermal region. These characteristics are crucial for identifying *C. cordatum*. The findings of this study highlight the

importance of conserving this species and taking measures to protect its existence.

## CONCLUSION

Anatomical characteristics can help to the authentic identification of a plant. The present study attempted to record the anatomy of stem, petiole, leaf, root and determination of leaf constants of *Cosmostigma cordatum*. Trichome, calcium oxalate crystals, stomatal number, stomatal index, palisade ratio, vein islet and vein termination values etc. are essential for species identification. *Cosmostigma cordatum* is a plant having numerous medicinal properties. According to the IUCN red list, in many parts of the world *Cosmostigma cordatum* is one of the highly threatened species. In this context the present work helps to identifying the anatomical features of this plant, underlines the need for taking serious measures to protect its existence. And also, the anatomical structures of the plant helps to understand the relationships between the taxa. All these findings are helpful for the conservation of plant and further studies.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## FUNDING

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## ABBREVIATIONS

**T.S:** Transverse Section; **NaOH:** Sodium Hydroxide.

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