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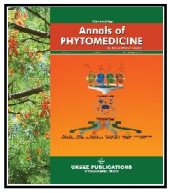

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Anatomical study of *Sarcostemma intermedium* Decne. from the Northern Western Ghats of India

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Abstract

The Apocynaceae is an ethnomedicinally important plant family. The genus *Sarcostemma* finds a prominent ethnomedicinal plant used in different Indian systems of medicine. It has been used for medicinal use such as mental disorders, fever, viral infections, cardiac diseases, body swelling and jaundice; it has also been reported that at the time of scarcity of fodder because of less precipitation, this plant is used as fodder for domestic stock. It has also been reported antibacterial bioprospecting activity. The plant, *Sarcostemma intermedium* Decne. is a succulent perennial leafless, twining shrub, with a green cylindrical stem and milky white latex. The plant bears pedicellate flowers which are present in a terminal and axillary umbel inflorescence. The plant shows distinct anatomical circular vascular bundles in the stem. It shows the presence of rosettes shaped calcium oxalate crystals, pitted parenchyma, pitted xylem medullary rays and laticifers cells with white-colored latex. Intra-axillary phloem present periphery of pith cells. The very important tannin secondary metabolite deposition was noted in the cortex and pith region of the succulent stem. The morphological, as well as anatomical significant characteristics of *S. intermedium*, will be used as a plant taxonomical identification technique which would be of great value in the standardization and authentication of this plant species.

1. Introduction

The Western Ghats (including Sri Lanka) is one of the biodiversity hotspots in India. The Northern Western Ghats (NWG) hotspot is also known as the 'Sahyadri range'(Myers *et al*., 2000). The region of Western Ghats consist a rich medicinal recourse, and these medicinal plant sources will be used for pharmacognostic and bioprospecting study (Rao, 2002). The indigenous major medicinal systems, such as Ayurveda, Siddha, Unani and Folk (tribal) are being used in India as well as in World. According to WHO, more than 21,000 plants, were used for treatments of diseases and abnormalities. Plant-based isolated bioactive chemical constituents are multifunctional that means isolated bioactive compounds in can be used treatment of different diseases and abnormalities (Khare, 2007; Kunwar and Bussmann, 2008). In India, richest source of traditional medicinal plant, there are more than 25000 effective plants based formulation used and near to 10000 plant-based formulations documented in the literature (Basha and Reddy, 2017; Priyadharshini *et al*., 2019). Most of the Apocynaceae members are medicinal and ethnomedicinal, were documented in Indian Pharmacopoeia and British Pharmacopoeia (Naik and Sellappan, 2019; Reddy *et al*., 2019). The Apocynaceae family covered basically three types of habitat like herbs, shrubby climbers, rare shrubs and trees. The family Apocynaceae is medicinally very important. The family is widely distributed in 410 genera and 18,268 species. In the *Sarcostemma* genus, 134 species were distributed in Asia, Africa, Australia and North America (The Plant List, 2013). *S. intermedium* succulent species growing on cliffs or slope on rocky surfaces and it is endemic plant to Central and Peninsular India especially to Western Ghats and Eastern Ghats of India, (Pullaiah *et al*., 2011; Datar and Watve, 2018). *S. intermedium* is known as Kallikodi, Phok, Pasandikodi, Mosurguduka and Nipati in local and regional languages. Laticifers cells have milky white colored latex that is poisonous and caustic (Vijayakumar *et al*., 2013). The *S. intermedium* synonym word is *Cynanchum sarcomedium* Meve and Liede. The accepted name for *Sarcostemma acidum* (Roxb.) Voigt is *Cynanchum viminale* (L.). This ethnomedicinal plant grown on arid rocky soil of slopes of rocky hill (Candolle, 1844; Indhumathi and Kalvimoorthi, 2010; Singh *et al*., 2001; IPNI, 2022).

Different volatile chemical components reported in Apocynaceae of various groups such as *Sarcostemma, Cynanchum, Vincetoxicum, Metastelma, Funastrum, Gonolobus, Oxypetalum* and *Orthosia* (Jürgens *et al*., 2008). *Sarcostemma* R. Br. genera are small succulent ethnomedicinal shrub plants. For many tribal peoples, this plant stem uses for medicinal treatment of various diseases and abnormalities (Meve and Liede-schumann, 2012). The *Sarcostemma* genus has a prominent ethnomedicinal plant group used in Indian medicinal systems. The distinctive tribal ethnic peoples used different species of *Sarcostemma* for the treatment of various human diseases and abnormalities (Dhivya *et al*., 2017). The different ethnic communities in India have also reported to use different species of *Sarcostemma* in the treatment of various human diseases and

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abnormalities. It is used as traditional medicine for various disorders, mostly for body swelling and jaundice. *S. secamone* has been used in treatment for gargling in throat and mouth infections. *S. acidum* is a stem extract used in the treatment of mental disorders, fever, viral infections and cardiac diseases. Methanolic solvent extract of *S. intermedium* showed significant antibacterial activity (Shailesh and Seema, 2010); Dahiya *et al*., 2014). *S. acidum* showed anthelmintic property (Madhavan and Tharakan, 2020). *S. secamone* is used in therapeutic treatment as jaundice, antiseptic, depurative, galactagogue and vulnerary. *S. brevistigma* is medicinal treatmen-temetic and insecticidal (Khare, 2007). Representation of morphological as well as anatomical characteristics is useful for appropriate identification, nomenclature and classification of medicinal plants (Kumar *et al*., 2021). In the ancient era, researchers used plant anatomy techniques to distinguish plant species as well as in the modern era, plant anatomy is a modern technique used for the identification of various medicinal plant species (Dilarom and Dilfuza, 2021).Various different types of secondary metabolites are recorded in the plant kingdom, especially in medicinal plants such as flavonoids, tannins, alkaloids, phenols and saponins; these phytochemical used against many diseases (Idris *et al*., 2021). The importance attributes such as laticiferous tubes and intraxylary phloem present in leaf and stem of Apocynaceae members such as *Gymnema sylvestre*, *Ceropegia*, *Asclepias obtusifolia* and *A. syriaca* (Metcalfe and Chalk, 1965). During the study of *S.intermedium*, we noted various anatomical variations in transverse section of the stem such as epidermis layer, hypodermis, cortex, phloem cell layer, phloem medullary rays, xylem medullary rays and xylem vessels. These anatomical sections were observed under the bright-field light compound microscope and their characters were documented. Starch grains, laticifers and trichomes were reported in the stem of *S. brevistigma* and *S. secamone* (Metcalfe and Chalk, 1965; Dhivya *et al*., 2017; Khandelwal and Sethi, 2019).

2. Materials and Methods

2.1 Taxonomy of *S. intermedium*

This plant specimen identification by using Flora of the Presidency of Bombay and Flora of Maharashtra. The taxonomical description is shrubs; stems a lot of spread, green. Flower in horizontal and terminal sessile umbels; pedicels pubescent; corolla 1.4 cm across, subrotate; corona staminal, two fold, external annular ring, membranous, inward 5 lobed, curves oval, intense; style peak elliptical, delivered past anthers (Figures 1a, b). Studied plant taxonomy compared with *S. acidum* are straying shrubs, much branched, green. Flowers are many, in terminal sessile umbels, cream coloured; pedicels sparingly pubescent; calyx puberulous corolla 1.8 cm across, rotate; corona staminal, double, outer cupular, crenately 10 lobed on margins, inner of 5 erect, thick and fleshy lobes. Follicles are 10 cm long, solitary, lanceolate, much stretched, green. Flowers many, in terminal sessile umbels, cream hued; pedicels sparingly pubescent; calyx puberulous corolla 1.8 cm across, pivot; crown staminal, twofold, external cupular, crenately 10 lobed on edges, internal of 5 erect, thick and plump curves. Follicles are 10 cm long, singular, lanceolate (Cooke, 1908; Singh *et al*., 2001).

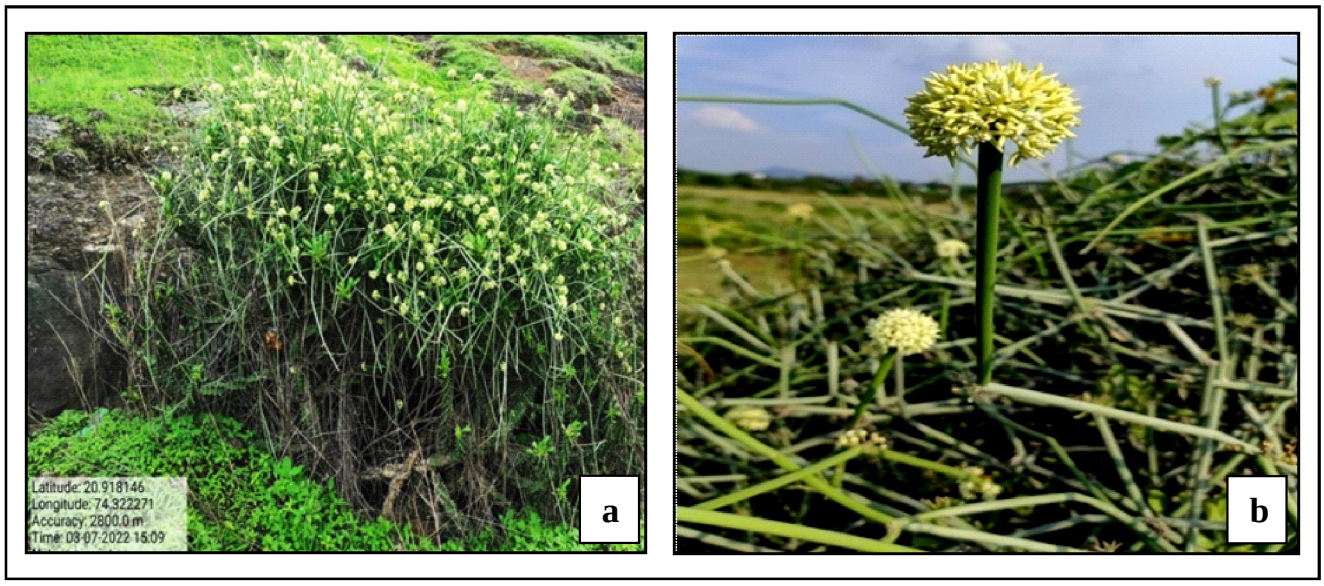


Figure 1(a-b): Living plant of *S. intermedium*.

2.2 Study area and collection

The stem of *S. intermedium* was collected from Nashik and Dhule (red colored arrow and circle indicating the specimen collection site) district during the January-August, 2022 of Maharashtra state, India (Figure 2). The plant was correctly identified using Flora of Maharashtra and Flora of Bombay Presidency and authenticated by BSI accession number CAL0000009470.

2.3 Anatomical study of stem

Made a thin sliced from fresh mature stem samples for anatomical study (8 mm to 10 mm) from plant species. Prepared hand sections and were stained using 0.1% safranin and mount in glycerin and examined under a bright-field compound microscope (LABOMED Microscope Lx 300) (Chamberlain, 1920; Khandelwal and Sethi, 2019; Pratap *et al*., 2021). Measure a antatomical perceptions of length, width, breadth and number of layers (mm.) (Salisbury's, 1927; Evert, 2006).

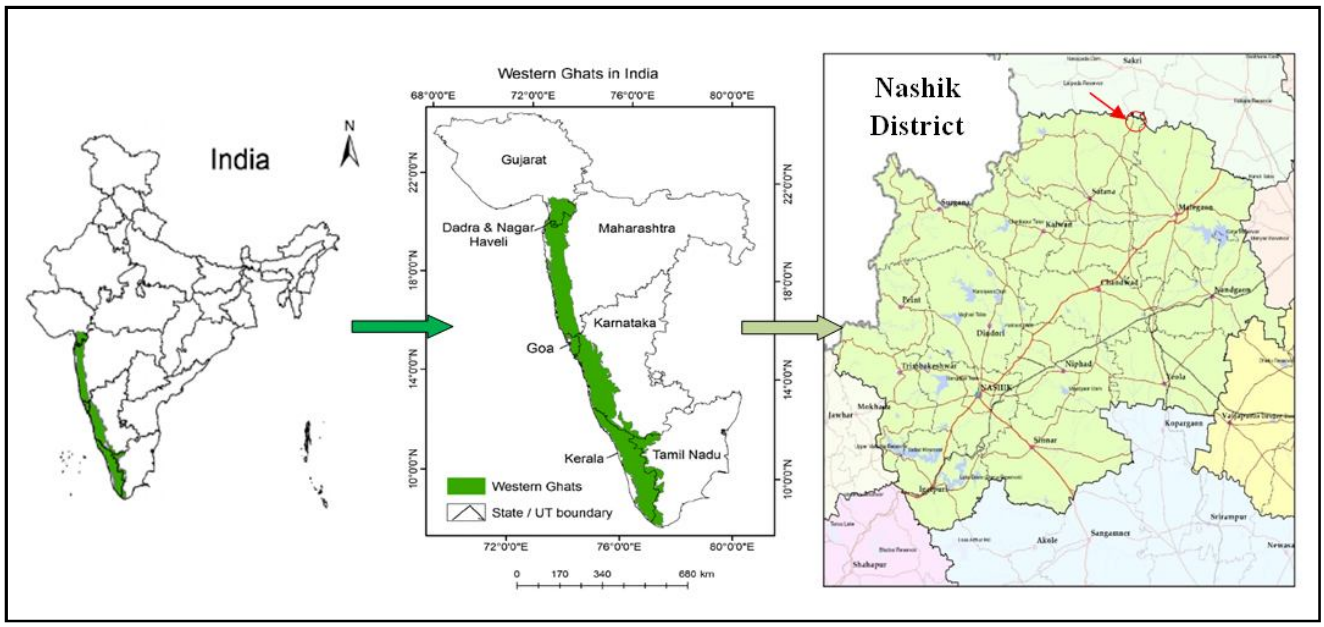


Figure 2: Map showing the collection site of *S. intermedium*.

3. Results

3.1 Anatomical study of stem

Transverse section of the succulent stem of *S. intermedium* (Figures 3 a-m) (Tables 1,2 and 3).

3.1.1 Epidermis

Superficial and strongly supportive layer observed in the species. It was a single layer (Figure 3b).

3.1.2 Cortex

The cortex cells are arranged in compact form. In the primary cortex, the region has 1-2 palisade cells layered (Figure 3 j) and collenchymas cells contain chlorophyll pigments for photosynthesis physiological significant processes. Mature secondary cortex region collenchymas cell. The total cortex layer is 34-36. Stone cells are frequently observed in the cortex region (Figures 3b, e).

3.1.3 Stone cells

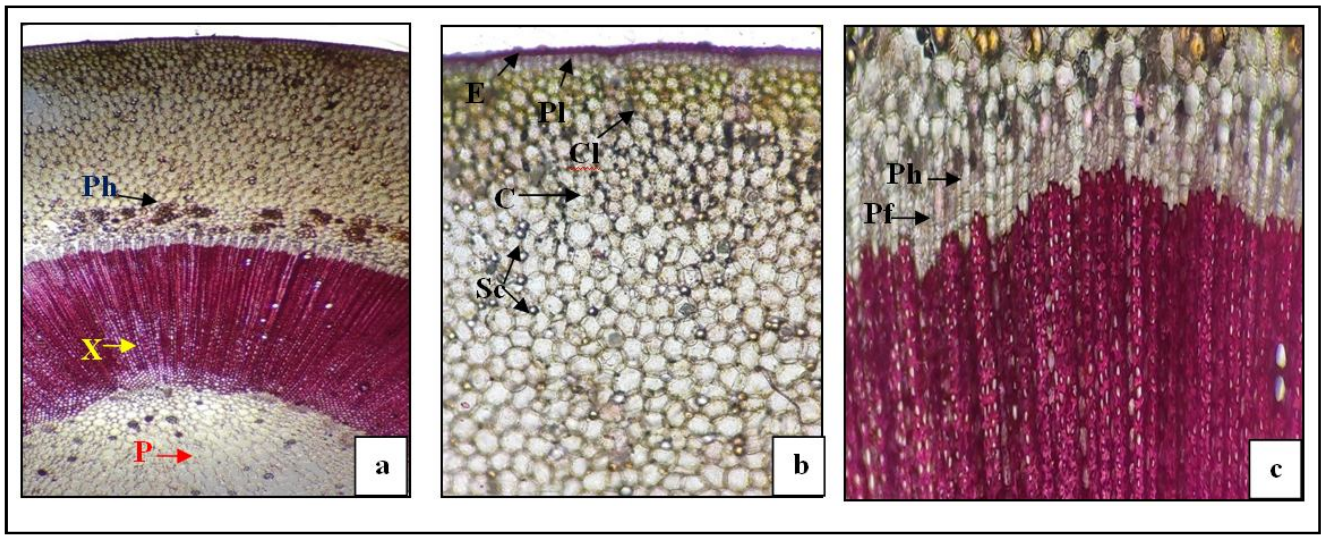
Strong and tough supportive 5-10 cell layer in the ring form noted (Figure 3e).

3.1.4 Vascular tissues-phloem

Phloem observed in this species is 5-10 layered. Phloem medullary rays observed in this species, they are 5-6 layered. The cluster of calcium crystalline structures deposition present in the phloem medullary rays (Figure 3c).

3.1.5 Vascular tissues-xylem

Diffuse porous wood observed, *i.e.*, tracheary elements present (tracheids and vessels). Medullary rays are broader and expanded at the distal end. During the study, significance observations noted, there are pitted type medullary rays with tannin deposition. Vessels are arranged in vertical rows. Vessels varying in size 29.81 ìm. Medullary rays mostly are 40 to 50. Well developed xylem fibers were arranged in vertical rows and in groups (Figures 3a, f, g).



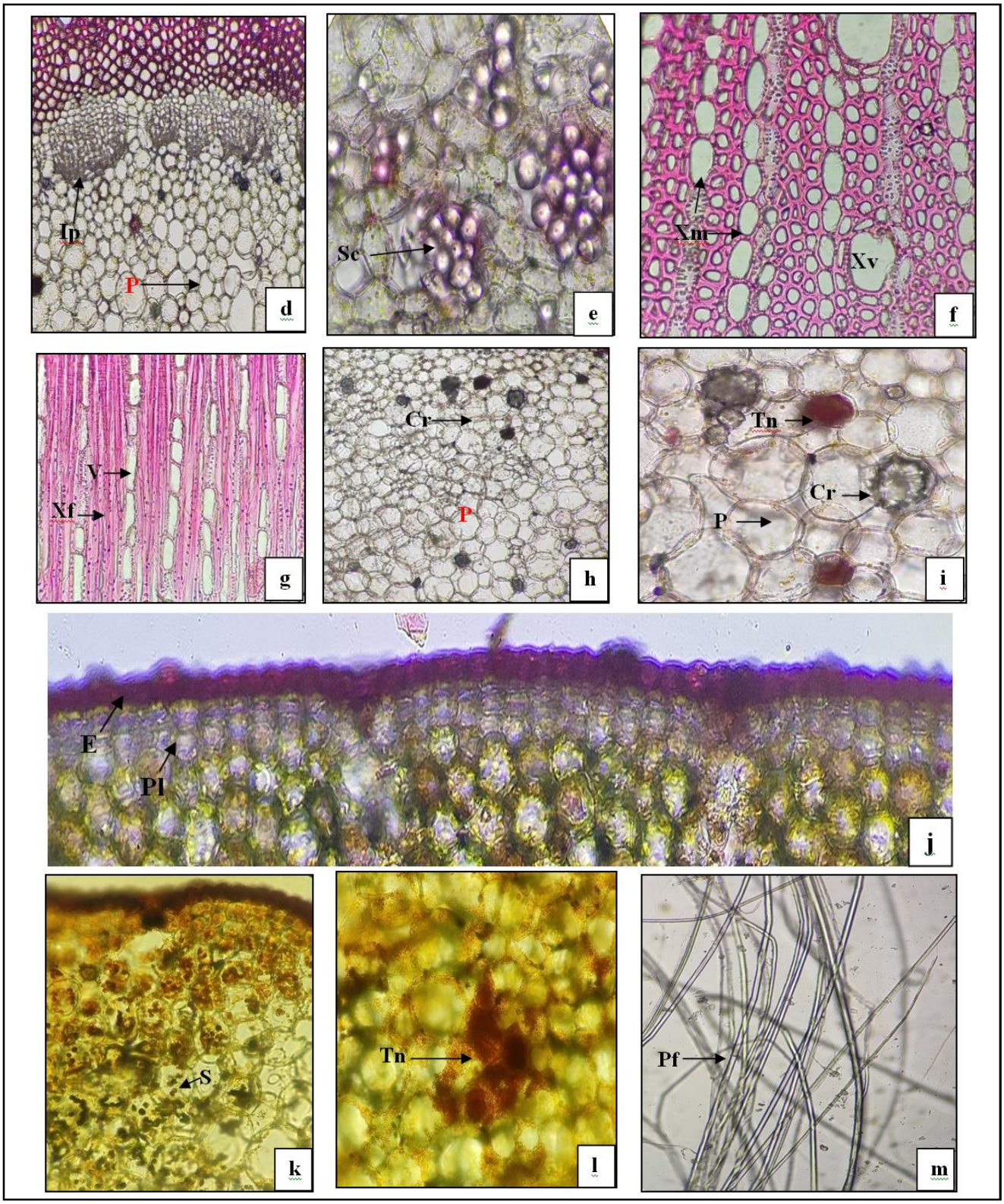


Figure 3(a-m): Stem anatomy of *S. intermedium*.

E = epidermis, C=Cortex layer, H=Hypodermis, P = pith, Ph = phloem, X = xylem, Xv= Xylem vessels, Ip= Intraaxillary phloem, Xm= Xylem medullary layer, Pm= Phloem medullary layer , V= vessels, Sc= Stone cells, Cr= Crystalliferous cells (roseate and solitary crystals), Tn= Tannin deposition, S= Starch, Pf= Phloem fiber, Xf= Xylem fiber, Pl= Palsied layer, Cl= Collenchymas cells.

3.1.6 Intraaxillary phloem

It is a contentious ring like structure present periphery of pith cells. Intra-axillary phloem is 8 to 10 layered (Figure 3d).

3.1.7 Pith

Pith is a soft, large pitted parenchymatous cell with thick-walled. Pith region of stem observed calcium solitary crystals and tannin deposition (Figures 3a, d, h, i).

Table 1: Stem anatomical observations of *S. intermedium*

Characters	Number of layers
Epidermis layer	01
Cortex layer	34 - 36
Stone cells ring	05-10
Phloem cell layer	05-10
Phloem medullary layer	05-06
Xylem medullary layer	40-45
Intraxylary phloem layer	08-10
Intraaxillary phloem medullary rays layer	03-05
Pith cell	Parenchyma cell-pitted, calcium crystals and tannin deposition

Table 2: Transverse section of stem anatomical characteristics of *S. intermedium*

Characters	*S. intermedium*
Epidermis layer	2.6 ± 0.51 (mm)
Vessel diameter	5.27 ± 0.99 (mm)
Medullary rays length	5.45 ± 0.66 (mm)
Pith cell diameter	13.27 ± 1.82 (mm)

*Each value expressed as mean ± S.D of 10 replicate.

Table 3: Transverse longitudinal section of stem anatomical characteristics of *S. intermedium*

Characters	*S. intermedium*
Vessel length	29.81 ± 10.67(mm)
Breadth	4.90 ± 1.15 (mm)
Medullary rays length	4.18 ± 1.05 (mm)
Breadth	1.76 ± 0.04 (mm)

*Each value expressed as mean ± S.D of 10 replicates.

4. Discussion

Many of the medicinal and ethnomedicinal plants documented their significant medicinal treatments in indigenous systems of medicines such as Ayurveda, Siddha and Unani. The studied plant is also used for the treatment of diseases and abnormalities (Pratap *et al*., 2021). Starch granules are one of the non-nitrogenous stored forms observed in studied medicinal plant species. Tapioca starch grain type observed in studied medicinal plant species (Stevens, 1907; Wallis, 1939; Jumaboev *et al*., 2022). Stem anatomical characteristics of *S. intermedium* were abundant crystalliferous cells noted during the study. Ray cells frequently contain roseate crystals. In the cortex, phloem and pith region of stem observed solitary crystals (Figures 3b, c, h, i) (Rao and Das, 1979; Zobel, 1985; Evert, 2006). Plentiful roseate calcium oxalate crystals are present in the vegetative living mesophyll cells of *Mollugo nudicaulis*. Abundant roseate type calcium oxalate crystals were observed in *S. intermedium* in the pith region of the stem (Evert, 2006; Rudall, 2007; Khandelwal and Sethi, 2019; Pratap *et al*., 2021). The important attributes such as laticiferous tubes and intraxylary phloem presence reported in the stem of *S. intermedium*. Intraxylary phloem present on periphery of pith cells (Metcalfe and Chalk, 1965). The stone cells were found in the secondary cortex randomly. A ring of stone cells below the secondary cortex region of the stem. Stone cells' important function is the thickening and lignification of the walls of thin-walled parenchyma cells. Stone cells provide a strengthening and toughness of parenchyma cells. Sclerenchyma cells are classified into two categories, fibers and sclereids (Figure 3 e) (Rao, 1957; Metcalfe and Chalk, 1965; Evert, 2006).

5. Conclusion

The anatomical sections of the succulent stem (transverse section and transverse longitudinal sectionstem) of the species were examined under the light compound binocular microscope and observed characters were documented. The anatomical characteristics observed during the study such as the arrangement of epidermis cells, cortex tissues, stone cells, xylem vessels, xylem medullary rays, phloem fibers, intraxylary phloem and phloem rays. Stem anatomical characteristics of *S. intermedium* were a roseate type of crystalliferous cells and an abundance of tannin. In the cortex and pith region of succulent stem observed solitary calcium crystals as well as tannin deposition. Many *Sarcostemma* taxa belong to the Apocynaceae family, were in that most significant anatomical character help to resolve this problem, easy to distinguish within the same genus. On the basis of preliminary study, the species can be differentiated from other species of the same family based only on anatomical characteristics. A study has been carried out to authenticate the identity of this species based on anatomical characteristics.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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