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ASSESSMENT OF ANATOMICAL CHARACTERISTICS IN SELECTED SPECIES OF OXALIS L. (OXALIDACEAE) FROM THE SOUTHERN PART OF INDIA

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Oxalis L. is a widespread genus in the family Oxalidaceae, comprising 500 species, with notable species diversity in tropical regions such as Brazil, Mexico, and South Africa, and represented by ten species in India. The present study investigated the stem, petiole, leaf, and root anatomical features of five different species of the genus *Oxalis*. The taxa selected for the present study are *O. corniculata*, *O. latifolia*, *O. pes-caprae*, *O. spiralis*, and *O. triangularis* from South India. Out of these five species, two of them are acaulescent. The samples collected were studied anatomically using the conventional method. The results of anatomical studies showed small but significant differences that helped to differentiate these five species. Among these, the pericycle characteristics of the stem emerge as a crucial distinguishing feature, particularly for the caulescent species. The number of cells in the cortex and the number of vascular bundles can also be used to identify species. This study aims to examine the internal characteristics of the species, with a focus on identifying subtle anatomical variations that may be linked to the morphological differences.

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Keywords: Stem anatomy; petiole anatomy; leaf anatomy; root anatomy; Oxalis; Oxalidaceae; South India

ارزیابی ویژگی های تشریحی در گونه های منتخب جنس .Oxalis L ،(Oxalidaceae) از بخش جنوبی هند

ام پی جاسیلا : دانشجوی دکتری، مرکز مطالعات رشد گیاهان و تحقیقات گیاه‌شناسی دانشکده خودگردان سنت‌جوزف، دواگیری، کوژیکود، کرالا، هند

توماس بینو: استادیار، بخش گیاه‌شناسی ، دانشکده خودگردان دواگیری، کالیکوت، کرالا، هند

.Oxalis L جنسی با پراکندگی وسیع از خانواده Oxalidaceae است که ۵۰۰ گونه دارد و گستردگی وتنوع زیادی در مناطق گرمسیری، به‌خصوص در برزیل، مکزیک و جنوب آفریقا دارد. در هند ۱۰ گونه از آن حضور دارد. مطالعه حاضر به بررسی آناتومیکی ساقه دمبرگ، برگ و ریشه پنج گونه مختلف از جنس Oxalis می‌پردازد. گونه‌های انتخاب شده عبارتند از:

O. corniculata, O. latifolia, O. pes-caprae, O. spiralis, and O. triangularis

از میان گونه‌ای بررسی شده، دو گونه بدون ساقه هستند. نمونه‌های جمع‌آوری شده با روش متداول مطالعات آناتومیکی بررسی شده‌اند.نتایج بررسی‌ها تفاوت‌های کم و بیش مفیدی را نشان داد که می‌تواند در تفکیک و شناسایی گونه‌ها مورد استفاده قرار گیرد. از میان صفات بررسی شده شاخص لایه زاینده ساقه (pericycle) یکی از مهمترین صفات تفکیک کننده به‌خصوص در گونه‌های ساقه‌دار است و همچنین تعداد سلولهای لایه پوست

(cortex) و تعداد دستجات آوندی می‌تواند برای شناسایی گونه‌ها مورد استفاده قرار گیرد. هدف از این مطالعه بررسی ویژگی‌های داخلی گونه‌ها با تمرکز بر تفاوت‌های آناتومیکی آنهاست که می‌تواند در ارتباط با صفات مورفولوژیکی برای شناسایی و تفکیک گونه‌ها مورد استفاده قرار گیرد.

INTRODUCTION

Oxalidaceae R.Br. is a small-sized family comprising about 5 genera and 565 species (Mabberley, 2017) distributed mainly in the tropics and subtropics of both hemispheres but extending into temperate regions (Huang *& al.*, 1998). Historically, the Oxalidaceae family was included in the order Geraniales, together with other primitive dicotyledonous families. The Angiosperm Phylogeny Group (APG, 1998) proposed the placement of Oxalidaceae within a new order, the Oxalidales within the Eurosides I. Later the position of Oxalidales within the Eurosides I of Rosides was confirmed by APG IV (2016).

Oxalis is the largest genus in the family Oxalidaceae with about 500 species (Mabberley, 2008). It has a cosmopolitan distribution, except for polar regions; species diversity is especially abundant in tropical Brazil, Mexico, and South Africa. It is represented by ten species in India, of which eight are known to exist in the Indian Peninsula (Manna, & al., 1997).

Linnaeus (1753) first described the genus Oxalis, with 13 species, and *O. acetosella* designated as the type species. Most species in the genus are considered garden weeds or are cultivated as ornamental plants, thriving in moist environments like lawns and waste areas. The genus is one of the bulbous angiosperm taxa (Judd & al., 1999). *Oxalis* is taxonomically difficult as it comprises characters that are extremely variable morphologically (López and Múlgura, 2011).

Members of *Oxalis* are herbs, rarely woody, often with underground bulbs or tubers. Leaves radical or cauline, usually digitate, 3-many foliate. 1-many flowered axillary peduncles, usually umbellate, regular and pentamerous. Disk without glands. Stamens 10, filaments free or united at the base. Ovary of 5, with one -many ovules. Capsule loculicidal. Seeds with an outer fleshy coat bursting elastically.

Anatomical characteristics of leaves, stems, and roots, are often used more frequently than floral ones because vegetative parts can provide more detailed and diverse information than focusing on the reproductive organs. When there is a need for more data to resolve a taxonomic question, looking at the internal structures of non-reproductive parts may reveal important differences that are not apparent in flowers. (Stuessy, 2009). Leaf, petiole, root, and stem anatomy offer critical diagnostic traits for plant identification, classification, and understanding of evolutionary relationships. These structures help distinguish species, understand ecological adaptations, and reconstruct phylogenetic histories (Judd & al., 2008).

Anatomical features have played an increasingly important role in the elucidation of phylogenetic relationships in *Oxalis* (Singh, 1999). Despite their widespread distribution, South Africa, Tropical Brazil, and Mexico are the primary hubs of *Oxalis* species diversity, hence not that much research was completed on the genus *Oxalis* from India. This study seeks to explore the internal characteristics of the species, with an emphasis on identifying subtle anatomical differences that could be associated with the morphological variations.

MATERIALS AND METHODS

Sample collection sites and period

The samples were collected from various locations in Kerala and Tamil Nadu, India, from December 2023 to March 2024.

Anatomical investigation

Fresh and healthy *Oxalis* plants were collected and identified based on their morphological characteristics by referencing flora, relevant literature, and identification keys. The present study was mainly based on live materials. The study encompasses five *Oxalis* species: *O. corniculata* L., *O. latifolia* Kunth., *O. pes-caprae* L., *O. spiralis* G. Don. and *O. triangularis* A. St.-Hil. (Fig. 1). The voucher specimens are deposited in the herbarium at St. Joseph's College (Autonomous), Devagiri, Calicut (DEV).

Fresh root, stem, leaf, and petiole samples were used for anatomical investigation. The collected materials were cleaned with tap water. Transverse sections of the fresh specimens were taken manually using sharp razor blades. The sectioning process was repeated 3-5 times until a good section was obtained. The sections were stained with a 1% aqueous solution of safranin for about 2 minutes, and excess stain was rinsed off with distilled water (Johansen, 1940) Semi-permanent slides were prepared by mounting the sections in glycerine on glass slides. Well-stained sections were examined under a light microscope and photographed using a Leica ICC50 E camera attached to a Leica 750 binocular research microscope. Anatomical features were described according to the terminology of Esau (1960)

Fig: 1. Habit. 1, *Oxalis corniculata*; 2, *O. latifolia*; 3, *O. pes-caprae*; 4, *O. spiralis*; 5, *O. triangularis.*

RESULTS AND DISCUSSION

Stem anatomy

Plants of *O. latifolia* and *O. triangularis* are acaulescent. The transverse section of the caulescent species of *Oxalis* stem is rounded and shows the following tissue arrangement from the periphery toward the center: dermal, ground, and vascular tissue systems.

Dermal tissue: consists of a single-layered epidermis, with thin-walled and compactly arranged cells. The outer surface of the epidermis is cuticularized. Trichomes are present in *O. corniculata* and *O. spiralis*.

But, absent in *O. pes-caprae*. In both *O. corniculata* and *O. spiralis*, the trichomes are unicellular and either glandular or non-glandular (Fig. 2). Shekhawat & al., (2019) also reported the presence of unicellular, glandular, and non-glandular trichomes in *O. corniculata*.

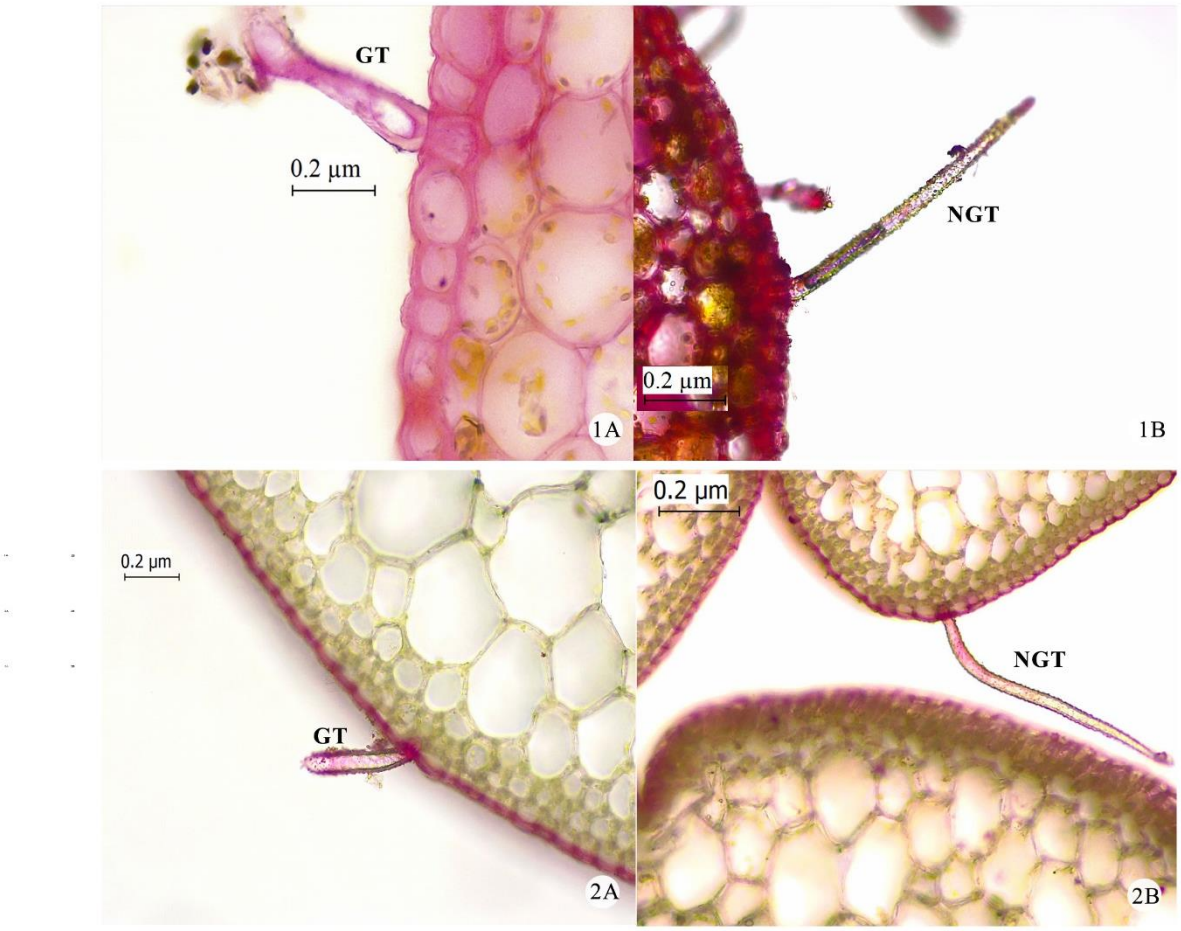

Fig. 2. Trichome Morphology. 1, *Oxalis corniculata*: A**,** Glandular; B, non-glandular; 2, *O. spiralis*: A, glandular; B, non-glandular.

Ground tissue: Consists of the Hypodermis, cortex, endodermis, pericycle, and pith. Hypodermis is single-layered and chlorenchymatous in *O. pes-caprae* and 1-2 layered, collenchymatous in *O. spiralis*. Hypodermis is absent in *O. corniculata*. Similar findings were observed by Al-Hussaini (2018) and Toma & al. (2007). The cortex is made up of 4-5 layers of parenchyma cells in *O. corniculata*, 8-10 layers in *O. pes-caprae*, and 5-6 layered in *O. spiralis*, Cortex is characterized by the presence of calcium oxalate crystals. Large, loosely packed parenchyma cells containing solitary crystals are also reported in *Averrhoa bilimbi* and *A. carambola* (Hari & al., 2020). Endodermis is single-layered, barrel-shaped, compactly arranged in all the species. Pericycle is single-layered and parenchymatous in *O. spiralis*, 1-2 layered and sclerenchymatous in *O. pes-caprae*, and 3-4 layered, sclerenchymatous in *O. corniculata*. This corresponds with Metcalfe and Chalk, 1950. These cells separate the cortex area of the vascular cylinder as it protects the phloem cells from the external pressure effect (Al-Hussaini, 2018). In all the species pith is composed of multi-layered, thin-walled parenchymatous cells that are somewhat polygonal or rounded in shape. The cortex and pith region are characterized by druse crystals and pigmented bodies.

Vascular tissue: Vascular bundles are arranged as a single ring. 12-14 vascular bundles are present both in *O. corniculata* and *O. spiralis*. Whereas it is 6-7 in *O. pes-caprae*. The presence of 13 big and 4-5 small

vascular bundles is reported in *O. corniculata* by Toma & al., 2007. Vascular bundles are conjoint, collateral, and open, and consist of a phloem and xylem endarch. In the xylem, the protoxylem is arranged towards the center and the metaxylem is arranged towards the periphery (Tab.1), (Figs. 3-4).

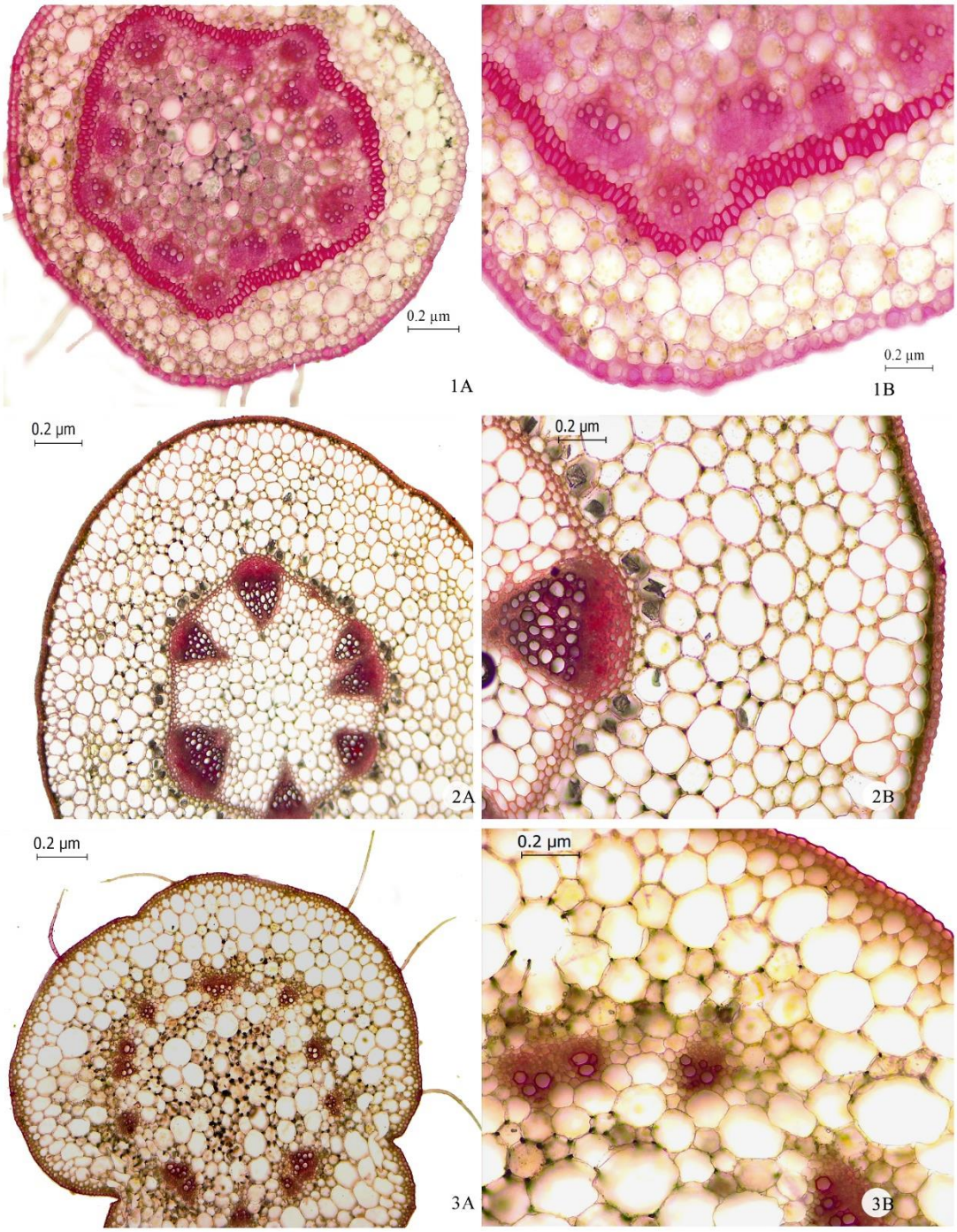

Fig. 3. Stem anatomy of *Oxalis* species. A, Ground Plan (4X); B, portion enlarged (10X). 1**,** *O. corniculate*; 2**,** *O. pes-caprae*; 3, *O. spiralis*.

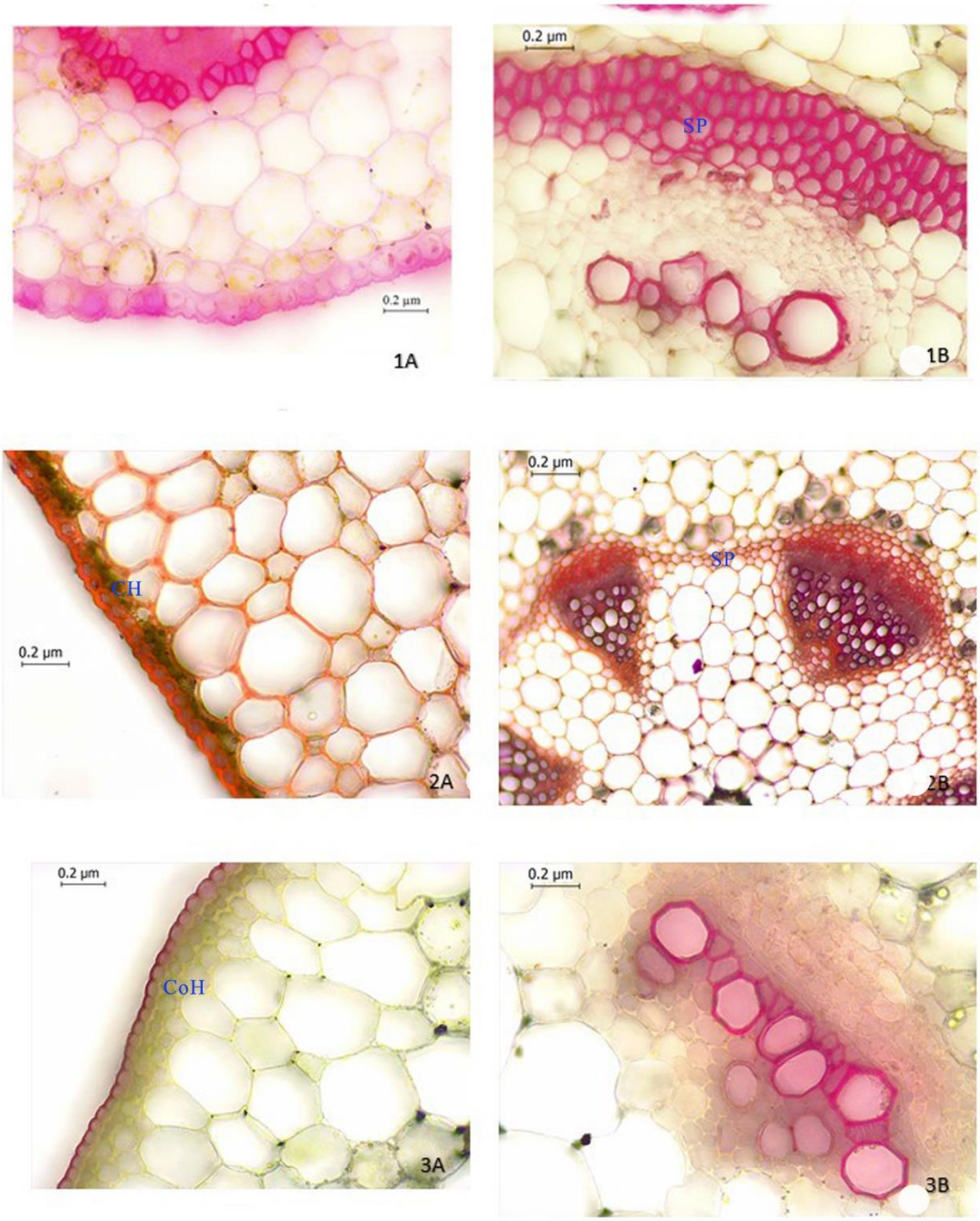

Fig. 4. Stem Anatomy of *Oxalis* species. A**,** Portion enlarged (20X); B, portion enlarged (40X). 1, *O. corniculata*; 2, *O. pes-caprae*; 3, *O. spiralis*. SP**:** sclerenchymatous Pericycle; CH: Chlorenchymatous hypodermis; CoH**:** collenchymatous hypodermis.

Petiole anatomy

The petiole is almost rounded in cross-section. The transverse section of the petiole shows the following tissue arrangement from the periphery towards the center; dermal, ground, and vascular tissue systems. It is somewhat similar to stem anatomy.

Tab 1. Stem anatomical characters of caulescent species of *Oxalis* L.

Character	***O. corniculata* L.**	***O. pes-caprae* L.**	***O. spiralis* G. Don.**
Outline of the stem	Round	Round	Round
Epidermis	Single-layered, trichomes present	Single-layered, trichomes absent	Single-layered, trichomes present
Hypodermis	absent	Single-layered, chlorenchymatous	1-2 layers of living collenchymatous
Cortex	4-5 layered parenchymatous	8-10 layered parenchymatous	5-6 layered parenchymatous
Endodermis	Single layered	Single layered	Single layered
Pericycle	3-4 layered, sclerenchymatous	1-2 layered sclerenchymatous	Single-layered and parenchymatous
No. of vascular Bundles	12-14 vascular bundles	6-7 vascular bundles	12-14 vascular bundles
Pith	Multi-layered parenchymatous pith	Multi-layered parenchymatous pith	Multi-layered parenchymatous pith

Dermal tissue: Consists of epidermis which is single-layered, made up of thin-walled and compactly arranged cells. The outer surface of the epidermis is cuticularized. Trichomes are present in all taxa studied. **Ground tissue**: Consists of the hypodermis, cortex, endodermis, pericycle, and pith. Hypodermis is absent in *O. corniculata*, whereas it is single-layered and chlorenchymatous in *O. latifolia*, *O. pes-caprae*, *O. spiralis* and *O. triangularis*. The cortex is made up of 4-5 layered parenchyma cells in *O. corniculata* and *O. latifolia*, 3-4 layered in *O. pes-caprae.*, *O. triangularis* and 2-3 layered in *O. spiralis*. Endodermis is single-layered. Pericycle is 1-2 layered, sclerenchymatous in *O. corniculata*. In all the other species endodermis and pericycle are single-layered. The cortex layer is followed by a layer of small cells representing the pericycle area, Consistent with Nasrallah (2007), *O. corniculata* is encircled by a continuous ring of sclerenchyma cells. Pith comprises multi-layered, thin-walled parenchymatous cells that are somewhat polygonal or rounded in shape, but in *O. pes-caprae*, it appears as a hollow cavity. Druse crystals, pigmented bodies and prismatic crystals characterize the cortex and pith region.

Vascular tissue: 4-5 vascular bundles are present in *O. corniculata*, *O. pes-caprae*, and *O. triangularis*, whereas it is 3-4 bundles in *O. latifolia* and 5-6 in *O. spiralis*. Vascular bundles are Conjoint, Collateral, and open, and consist of phloem and xylem. The xylem is the end arch. In the xylem, protoxylem is arranged towards the center and the metaxylem towards the periphery (Tab. 2), (Fig. 5-6).

Tab 2. Petiole anatomical characters of five examined species of *Oxalis*.

Character	***O. corniculata***	***O. latifolia* Kunth.**	***O. pes-caprae***	***O. spiralis***	***O.triangularis* A.St.-Hil.**
Epidermis	Single-layered, trichome present	Single-layered, trichome present	Single-layered, trichomes present	Single-layered, trichome present	Single-layered, Trichome present
Hypodermis	absent	Single-layered, chlorenchymatous	Single-layered, chlorenchymatous	1-2 layers of living Chlorenchymatous	Single-layered, chlorenchymatous
Cortex	4-5 layered parenchyma	4-5 layered parenchyma	3-4 layered parenchymatous	2-3 layered parenchymatous	3-4 layered parenchymatous
Endodermis	Single layered	Single layered	Single layered	Single layered	Single layered
Pericycle	1-2 layered, sclerenchymatous	Single-layered and parenchymatous	Single layered sclerenchymatous	Single-layered and parenchymatous	Single-layered and parenchymatous
No. of vascular Bundles	4-5 vascular bundles	3-4 vascular bundles	4-5 vascular bundles	5-6 vascular bundles	4-5 vascular bundles
Pith	Multi-layered parenchymatous pith	Multi-layered parenchymatous pith	Multi-layered parenchymatous/hollow cavity in pith	Multi-layered parenchymatous pith	Multi-layered parenchymatous pith

Leaf anatomy

The leaf is dorsi-ventral and has reticulate venation.

Epidermis: Consists of an upper and lower epidermis comprising uni-seriate compactly arranged thin-walled cells. The upper and lower epidermal cells were nearly identical; however, the lower epidermis cells were smaller than those of the upper epidermis, which is uniseriate and consists of a single layer of large, irregularly sized cells. These findings correlate with the findings of Al- Al-Hussaini (2018). Stomata are distributed in the lower epidermis.

Mesophylls: Differentiated into single-layered palisade and spongy parenchyma layers. The lower midrib region consists of a layer of large parenchyma cells in *O. corniculata* and *O. pes-caprae*, 1-2 layered in *O. latifolia*, 2-3 layered in *O. spiralis* and 3-4 layered in *O. triangularis*. Calcium Oxalate crystals are found in the form of prismatic shapes, and other cubical shapes. Single crystals, druses and sandy crystals are also found in the leaf tissues. R.D. Sá & al., (2017), also reported the presence of prismatic crystals in the parenchyma of the leaf midrib.

Vascular bundle: Conjoint and collateral. Collateral vascular bundles are common in the Oxalidaceae family (Fig. 7), as also reported by Metcalfe and Chalk (1950).

ROOT ANATOMY

Transverse section of the root shows a well-differentiated periderm with wavy margins. The cortex and epidermis may rupture as a result new protective layer of periderm is formed. Cork or phellem is 2-3 layered, also reported by Toma & al., (2007). A secondary cortex or phelloderm is present which is variable in all species studied. Parenchymatous and 6-7 layered in *O. corniculata*, well differentiated in *O. latifolia* and *O. triangularis*, 8-9 layered in *O. spiralis* and *O. pes-caprae*. Many layered phelloderm is also reported in *O. corniculata* (Toma & al., 2007). The vascular bundles contain both the xylem and phloem in which the xylem is prominent and the phloem is present in the outer side of the xylem. The protoxylem is arranged towards the periphery and the metaxylem towards the center. Pith is absent in *O. corniculata*, *O. latifolia* and *O. triangularis*, whereas it is multi-layered and parenchymatous in *O. pes-caprae* and *O. spiralis*. Some of the roots exhibited an asymmetrical structure, with the cortex and phloem thicker on one side of the organ; this asymmetry is due to the pressure caused by the stones among which the root is growing (Toma *& al.,* 2007), (Fig 8).

Although anatomical characters of the genus *Oxalis* possess many common characteristics, some differences are helpful in taxonomic delimitation. The anatomical traits such as pericycle characteristics, cortex cell count, and vascular bundle arrangement, are critical for species recognition in *Oxalis*, particularly among caulescent species. These characteristics often correlate with its phylogeny, as closely related species tend to share similar structural traits; for instance, variations in stem anatomy, such as pericycle structure, cortex cell number, and vascular bundle arrangement, can reflect evolutionary relationships within the genus.

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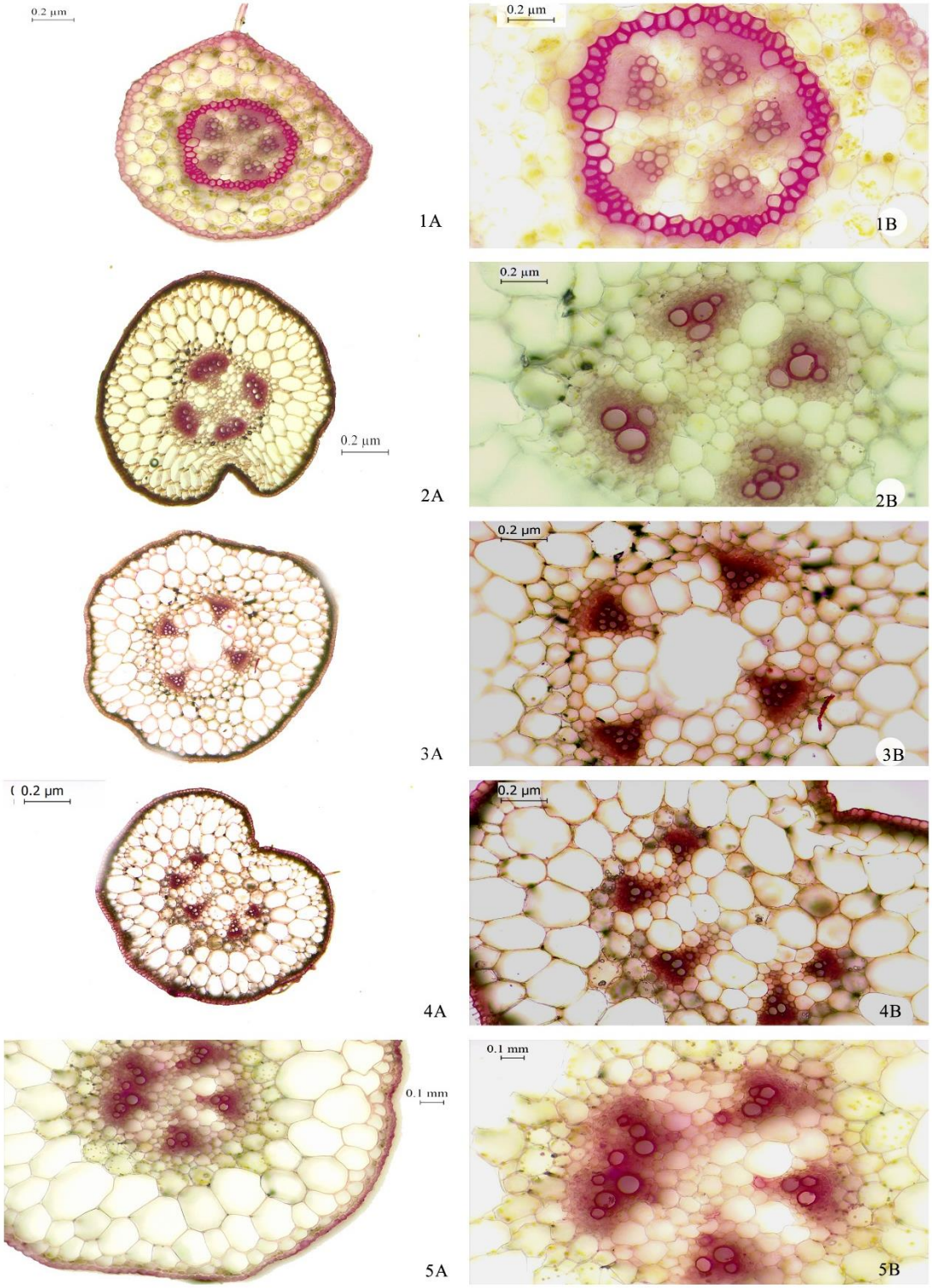

Fig. 5. Petiole anatomy of *Oxalis* species. A, Ground plan (4X); B, portion enlarged (10X). 1, *O. corniculate*; 2, *O. latifolia*; 3, *O. pes-caprae*; 4, *O. spiralis*; 5, *O. triangularis*.

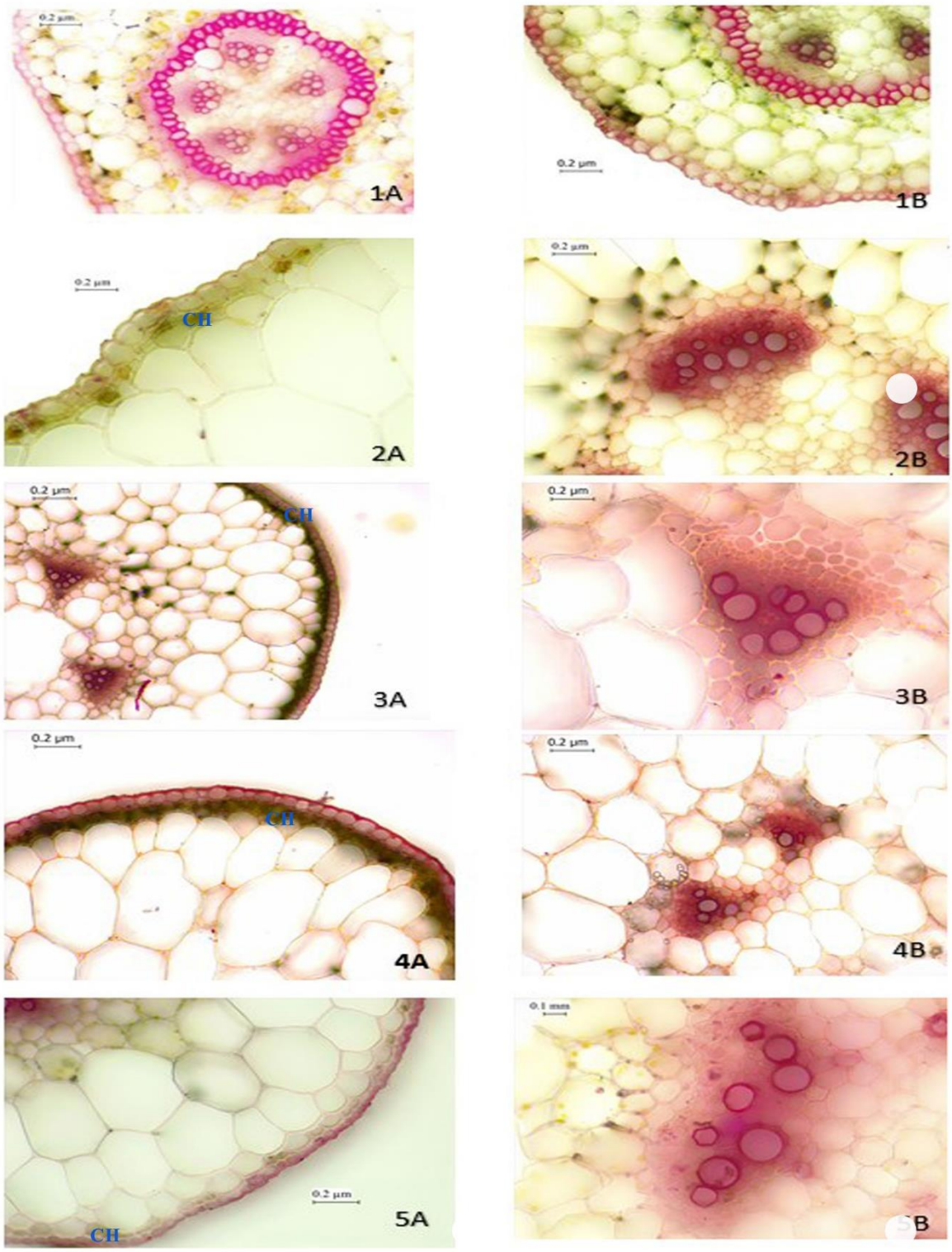

Fig. 6. Petiole Anatomy of *Oxalis* species. A, Portion enlarged (20X); B, portion enlarged (40X). 1, *O. corniculate*; 2, *O. latifolia*; 3. *O. pes-caprae*; 4, *O. spiralis*; 5, *O. triangularis*. CH=Chlorenchymatous Hypodermis.

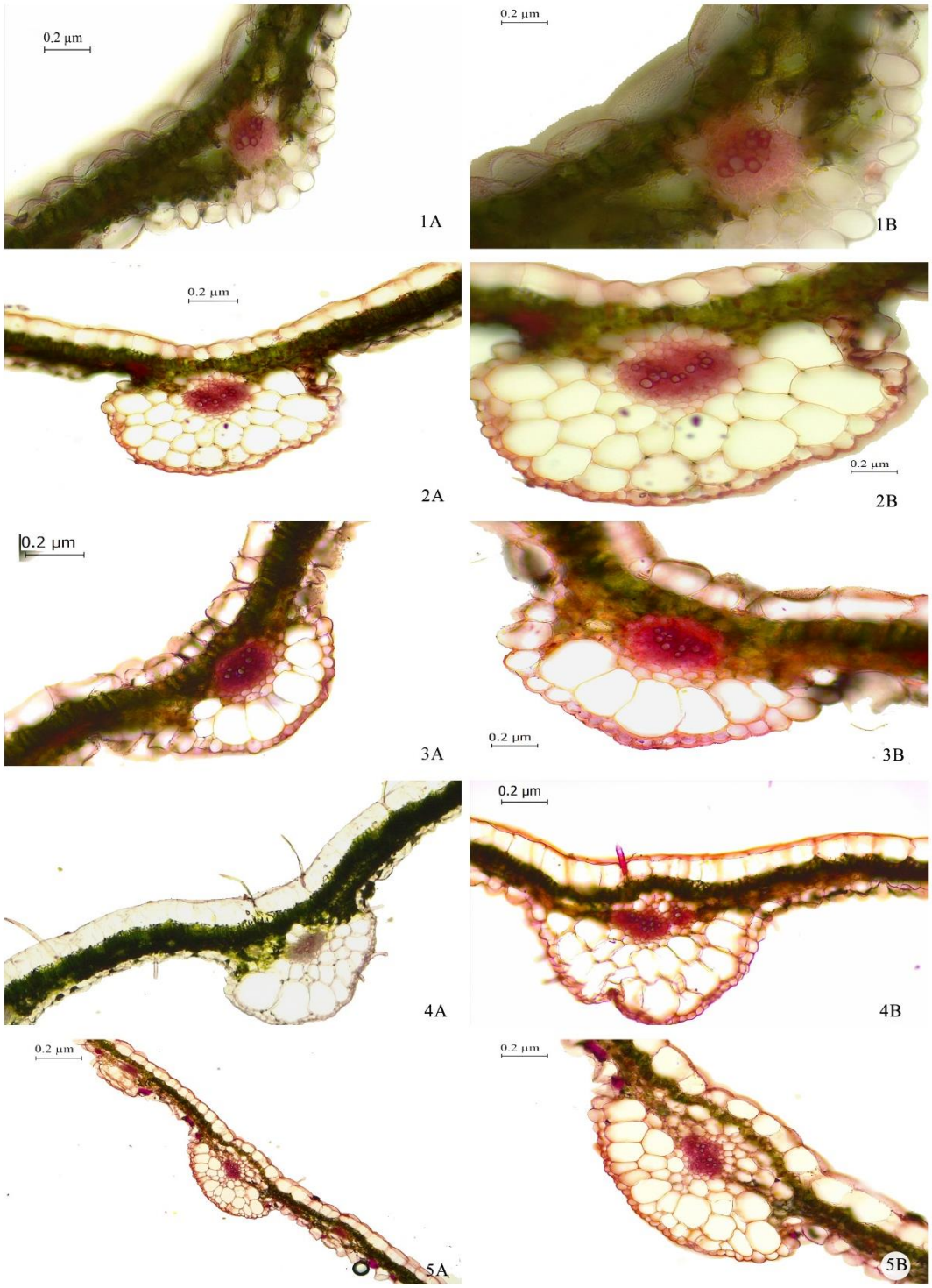

Fig. 7. Leaf Anatomy of *Oxalis* species. A, Ground plan (10X); B, Portion enlarged (40X). 1, *O. corniculate*; 2, *O. latifolia*; 3, *O. pes-caprae*; 4, *O. spiralis*; 5, *O. triangularis.*

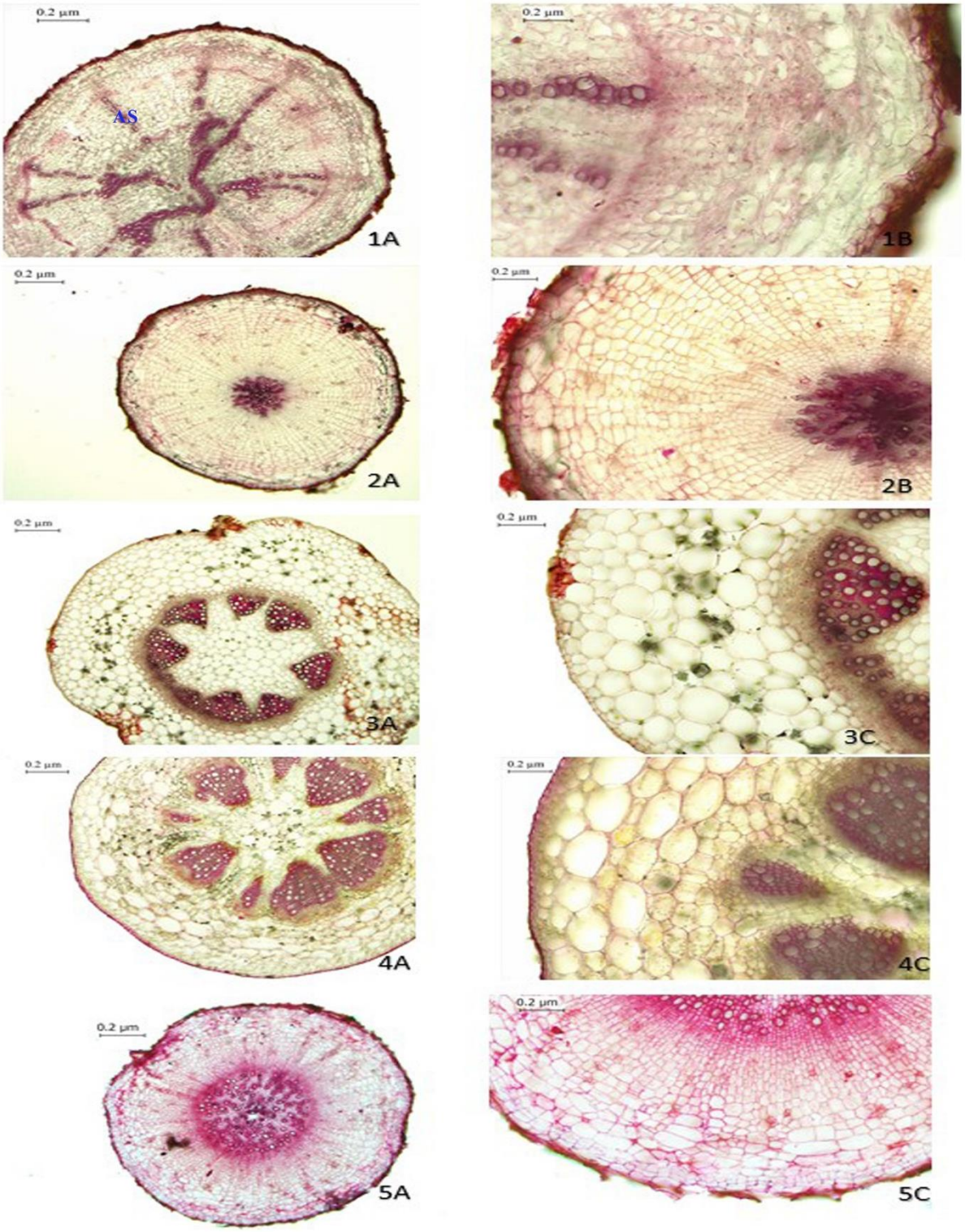

Fig. 8. Root Anatomy of *Oxalis* species. A, Ground plan (10X); B, portion enlarged (20X). 1, *O. corniculate*; 2, *O. latifolia*; 3, *O. pes-caprae*; 4, *O. spiralis*; 5, *O. triangularis*. AS: Asymmetry.

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