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Research Article

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Leaf anatomy studies of the genus *Saraca* L. (Fabaceae) in India

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ABSTRACT

Anatomical studies on leaves and petioles were carried out for four species of *Saraca* L. found in India, focusing on microscopic characters based on an anatomical key and a dendrogram (phylogenetic) tree is provided. The distinguishing characters of the leaf revealed the presence of mesophyll length of palisade and spongy parenchyma, size of xylem vessels, type, and number of stomata, presence and absence of crystals and oil glands. In the petiole, the overview shape, number of vascular bundles, types, layers of sclerenchyma, shape of epidermis, *etc*. are discussed. Among the thirty-five microscopic characters, the signified diversity of each species with qualitative and quantitative data of the anatomical character has been investigated through numerical cluster analysis method by to the UPGMA. An organized petiole anatomical character based on the anatomical keys can play a pivotal tool for taxonomic delimitation and comparison between each of the species.

KEYWORDS: Anatomy, Leaf, Petiole, Dendrogram, Key characters

INTRODUCTION

The genus *Saraca* L. comprises 12 species in worldwide, belonging to the family Fabaceae, and is distributed in South China to Tropical Asia respectively (POWO, 2024). In India, 4 species of *Saraca* have been reported, and the species are represented by *Saraca asoca* (Roxb.) W.J. de Wilde; *S. declinata* (Jack) Miq.; *S. indica* L. and *S. thaipingensis* Cantley *ex* Prain, in which *S. asoca* is the only species that grows in the wild as well as planted in gardens and, avenues whereas the remaining three species are seen only in botanical gardens (Sanjappa, 1992; Begum *et al*., 2014). The leaves of *Saraca* are compound; bracts ovate; bracteoles 2, at base of calyx tube; calyx petaloid; stamens 4-8, inserted on a short disc in the throat; ovary stalked and pod flat, coriaceous or woody, dehiscent (Pullaiah & Ramamurthy, 2001).

S. asoca, commonly known as the "Asoka" tree is highly valued for its ornamental qualities, particularly a cluster of bright red or orange flowers which bloom profusely during the spring season (Santapau & Hendry, 1973). The bioactive compounds of *S. asoca* have been isolated and identified in this study. *S. asoca* is native to India, Sri Lanka, and Myanmar and is revered for its cultural, religious, and medicinal uses. Most research work on *S. asoca* mentioned the secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids present in different parts and exhibit medicinal properties and have potential applications in drug development (Satish *et al*., 2014).

S. asoca is known for its medicinal properties in traditional systems of Indian medicine like Ayurveda, Siddha *etc*., where pharmacological studies to evaluate the biological activities of extracts and isolated compounds. This study includes investigations of the anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and anti-cancer activities. Understanding the pharmacological effects helps in validating their traditional uses and exploring new therapeutic applications (Borokar & Pansare, 2017; Maruthappan & Shree, 2010; Verma *et al*., 2010).

Information on the anatomical features of the genus *Saraca* emphasized the importance of the characteristic of sclerenchymatous cells surrounding the vascular bundles (Metcalfe & Chalk, 1950).

The taxonomic identity of the genus *Saraca* largely depends upon the floral characters and a little on the morphological features. There is no comprehensive work on the genus *Saraca* in India that distinguishes different species based on anatomical characters. The present study addresses the anatomy of petioles and leaves of 4 species of *Saraca*.

MATERIALS AND METHODOLOGY

In the present study, fresh specimens of petioles and leaves were collected from the botanical gardens of Bangalore and

Thiruvananthapuram (Table 1) and were made into herbaria and deposited in the herbarium of FRLH, National Medicinal Plants and Repository of Raw Drug, TDU, Bangalore, Karnataka.

Fresh samples of petioles and leaves were cut into small pieces and freehand sectioning was carried out. Detailed identification characters were noted with or without staining procedures (Johansen, 1940; Krishnamurthy, 1988). Stomatal number and index of the leaves were studied. All the photomicrographs were taken using an Olympus CX 33 light microscope.

During this study, anatomical characters such as petioles, leaves, midrib, epidermis, vascular bundles pattern, occurrence of cortical and fibers, stomata types, crystals, and trichomes in addition to the shape, size arrangements of tissues, are the main characters to distinguish the species as stated by Metcalfe and Chalk (1950).

To organize the species that are similar/and dissimilar to each other, clustering analysis of Mesquite version 3.18 was used. The distance from the character matrix of the 4 species with the investigations of 35 anatomical characters, are subjected to the clustering method UPGMA with the average similarity of distance. All the distinguished characters are converted into binary characters (0,1) in order to allow the use of cluster analysis techniques.

This is followed by detailed anatomical character-based key depending on the petiole anatomical characters investigated.

RESULT

Petiole

Transverse section of the petioles overall outline shows angular and rounded to oval shapes (Figure 1a, d, g & j). Epidermis is rectangular and tooth-like, a 1-layered cell with very thick and thin cuticle with unicellular trichrome. Cortical cells are 13-23 layers, closely arranged parenchymatous cells with brownish tannin content. The vascular system is either solenostele or ectophloic siphonostele with outer phloem cells. Fibers are 2-5 layers, thick-walled cells with very narrow lumen walls. Calcium oxalate crystals abundantly occur in the cortical region and phloem cells. Vascular bundle overview of the *S. ascoa* and *S. thaipingensis* in oval-shaped, C-shaped in *S. declinata* and, egg-shaped in *S. indica*. Xylem vessel shapes are hexagonal in *S. asoca* and *S. indica*; octagonal in *S. declinata* and *S. thaipingensis*. Prismatic calcium oxalate crystals in *S. declinata*, *S. indica*, *S. thaipingensis*, and solitary in *S. asoca*. More details of the microscopic characters are in Table 2.

Leaves Midrib

Transverse section of midrib overview shows slightly convex to convex in *S. declinata*, convex in the remaining three species (Figure 1b, e, h & k). Epidermis of adaxial and abaxial surface 1-layered, tooth-like, square, and rectangular shapes. Cortical fibers are oval-shape in *S. asoca* and *S. thaipingensis*; rounded in *S. declinata*; hexagonal in *S. indica*, and 3-7 layered with very thick-wall cells are in these four species. Continuous and patches in 3-7 layers, thick-walled cells of sclerenchyma cells covered with vascular bundles. Vascular bundle overview of the *S. asoca* and *S. declinata* in cat-face shape, *S. indica* in V-shape and *S. thaipingensis* in D-shape. Solitary calcium oxalate crystals in *S. declinata* and *S. indica*; square in *S. asoca* and prismatic in *S. thaipingensis*. Tannin content is less in *S. indica* and abundant in the remaining three species. More details of the microscopic characters are in Table 2.

Leaves Lamina

Transverse section of leaf-blade shows dorsi-ventral in all four species (Figure 1c, f, i & l). Epidermis cells of the adaxial and abaxial surfaces are 1-layer with square, rectangular, and tooth-like cells with a thin cuticle. Mesophyll cells are differentiated into palisade and spongy parenchyma cells. Palisade, cells are elongated and rectangular in shape, 2 rows except in *S. indica*. Spongy parenchyma cells are 4-layered in *S. declinata*, 5-layered in *S. asoca* and *S. thaipingensis*, and 7-layered in *S. indica*, with loosely arranged cells. Spongy parenchyma oval shape in *S. declinata* and *S. indica*; rounded in *S. asoca*; square in *S. thaipingensis*. Calcium oxalate crystals are present in all 3 species except *S. thaipingensis*, and oil glands absent in all 3 species except *S. asoca*. More details of the microscopic characters are in Table 2.

Stomata Type and Index

Anisocytic and paracytic stomata are present on the abaxial surface of the leaves. *S. asoca*, *S. declinate*, and *S. indica* have 2 types of stomata, and *S. thaipingensis* has anisocytic stomata only. Stomata index was calculated on leaf surfaces and is more on the abaxial surface epidermis of all 4 species and is totally absent on the adaxial surface. The stomatal number is less (20) in *S. asoca* and higher in *S. thaipingensis* (65), and the stomata index is least (7.3) in *S. asoca* and highest in *S. thaipingensis* (34.2).

Dendrogram

Table 2 shows a detailed investigation of 35 distinct characters of petioles, leaf midribs, and lamina of 4 species of *Saraca*.

Table 1: The data of samples collections

S. No.	FRLH Coll. No.	Species	Locality	Date of collection	Collected by
1	124318	*S. asoca*	Ethno-Medicinal Garden, FRLHT-TDU, Bangalore, Karnataka	20/11/2022	R. Patturaj & S. Noorunnisa Begum
2	122947	*S. declinata*	Lalbagh Botanical Garden, Bangalore, Karnataka	15/10/2019	K. Ravikumar & R. Patturaj
3	124319	*S. indica*	JCBTBGRI Botanical Garden, Thiruvananthapuram, Kerala	25/11/2022	K. Ravikumar & J. Stephen
4	123149	*S. thaipingensis*	Lalbagh Botanical Garden, Bangalore, Karnataka	15/10/2019	K. Ravikumar & R. Patturaj

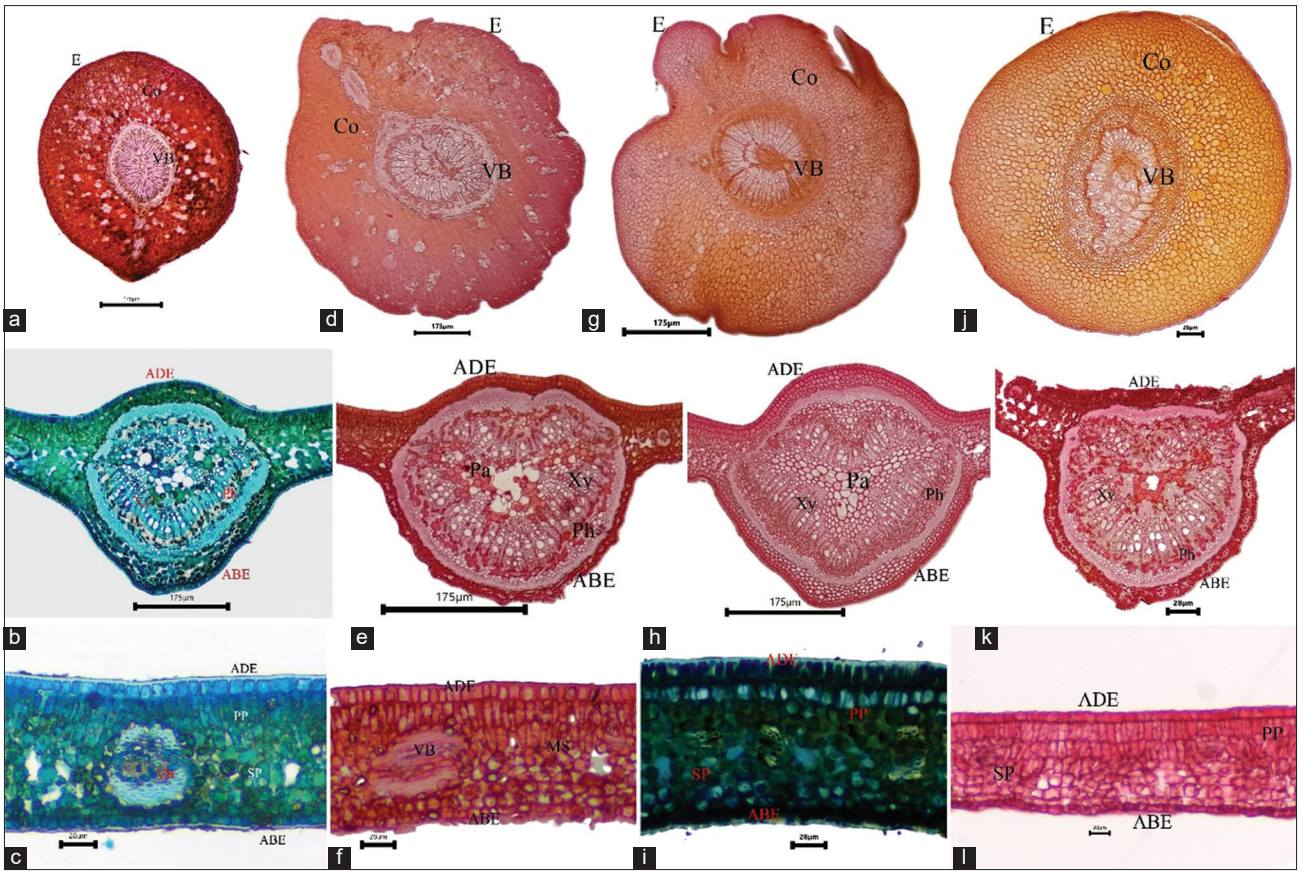

Figure 1: TS of petiole, leaf midrib and lamina of *Saraca*. a-c: *S. asoca*, d-f: *S. declinata*, g-i: *S. indica*, j-l: *S. thaipingensis*. (ABE-Abaxial Epidermis, ADE-Adaxial Epidermis, Co-Cortex, E-Epidermis, MS-Mesophyll, Pa-Parenchyma, Ph-Phloem, PP-Palisade Parenchyma, SP-Spongy Parenchyma, VB-Vascular Bundle, Xy-Xylem)

These characters are unique and are used to build the following cluster analysis of distances from the characters matrix methods of Unweighted Pair Group Method with Arithmetic mean (UPGMA), based on distinct differentiated anatomical characters. Cluster analysis aims to group a collection of data in such a way that anatomical characters in the same group are more similar to one another than the characters in other species. The hierarchical structure of UPGMA with the average similarity distances from the character matrix of these species on the dendrogram image (Figure 2). *S. indica* and *S. declinata* are the 2 species in same clade based on the anatomical characters, whereas *S. asoca* and *S. thaipingensis* are very distinct species based on the characters.

Detailed petiole anatomical characters based on an artificial key would help in species identification.

Petiole: Key based on anatomical characters

Overview of petiole angular shaped..... 1
1 a. Petiole vascular bundle egg-shaped..... ***S. indica***
1 b. Petiole vascular bundle C-shaped..... ***S. declinata***
Overview of petiole oval-rounded shaped..... 2
2 a. Trichomes present..... ***S. thaipingensis***
2 b. Trichomes absent. ***S. asoca***

DISCUSSION

Most of the research works on various plant taxa used the leaf anatomical characters for the taxonomic identification. The leaves are the most diverse organ of angiosperms, and their anatomical variation frequently correlates closely with generic and common, often phylogenetic variations related issues (Carlquist, 1961). Leaves have the most variations both in morphology and anatomy (Fahn, 1990). The conducting tissues in the petiole and main -nerves of the leaves are usually similar to the stem. The anatomical characters of leaves and petioles, along with other characters, have been considered to play a pivotal role in differentiating important species as an additional character because they are of primary taxonomic significance in plant identification and classification (Waly *et al.*, 2020).

To identify 4 species of *Saraca*, distinct morphological characters like leaf shape, margin, inflorescence, flower color, pods, seeds etc. were sufficient (Hooker, 1984). However, in the present study, apart from morphological characters the anatomical features in the leaves, and petioles such as the presence of epidermis, cortical cells with fibres, vascular elements, trichomes

Table 2: Anatomical characters of petioles and leaves of 4 species of *Saraca*

Parts	Characters	*S. asoca*	*S. declinata*	*S. indica*	*S. thaipingensis*
Petiole	TS Shape: round (0), oval (1), angular (2)	0	1	1	2
	Epidermis shape: rectangular (0), teeth like (1)	0	1	0	0
	Cortex: number of layers in parenchyma: > 20 (0), < 20 (1)	0	0	1	0
	Trichomes: present (0), absent (1)	1	1	1	0
	VB shape: oval (0), C shaped (1), egg shaped (2)	0	1	2	0
	Number of layers in sclerenchyma: > 4 (0), < 4 (1)	0	0	0	1
	Number of xylem vessels rows: > 30 (0), > 30 (1)	1	1	0	0
	Number of xylem rays: biseriate (0), uniseriate (1)	0	1	1	1
	Xylem vessels shape: hexagonal (0), octagonal (1)	0	1	0	1
	Cortical fibers: present (0), absent (1)	1	1	0	1
	Tannins content: less (0), abundant (1)	1	1	0	0
	Crystals type: solitary (0), prismatic (1)	0	1	1	1
Midrib	TS Midrib Shape: convex (0), slightly convex (1)	1	0	1	1
	Adaxial epidermis shape: rectangular (0), teeth like (1), square (2)	1	0	2	2
	Abaxial epidermis shape: rectangular (0), square (1)	1	1	0	1
	VB shape: cat face (0), V-shaped (1), D-shaped (2)	0	0	1	2
	Number of VB in midrib: > 4 (0), < 4 (1)	0	1	1	0
	Cortical fibers: oval (0), round (1), gonal (2)	0	1	2	0
	Cortical parenchyma shape: oval (0), round (1)	1	1	0	0
	Tannin content: less (0), abundant (1)	1	1	0	0
	Number of layers in sclerenchyma: > 4 (0), < 4 (1)	1	0	1	0
	Sclerenchyma cells arrangement: continuous (0), patches (1)	0	0	1	0
	Crystals type: solitary (0), prismatic (1), square (2)	2	0	0	1
Lamina	Adaxial epidermis shape: rectangular (0), teeth like (1), square (2)	2	0	1	2
	Abaxial epidermis shape: rectangular (0), square (1)	1	1	1	0
	Number of layers in palisade: > 2 (0), < 2 (1)	1	1	0	1
	Number of layers in spongy: > 5 (0), < 5 (1)	1	0	1	0
	Spongy parenchyma shape: oval (0), rounded (1), square (2)	1	0	0	2
	Length of palisade in mesophyll (Average): > 60 μm (0), < 60 μm (1)	1	1	1	0
	Length of spongy in mesophyll (Average): > 100 μm (0), < 100 μm (1)	1	0	1	0
	Stomata types: both (0), paracytic (1), anisocytic (2)	0	1	1	2
	Stomata number: > 30 (0), < 30 (1)	0	1	1	1
	Stomata index: > 30 (0), < 30 (1)	0	1	1	0
	Crystals type: solitary (0), prismatic (1), square (2)	2	1	1	0
	Oil glands: present (0), absent (1)	0	1	1	1

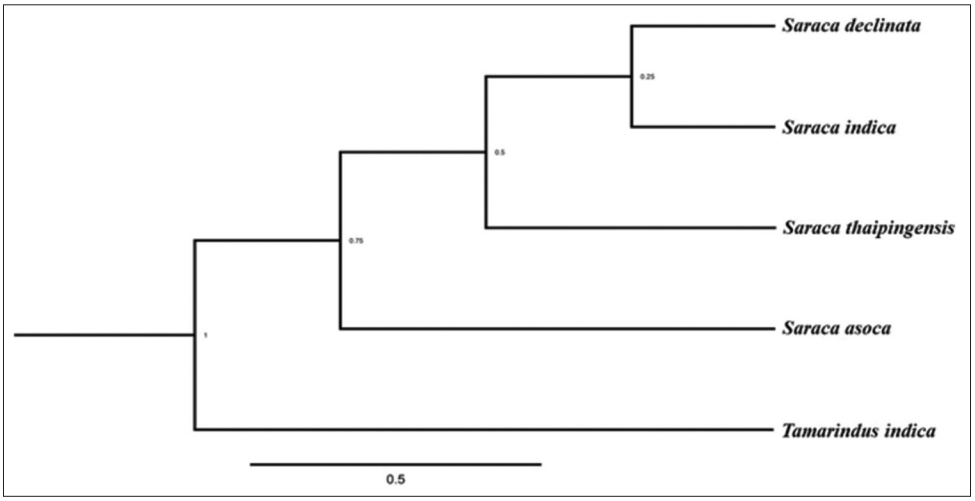

Figure 2: The dendrogram of the four *Saraca* species based on the anatomical characters

in the petiole, and stomata types are also important features to identify different species of *Saraca*.

Pereira *et al.* (2018) distinguished 3 species of *Bauhinia* based on the leaf anatomy characters, such as epidermis cells with straight, polygonal, anticlinal shape of each species. During this study, *S. thaipingensis* is differentiated from the rest of the species in the presence of square-shaped epidermis on both surfaces with a thin cuticle.

Apart from these, the dorsi-ventral shape of mesophyll cells is observed in all the species. The leaf anatomy also showed that distinctive spongy parenchymatous cells were seen among the 4 species under study as in the case of Ceasealpiniaceae

(Metcalfe & Chalk, 1950). The present study showed paracytic and anisocytic stomata in the abaxial surface of the 4 species of *Saraca*.

The petiole anatomical characters showed the presence of two angular-shaped out-lines in *S. indica*, one angular-shaped out-line in *S. declinata*, whereas in *S. asoca* and *S. thaipingensis*, it is absent. The anatomical characters are similar based on the dendrogram (phylogentic tree) and anatomical key characters.

CONCLUSION

The anatomy characteristics of the petiole epidermis showed a rectangular and tooth-like shape. More so, the leaves' midrib fibres are oval, rounded, and hexagonal shaped with thick-walled cells. The present study is significant in the broad differentiation of the 4 species of *Saraca*. This type of microscopic study will help to establish identity, purity, quality and safety of the species authentication of there for various conservation, biodiversity, and various industries there to species identification.

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