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THE SIGNIFICANCE OF ANATOMICAL CHARACTERS IN RESOLVING TAXONOMIC AMBIGUITY OF A TAXON: A CASE STUDY ON ROTALA L. (LYTHRACEAE) FROM INDIA

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Abstract
The genus *Rotala* L. is one of the largest genera in the family Lythraceae. In the present study, anatomical features of the stem and leaf of selected species of the genus *Rotala* were investigated. The selected taxa chosen for the present study include *R. baileyana*, *R. deniflora*, *R. macrandra*, *R. malabarica*, *R. malampuzhensis, R. mexicana, R. occultiflora,* and *R. tulunadensis.* Moreover, these species were also distributed in the North Malabar regions of Kerala. The results of this study revealed that all examined taxa shared nearly identical anatomical characteristics, but some showed few remarkable differences. According to the result, cortical cells and the nature of vascular bundles vary in different species. The species can be distinguished based on aerenchyma cells in the inner cortex, vascular bundles, the presence of calcium crystals, and the layer of palisade tissue. An identification key to the taxa was also provided based on the anatomical features of their leaf and stems.

Keywords: Anatomy; calcium crystals; India; Lythraceae; *Rotala*

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ارزش صفات تشریحی در حل مشکلات رده بندی؛ مطالعه موردی جنس **Rotala** از خانواده **Lythraceae** از هند

سوارنیکا ولامتاتا: کالج دولتی کاسارگاد، دانشکاه کنور، کرالا، هند
راجی وارانگومچواتیل: گروه گیاه‌شناسی کالج سنت مری، دانشگاه کالی کوت، کرلا، هند
بینو توماس: گروه گیاه‌شناسی کالج سنت ژوزف، دانشگاه کالی کوت، کرلا، هند

چکیده: جنس *Rotala* L. یکی از بزرگترین جنس‌های خانواده Lythraceae است. در مطالعه حاضر صفات تشریحی ساقه و برگ تعدادی از گونه‌های این جنس شامل *R. baileyana, R. deniflora, R. macrandra, R. malabarica, R. malampuzhensis, R. mexicana, R. occultiflora,* و *R. tulunadensis* مورد بررسی قرار گرفتند. این گونه‌ها در شمال منطقه مالبر در کرالا حضور دارند.

نتایج این بررسی نشان داد تقریباً همه گونه‌های مطالعه شده صفات تشریحی مشترکی دارند، اما بعضی گونه‌ها تفاوت‌های قابل ملاحظه‌ای را نشان می‌دهند. براساس نتایج این تحقیق سلول‌های پوسته و دستجات آوندی در گونه‌های مختلف اشکال متفاوتی دارند. گونه‌ها بر اساس سلول‌های آئرانشیمی کورتکس داخلی، دستجات آوندی و حضور کریستال‌های کلسیم و لایه‌های بافت پوششی کلروپلاستی از هم قابل تفکیک هستند. همچنین یک کلید شناسایی برای گونه‌های بررسی شده براساس صفات تشریحی برگ و ساقه ارایه می‌گردد.

INTRODUCTION

The genus *Rotala* L. (Lythraceae), has tropical and subtropical distribution and is represented globally by 44 species (Mabberley, 2005), with the highest distribution in tropical Asia (Cook, 1979). After Cook's revision, it is represented, as per the revised estimate, in India by 29 species, of which 24 are from Peninsular India (Joseph & Sivar 1988, 1989; Pradeep & al. 1990; Mathew & Lekshminarasimhan, 1990; Yadav & al. 2010; Prasad & al. 2012; Gaikwad & al. 2013; Prasad & Raveendran 2013 a, b; Sunil & al. 2013; Anto & al. 2014; Ratheesh Narayanan & al. 2014; Lemiya & Pradeep 2015; Nandakumar 2018; Prasanth & Sardesai 2022).

Species of the genus *Rotala* fall under two groups, based on the nature of their habitat, viz., obligate aquatics, which grow in shallow water, and semi-aquatics or terrestrials, which thrive in marshy lands. Most of the aquatic species are characterized by what is called 'Hippuris syndrome' (Cook 1978): erect, unbranched stems with simple, elongate leaves borne in symmetrical whorls, "heterophylly manifesting itself as variation in some leaves in each whorl and individual leaf shape and size." Among the Indian species, this syndrome is displayed by *R. verticillaris* L., *R. mexicana* (only where it grows as an emergent aquatic), *R. cookie* K.T. Joseph & Sivar., and *R. vasudevanii* K.T. Joseph & Sivar. Most other species have decussate leaves, while *R. floribunda* (Wight) Koehne has alternate ones, and *R. occultiflora* has leaves disposed in whorls of 3.

Leaf and flower arrangements have been taken as an important taxonomic character in this genus by many authors. Koehne (1903) divided the genus into two sections based on these characters: Sect. Hippiuridum with whorled/alternate leaves and flowers, and Sect. Enantiorotala with decussate leaves and flowers, but species like *R. mexicana*, and *R. myriophylloides* Welw. ex Hierndisplay that both their characteristics depending upon whether they are growing as aquatics or terrestrials. Consequently, Cook (1978, 1979) considered these characters to be direct responses to the aquatic environment and that these 'Hippuris mimics' do not constitute a single phyletic group. Cook (1979) has also found that the genus is very uniform and does not yield a satisfactory subgeneric classification into natural subgeneric groups.

Anatomical characteristics of vegetative and floral parts in flowering plants have been successfully employed to solve taxonomic problems and elucidate phylogenetic relationships. Plant micromorphological studies using SEM and TEM, supported by ultramicrotomy techniques, have proven to be powerful tools for investigating anatomical features of taxonomic significance. Anatomical characters are conserved and stable and thus used to solve many taxonomic ambiguities. Anatomical characters of stem, root, leaf, bark, stomata, sclereids, fibers, trichomes, cambium, and wood anatomy can be generally used. 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). Anatomical features can be used in taxonomic analysis for the identification of plants and establishing genetic relationships to solve taxonomic disputes.

The genus *Rotalia* includes herbaceous, annual plants that inhabit seasonal lateritic pools, depressions with rich humus deposits, paddy fields, and marshy areas. Leaves are alternate, opposite, or whorled. It may be petiolate or sessile, lanceolate or narrow needle-like, or bimucronate at apex, margins are entire, and the color ranges from bright green to reddish, based on light intensity and nutrient availability. Upper epidermal cells have sinuate walls, with the stomata on both surfaces. Flowers are bracteate and bracteolate, with calyx lobes 3-5, small, corolla lobes 3-5 or absent in some, and nectar scales are seen in most species. Stamens 3-5, inserted at the base of the calyx tube. Ovary ellipsoid, style short, stigma capitate, capsule globose, seeds ovoid, brownish, 10-15 per fruit. Nodal anatomical and epidermal features of some species in the genus *Rotala* have been studied by some authors (Kshirsagar A. A. 2017, Sarojini 1998). Several authors also commented on the usefulness of anatomical characters for resolving the taxonomic ambiguities of some plant taxa. Moreover, the anatomical features of *Rotala* have not been fully explored so far; hence, the present study mainly focused on the anatomical characterization of selected species of *Rotala* from the North Malabar Region of Kerala to fill this gap.

MATERIALS AND METHODS

Sample collection sites and period

Rotala species were collected from various locations in Kerala for anatomical studies from June 2024 to January 2025 (Table 1).

Fresh plant specimens of selected species of *Rotala* were preserved in formalin or FAA for anatomical characterization. The plant taxa selected for the present study include *R. tulunadensis*, *R. malampuzhensis*, *R. occultiflora*, *R. malabarica*, *R. Mexicana*, *R. densiflora*, *R. baileyana*, and *R. macrandra*. Identification of selected taxa was done by observing their morphological characteristics with the help of available floras and literature and it was also compared with authenticated herbarium specimens were deposited in the herbarium of University of Calicut (CALI), the voucher specimens were also deposited in the herbarium of St. Joseph's College (Autonomous), Devagiri, Calicut (DEV) for future reference. Images of some studies *Rotala* species are shown in Fig. 1. The studied species are listed in Table 1.

Anatomical studies

The samples for the anatomical studies were collected from different areas of the North Malabar region of Kerala. The collected materials were cleaned using tap water. The stems and leaves of the collected plants were cut into suitable dimensions. Freehand sectioning of the material was done by using a razor blade. The leaves were cut into delicate sections with the help of pith, then these sections were transferred into safranin for one minute for staining (Johansen 1940). The stained sections were washed with distilled water, mounted by using a drop of glycerin on the slide. Then it was examined under a stereo microscope at a magnification of 4x for a ground plan of the stem, 10x for the epidermis, cortex, pith, and 20x for the epidermis, cortex, and pith for stems, and 10x for leaves. The photographs of the transverse sections of both stem and leaves were captured using Leica DFC 290, a camera attached to Leica DM 100 trinocular research microscope. The anatomical features were described according to the terminology of Esau (1960).

Table1. Collecting data on the *Rotala* species selected for the anatomical studies.

No	Taxa	Habitat	Geographical coordinates	Details of plant specimens studied/examined
1	*Rotala baileyana* Rogi, Joby, Rogimon, Nisha & I. Antony.	Permanent lateritic pool	12º26'49" N 75º18'37"E	Kayyur, Kasaragod District, Kerala State, Suvarnika. V., 10407, DEV, 10/10/2024
2	*R. densiflora* (Roth ex Roem. &Schult.) Koehne	Dry paddy field	11º 84'67" N 76º06'31" E	Kattikkulam, Wayanad District, Kerala State, Suvarnika. V., 10406, DEV, 10/01/2024
3	*R. macrandra* Koehne	Wet paddy field	12º39'69" N 75º39'69" E	Ravaneshwar, Kasaragod District, Kerala State, Suvarnika. V., 10408, DEV, 06/01/2025
4	*R. malabarica* Pradeep, K.T. Joseph & Sivar.	Seasonal lateritic pool	12º28'87" N 75º23'80" E	Karinthalam, Kasaragod District, Kerala State, Suvarnika.V., 10404, DEV, 10/10/2024
5	*R. malampuzhensis* R.V. Nair ex C.D.K. Cook	Seasonal lateritic pool	12º26'49"N 75º18'37" E	Kayyur, Kasaragod District, Kerala State, Suvarnika. V., 10402, DEV, 25/09/2024
6	*R. mexicana* Schltdl. & Cham.	Wet lateritic soil	12º 23'13" N 75º27'54"E	Velichamthodu, Cheemeni, Kasaragod District, Kerala State, Suvarnika.V., 10405, DEV, 24/09/2024
7	*R. occultiflora* Koehne	Seasonal lateritic pool	12º28'87" N 75º23'80" E	Karinthalam, Kasaragod District, Kerala State, Suvarnika.V., 10403, DEV, 13/09/2024
8.	*R. tulunadensis* K.S.Prasad, P.Biju, Raveendran & K.G. Bhat	Permanent lateritic pool	12º27'68" N 75º15'92"E	Angakkalari, Nileshwar,Kasaragod District, Kerala State, Suvarnika.V., 10401, DEV, 10/10/2024

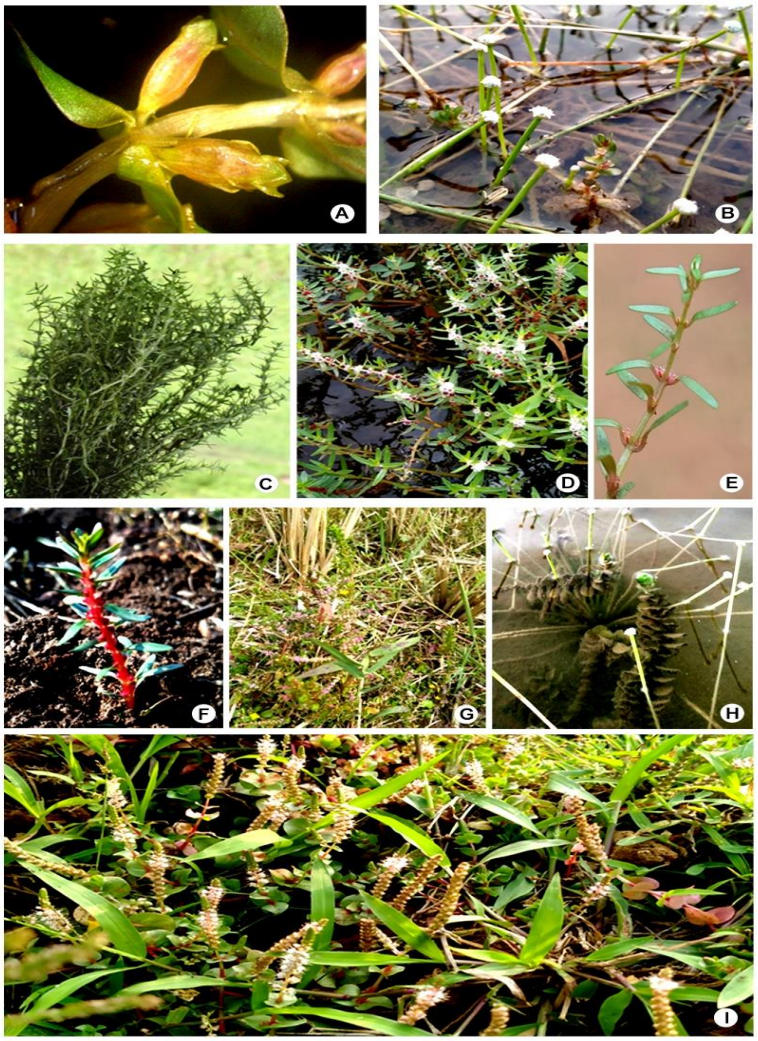

Fig. 1. Habit of selected *Rotala* spp. from the study area: A & B, *R. tulunadensis;* C, *R. occultiflora;* D, *R. malabarica;* E, *R. mexicana;* F, *R. malampuzhensis;* G, *R. densiflora;* H, *R. baileyana;* I, *R. macrandra*.

RESULT AND DISCUSSION

General anatomical characters of the genus *Rotala* are discussed below:

Stem anatomy

Epidermis

The epidermis is undulate, made up of single-layered parenchymatous cells (Fig. 2). Spherical and oval collenchyma cells were seen in the epidermis of *R. malabarica* and *R. malampuzhensis* (Fig. 3). Some cells of the epidermis are slightly larger with thick cuticles. The stem of *R. mexicana* is irregular, and it shows ridges and grooves. Cells are bead-shaped, oval, and parenchymatous.

Cortex

The cortex is differentiated into the hypodermis, middle cortex, and inner cortex. Hypodermis is made up of parenchymatous cells, without any intercellular spaces, which may be larger at the region where the vertical cells of the middle cortical cells meet. Starch granules are seen in the cortical region. Large and spherical parenchymatous cells are seen in the hypodermal regions of *R. malabarica*, *R. occultiflora*, and *R. mexicana*. While in *R. baileyana* and *R. tulunadensis*, the hypodermis is rectangular (Fig. 3). 2-3 layered oval to spherical parenchymatous hypodermis is present in *R. macrandra* (Fig. 3).

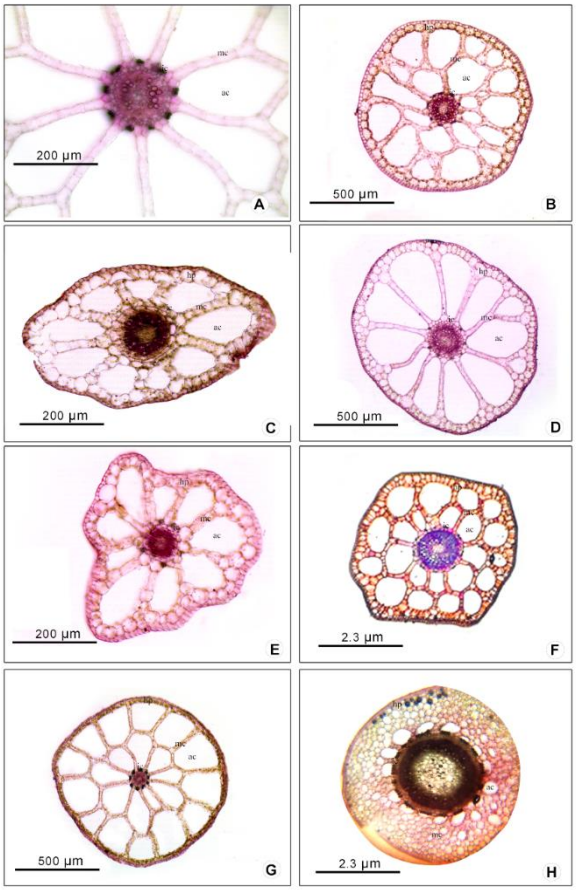

Fig. 2. Ground plan of the stem. A, *R. tulunadensis*; B, *R. malampuzhensis*; C, *R. occultiflora*; D, *R. malabarica*; E, *R. mexicana;* F, *R. densiflora;* G, *R. baileyana*; H, *R. macrandra*. hp=hypodermis; mc=middle cortex; ac=air cavity; ic=inner cortex. Scale bars=2.3, 200 & 500 µm.

Variously sized air chambers are present in the middle cortex. The cells of the middle cortex are aerenchymatous and are arranged vertically. In the region where two vertically elongated aerenchyma cells meet, or at the corners of air cavities, the cells are large and triangular in appearance. The inner cortex is single-layered, with 9-10 large spherical cells alternating with small parenchyma cells. Large air cavities are more prominent towards the inner cortex in *R. macrandra* (Fig. 2, H). Calcium crystals are also seen in the inner cortical region of all selected species.

Endodermis and Pericycle

Endodermis is single-layered, large, and parenchymatous. Bead-shaped to polygonal in *R. tulunadensis* and *R. baileyana*, while it was elongated and bead-shaped in *R. occultiflora* and *R. mexicana* (Fig. 4). A single layer of pericycle is found in *R. occultiflora, R. baileyana*, *R. malampuzhensis,* and *R. mexicana*, but in taxa like *R. malabarica, R. densiflora, R.macrandra,* and *R. tulunadensis,* the pericycle was absent (Fig. 4).

Vascular bundles

Numerous vascular bundles were present in all studied taxa. Phloem cells are mostly parenchymatous (Fig.4). Xylem cells are differentiated into metaxylem and protoxylem, respectively, with an end arch arrangement in the stem. Intraxylary phloem cells are a feature of all species examined.

Pith

Pith consists of large, polygonal, thin-walled parenchymatous cells without intercellular spaces, in all studied species (Fig. 4).

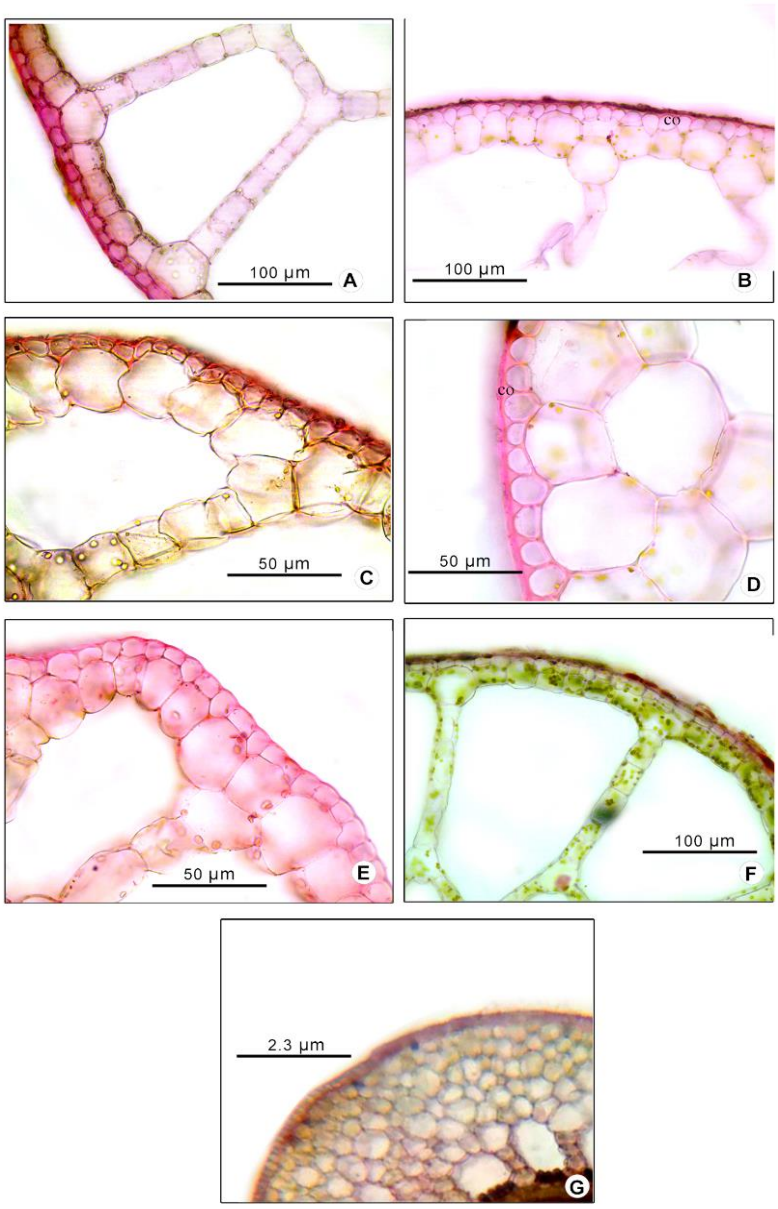

Fig.3. Epidermis of *Rotala* spp. A, *R. tulunadensis*; B, *R. malampuzhensis*; C, *R. occultiflora*; D, *R. malabarica*; E, *R. mexicana*; F, *R. baileyana*; G, *R. macrandra*. co=collenchymatous epidermis. Scale bars=2.3, 50 &100 µm.

Leaf anatomy

Epidermis

The epidermis consists of single-layered, large, thin-walled parenchymatous cells with cuticles. Epidermal hairs are absent in all species studied. Upper epidermal cells are larger than those of the lower epidermis. In *R. mexicana*, both the upper and lower epidermis become equal-sized (Fig. 6, D3). A groove is present in the midrib region of *R. malabarica* (Fig. 6, D2). Stomata were present on both the lower and upper epidermis. The cells of the lower epidermis are smaller in size, spherical, or barrel-shaped. The epidermis was collenchymatous in *R. malampuzhensis* and *R. malabarica*. In *R. tulunadensis* and *R. baileyana* the leaves are broad and delicate. In *R. baileyana*, the upper surface of the leaf shows a pale red color. The narrow needle-like leaf of *R. occultiflora* and its cross section showed larger spongy tissues towards the mid-rib region (Fig. 5, C1).

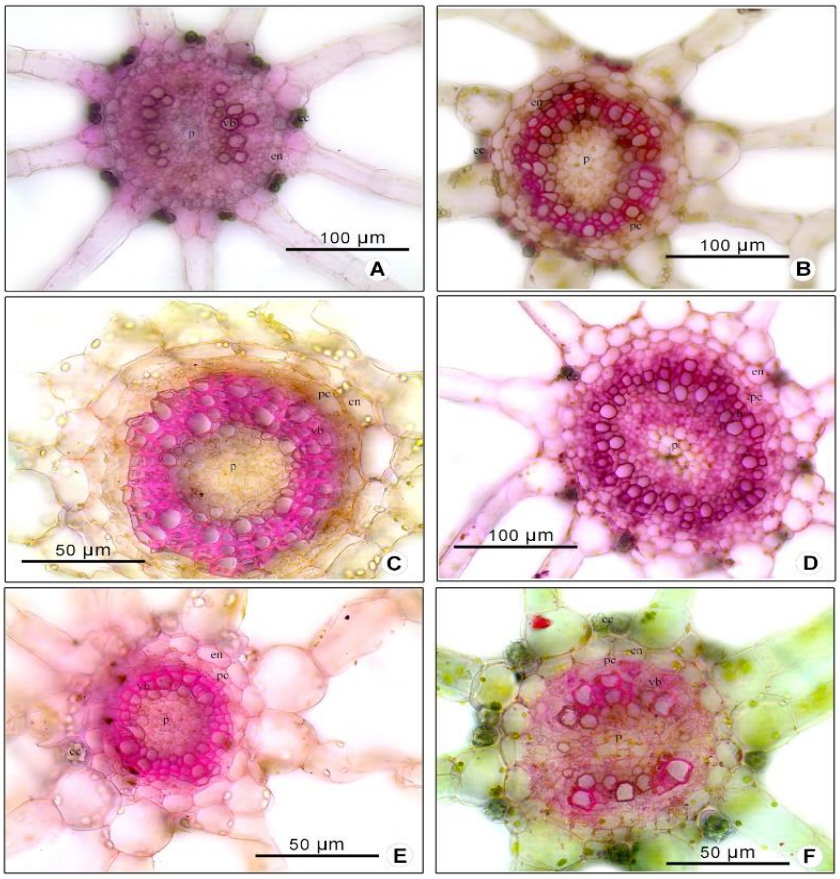

Fig. 4. Stellar regions of *Rotala* spp. A, *R. tulunadensis*; B, *R. malampuzhensis*; C, *R. occultiflora*; D, *R. malabarica*; E, *R. mexicana*; F, *R. baileyana*. cc=calcium crystal; en=endodermis; pc=pericycle; vb=vascular bundle; p=pith. Scale bars=50 & 100 µm.

Mesophyll

Mesophyll cells are differentiated into palisade and spongy tissue. The palisade is 1-2 layered. It is single-layered in *R. Mexicana*, and *R.malabarica*. The palisade cells are elongated in *R. malabarica*. Spongy cells are 1-2 layered, small, spherical, irregularly arranged with intercellular spaces, and contain chloroplast. 3-4 layered spongy cells were seen in *R. macrandra* (Fig. 7, E3). Calcium oxalate crystals are present in mesophyll regions. The hypodermis of the midrib region of *R. malabarica* is composed of 2-3 layers of spherical spongy parenchyma cells with intercellular spaces towards its lower side (Fig. 6, D2). In *R. malabarica*, the mesophyll region has both spherical and oval parenchymatous cells. Air canals are present in the mesophyll regions of *R. tulunadensis* (Fig. 5, A1) and *R. baileyana* (Fig. 7. E2).

Vascular bundles

A single vascular bundle is present in the midrib region of all selected taxa for the present study. Vascular bundles are composed of xylem and phloem. The phloem cells are oriented towards the lower epidermis, and the xylem cells are oriented towards the upper epidermis. The cells close to the vascular bundle in the midrib region are large-sized, spherical collenchyma with chloroplasts (Figs. 5-7). Tracheary elements are also present in all selected taxa. Moreover, the vascular bundles are intraxylary phloem nature. There are 10-12 vascular bundles in *R. densiflora* (Fig. 7, E1).

It was noticed that some anatomical characters can be used for species delimitation in the genus *Rotala*. According to the present study, different species of *Rotala* can be distinguished

By the nature of their epidermal cells, size of inner cortical cells, presence or absence of pericycle, peculiarity of vascular bundles, and mesophyll cells, etc. All these characters are used as key anatomical features for taxonomic delimitation rather than their morphological characteristics.

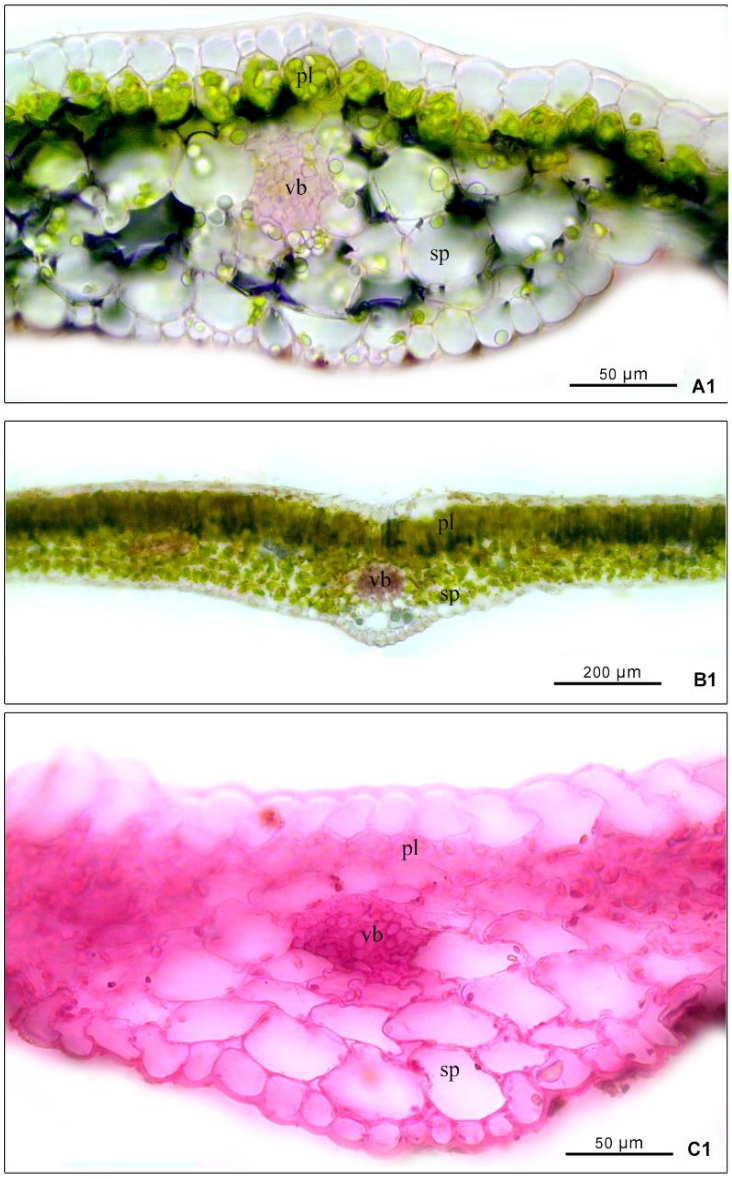

Fig. 5**.** Leaf anatomy of *Rotala* spp. A1, *R. tulunadensis*; B1, *R. malampuzhensis*; C1, *R. occultiflora*. pl=palisade tissue: sp=spongy tissue; vb=vascular bundle. Scale bars=50, 100 µm.

Key to the identification of selected *Rotala* species for the present study

1. Stem epidermis is parenchymatous 2
1. Stem epidermis is collenchymatous 3
2. Leaf lamina is mucilaginous, expanded, and the upper surface is pale red ..... *R. baileyana*
2. Leaf lamina is not mucilaginous, not expanded, and the upper surface is not pale red 4
3. Pericycle absent in the stem ..... *R. malabarica*
3. Pericycle present in the stem .... *R. malampuzhensis*
4. Undulate stem with deep ridges ..... *R. mexicana*
4. Undulate stem without deep ridges 5
5. Stem is mucilaginous 6
5. Stem is not mucilaginous 7
6. Pericycle absent in stem ..... *R. tulunadensis*
6. Pericycle present in stem ..... *R. occultiflora*
7. Stem is quadrangular, leaf with 5-6 layers of spongy tissues ..... *R. densiflora*
7. Stem is not quadrangular, leaf with 3-4 layers of spongy tissues ..... *R. macrandra*

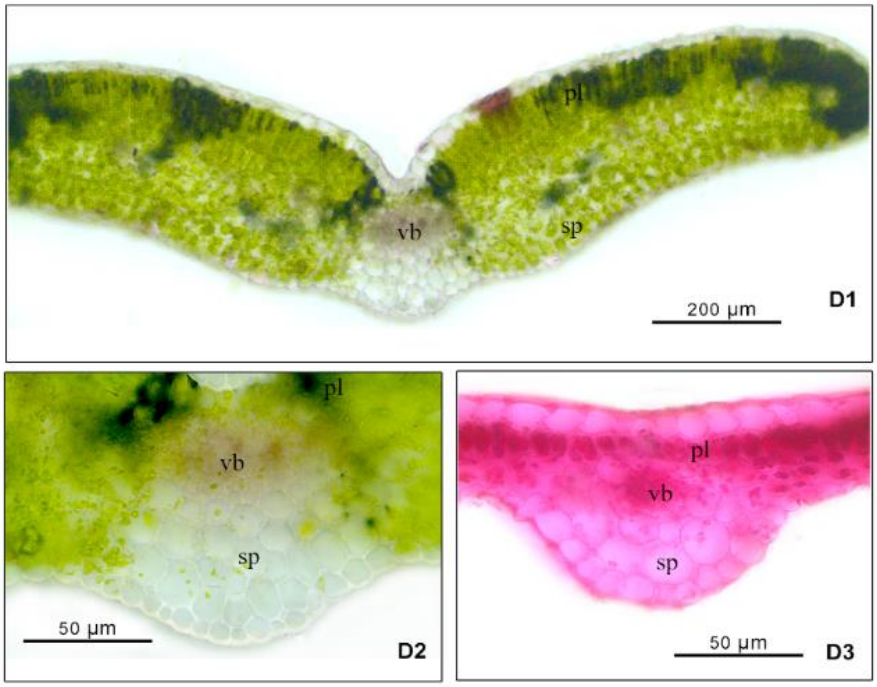

Fig. 6**.** Leaf anatomy of *Rotala* spp. D1 & D2, *R. malabarica;* D3, *R. mexicana.* pl=palisade tissue; sp=spongy tissue; vb=vascular bundle. Scale bar=50, 200 µm.

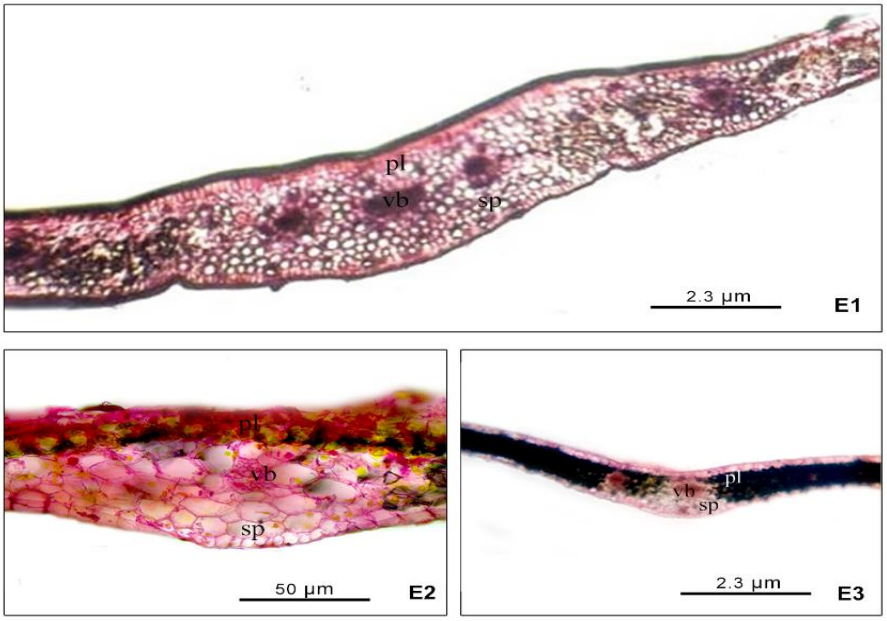

Fig. 7. Leaf anatomy of *Rotala* spp. E1, *R. densiflora*; E2, *R. baileyana*; E3, *R. macrandra*. pl=palisade tissue; sp=spongy tissue; vb=vascular bundle. Scale bar=2.3, 50 µm.

DISCUSSION

Baas & Zweipfenning (1979) reported the taxonomic significance of wood anatomy in 18 genera of Lythraceae. They pointed out that Lythraceae is hypothesized to be derived from a prototype with scanty paratracheal parenchyma, heterogenous uniseriate and multiseriate rays, septate libriform fibers with minutely bordered pits, and vessels with simple perforations. These characteristics still apply to some members of the Lythraceae. The midrib anatomy revealed useful taxonomic information for the recognition of species in the genus *Lagerstroemia* of Lythraceae. Furthermore, Metcalfe and Chalk (1950) reported the presence of sclereid elements of various kinds in the parenchymatous tissue of the leaf and axis of *Lagerstroemia* species.

The relevance of calcium oxalate crystals in the cortical tissues and also in the vascular bundles is an important anatomical feature of the family Lythraceae. Leaves of many members of Lythraceae show dorsiventral leaf lamina (usually), or rarely bifacial, mucilaginous epidermis in aquatic plants, stomata mainly confined to one surface (abaxial), or on both surfaces; anomocytic, eglandular or glandular, adaxial hypodermis present or absent, the mesophyll cells without sclerenchyma containing crystals raphides (Watson & Dallwitz, 1992).

Stem or wood anatomy of Lythraceae also revealed that the young stems are cylindrical or tetragonal in section, secretory cavities are absent, the cork cambium is characteristically present, and nodes may be unilacunar or tri-lacunar. Primary vascular tissues in a cylinder; bicollateral, internal phloem is seemingly nearly always present. The wood ring- porous to diffuse -porous, the vessels are small to medium; solitary, radially paired, and the axial xylem with fiber tracheids; tyloses are also present (Watson & Dallwitz, 1992).

In this study, we found that, in most species, the upper epidermal cells of leaves are larger than those of the lower epidermis; in *R. mexicana*, both the upper and lower epidermis are equal-sized. The leaf lamina is expanded and mucilaginous in *R. tulunadensis* and *R. baileyana*, but in all other species, it is narrow and linear. In the present study, we also noticed that the palisade parenchyma of the mesophyll of *R. malabarica* is much elongated than the other studied species. In all species, trichomes are absent. There are 10-12 vascular bundles in the leaves of *R. densiflora*.

Almost all members of Lythraceae possess Calcium oxalate crystals that are often related to the cortex and endodermis of stems and mesophylls of leaves. Studies emphasized that the epidermal cells and cortical cells in Lythraceae are amazingly complex. The current observation on *Rotala* showed the same results. Selected *Rotala* species also showed different types of epidermis and cortex. In the family Lythraceae, intraxylary phloem is a characteristic anatomical trait (Metcalf & Chalk, 1950). In all such studied taxa, the intraxylary phloem is characteristically present.

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