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Petiole anatomical characteristics of true mangrove species in Kerala

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Abstract
Petiole anatomy of 15 mangrove species in Kerala was investigated to identify and determine its systematic significance that may be useful in species identification and classification. Variations in petiole anatomical attributes were obvious that they could be used as systematic evidence to taxonomically delineate these taxa even at species level. The use of petiolar anatomical features in systematic description of mangrove species is maiden and innovative and reported for the first time. Findings in this study have shown some common characters and variations in the petiole anatomical characteristics. Petiole anatomical characteristics observed included petiole outlines, patterns of petiole vascular bundles, presence of sclerenchyma cells, presence of phloem sclerenchyma cells, presence of tanniferous and idioblast cells, presence and types of crystals, presence of lignified parenchyma cells, and presence and types of trichomes. Results showed that petiole anatomical characteristics can be used as additional data in identification and classification of species in the genus.

Keywords: Petiole, anatomy, mangrove, salt gland, trichome

Introduction
Mangrove form a rather uniform, evergreen fringe of forest which is most profusely developed on low muddy shores, in estuaries and lagoons and is less abundant along sandy or rocky beaches and on old coral reefs covered with a thin sheet of sand or mud. Plant anatomy, which is a study of microscopic characteristics of all plant organs using light, scanning and transmission electron microscopy techniques not only provide data which facilitate problem solving in plant taxonomy, but would also enhance the understanding of complex correlations between plant structural anatomy, physiology and growth functions [1, 2, 3].
Angiosperms are endowed with external morphological characters of significant taxonomic value which can be easily observed with the naked eye or with simple hand lens. Morphological attributes of vegetative organs have often constituted the mainstay of taxonomic studies in plants [4, 5, 6] and are very important in classification.
The use of anatomical methods in taxonomic investigations cannot be over emphasized. Although no character is absolutely immutable, some are more fixed than the others and it is on those that are less plastic that the systematic anatomist rely because they are not really affected by environmental conditions [7] comparative plant epidermal studies have been found to be reliable in taxonomy [8, 9, 10, 11] and systematics have all stressed the taxonomic importance of anatomical features which along with other characters are useful for identification and classification of plants [12, 13, 14].
The present study reports on the use of petiole anatomy in establishing the taxonomic relationships among 15 species of mangroves in kerala.

Materials and methods
Transverse sections of the petiole of *Acanthus ebracteatus* Vahl., *Acanthuus ilicifolius* L., *(Acanthaceae), Aegiceras corniculata* (L.) Blanco., (Myrsinaceae), *Avicennia marina* (Forssk.) Vierh., *Avicennia officinalis* L., (Avicenniaceae), *Bruguiera cylindrica* (L.) Blume., *Bruguiera gymnorrhiza* (L.) Lam., *Bruguiera sexangula* (Lour.) Poir., *Kandelia candel* (L.) Druce., (Combretaceae) *Rhizophora apiculata* Blume., *Rhizophora mucronata* Lam., (Rhizophoraceae), *Excoecaria agallocha* L., (Euphorbiaceae), *Sonneratia alba* Sm., *Sonneratia caseolaris* (L.) Engl. (Sonneratiaceae) were taken and stained with Toluidine blue 0.Microscopic observation by trilocular Labomed compound microscope model No. 100934409 and images were photographed Olympus digital camera.

Result and Discussion
In *S. alba* adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate, very small cells and highly cutinized. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma. The outer layer of ground tissue showed the presence of phenolic depositions. Numerous vascular bundles were present, amphicribal, arranged in the form of arc and it consists of crystalliferous cells. The pith consists of thin walled parenchyma, idioblast with phenolic depositions. The metaxylem and protoxylem vessls were arranged radially. The starchy phloem is seen around the xylem. Distinct lacuna present in pith. (Plate A)
In *S. caseolaris* adaxial outline were concave surface and convex in abaxial side. The epidermis were uniseriate, very small cells and highly cutinized. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma. The outer layer of ground tissue showed the presence of phenolic depositions. Numerous vascular bundles were present, amphicribal, arranged in the form of arc. The pith consists of thin walled parenchyma, idioblast, and crystalliferous cells with phenolic depositions. The

metaxylem and protoxylem vessls were arranged radially. The starchy phloem is seen around the xylem. Distinct lacuna present in pith. (Plate B)

In *B. cylindrica* adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate, very small cells and highly cutinized. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma with numerous lacuna. Numerous vascular bundles were present, amphicribal, crescent shaped and it consists of crystalliferous cells. The pith consists of thin walled parenchyma, with phenolic depositions. The metaxylem and protoxylem vessls were arranged radially. The starchy phloem is seen around the xylem. (Plate C)

In *B.gymnorrhiza* arc shaped in outline. Adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate with phenolic depositions, very small cells and highly cutinized. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma with numerous lacuna. The outer layer of ground tissue showed the presence of phenolic depositions. Numerous vascular bundles were present, amphicribal, crescent shaped and it consists of crystalliferous cells. The pith consists of thin walled parenchyma, with phenolic depositions. The metaxylem and protoxylem vessls were arranged radially. The starchy phloem is seen around the xylem. (Plate D)

In *B. sexangula*, adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate with phenolic depositions, very small cells and highly cutinized. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma with numerous lacuna. The outer layer of ground tissue showed the presence of phenolic depositions. Numerous vascular bundles were present, amphicribal, crescent shaped and it consists of crystalliferous cells. Centre portion of vasculature possess groove because of curving nature. The pith consists of thin walled parenchyma, with phenolic depositions. The metaxylem and protoxylem vessels were arranged radially. (Plate E)

In *K. candel* adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate with phenolic depositions, very small cells and highly cutinized. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma with numerous lacuna. The outer layer of ground tissue showed the presence of phenolic depositions. Numerous vascular bundles were present, amphicribal, vasculature arc shaped with one end very much incurved and it consists of crystalliferous cells. The pith consists of thin walled parenchyma, with phenolic depositions. The metaxylem and protoxylem vessls were arranged radially. (Plate F)

In *R. apiculata* adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate with phenolic depositions, very small cells and highly cutinized. Cork warts present in adaxial and sclereids present in abaxial side. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma with numerous lacuna. The outer layer of ground tissue showed the presence of phenolic depositions. Numerous vascular bundles inward in nature amphicribal, and it consists of crystalliferous cells. The pith consists of thin walled parenchyma, with phenolic depositions. The metaxylem and protoxylem vessls were arranged radially. (Plate G)

In *R. mucronata* adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate with phenolic depositions, very small cells and highly cutinized. Cork warts present in adaxial and sclereids present in abaxial side. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma with numerous lacuna. The outer layer of ground tissue showed the presence of phenolic depositions. Numerous vascular bundles inward in nature amphicribal, and it consists of crystalliferous cells. The pith consists of thin walled parenchyma, with phenolic depositions. The metaxylem and protoxylem vessls were arranged radially. (Plate H)

A study on petiole anatomy of the three species of Rhizophoraceae in Nigeria to find out stable characters for delimiting the species. Study showed three taxa show generic closeness. The species were discriminate based on the number of the vascular bundles of the petiole are distinct for each species [15].

A study on petiole anatomical characteristics of seven members selected Rhizophoraceae in Malaysia revealed anatomical characters can be very useful in to prove the relationship between plant species and its extreme climate and exposed environment such as presence of aerenchyma cells, cutinized epidermal cells and variations in the presence of parenchyma cells which occurred fairly uniformly among all the species studied [16].

In *E. agallocha* adaxial outline were concave surface and convex in abaxial side. The epidermis was uniseriate, very small cells and cutinized. The hypodermis and cortex were not clearly differentiated. The cells were round, thin walled parenchyma. Numerous vascular bundles were present, amphicribal, arranged in the form of arc and it consists of crystalliferous cells. The metaxylem and protoxylem vessls were arranged radially. The starchy phloem is seen around the xylem. (Plate J)

In *A. corniculata* arc shaped in outline. The epidermis was uniseriate with phenolic depositions, very small cells and highly cutinized. The hypodermis and cortex were not clearly differentiated. Ground tissue consists of tannins, brachy sclereids. Pericycle consists of isolated arches of sclerenchymatous fibers. Numerous vascular bundles inward in nature, amphicribal, and crescent shaped and it consists of crystalliferous cells. The pith consists of thin walled parenchyma, with phenolic depositions. The metaxylem and protoxylem vessls were arranged radially. (Plate I)

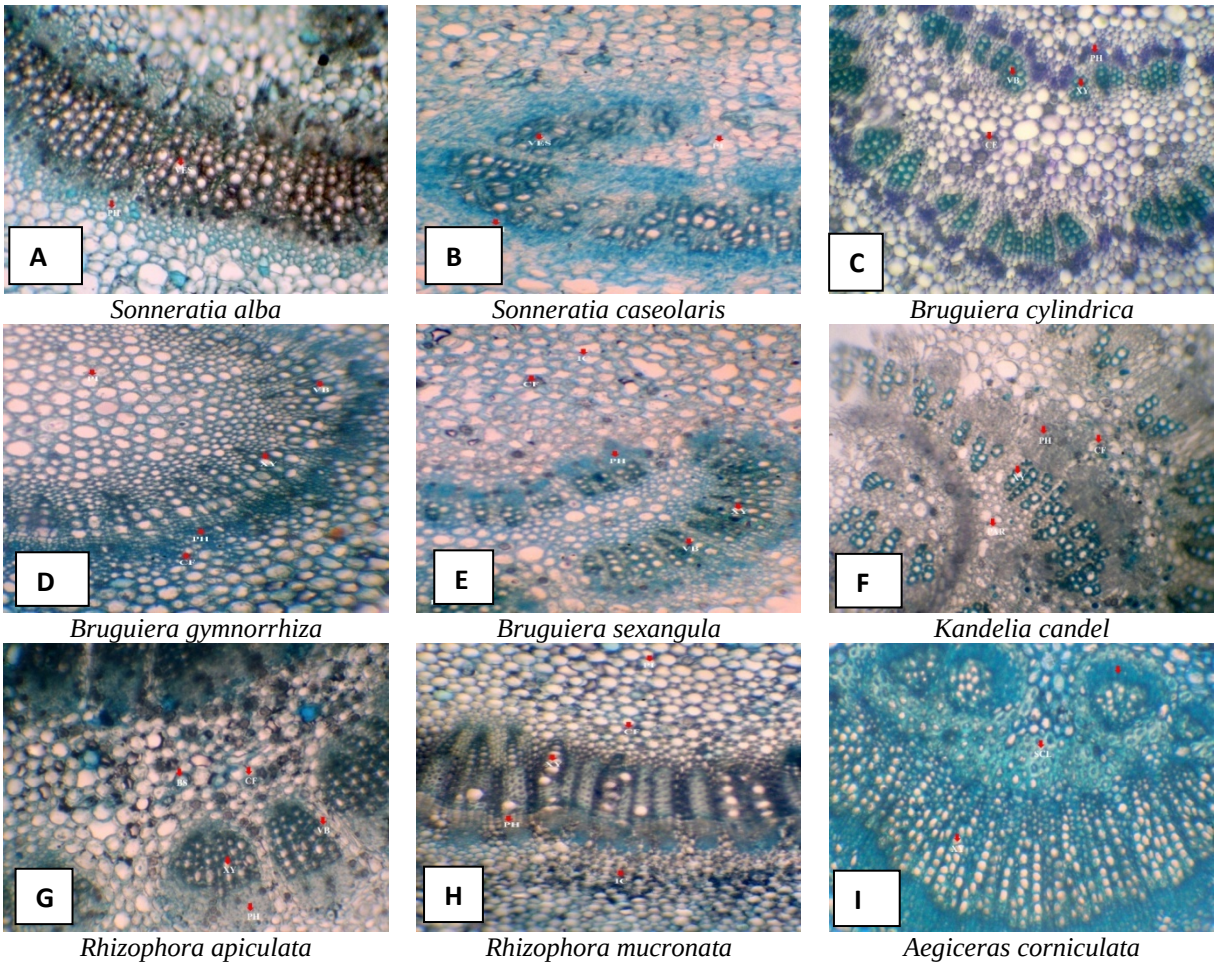
In *A. ebracteatus* slightly concave at the adaxial side and convex at the abaxial side, epidermis uniseriate. Circular arrangement of vascular bundles, amphicribal, arranged in the form of an arc, endodermal and pericylic differentiation not prominent. Lateral bundle observed in a clear circular fashion, it clearly delimited with endodermis and pericyclic and cells showing brachy sclereids in the periphery. Central portion occupy with more xylem elements and were slightly modulated. Peripheral phloem showing presence of phenolic depositions. Glandular trichomes and simple, uniseriate multicellular hairs present. Pith consists of thin walled parenchyma cells. (Plate K)

In *A. ilicifolius*, slightly concave at the adaxial side and convex at the abaxial side, epidermis uniseriate, cortex composed of 7-8 layers of polygonal angular collenchymas and chlorophyllated thin walled parenchyma cells. Circular arrangement of vascular bundles, amphicribal, arranged in the

form of an arc. endodermal and pericylic differnation not prominent. Lateral bundle observed in a clear circular fashion, it clearly delimited with endodermis and pericyclic and cells showing brachy sclereids in the periphery. Central portion occupy with more xylem elements and were slightly modulated. Peripheral phloem showing presense of phenolic depositions. Glandular trichomes and simple, uniseriate multicellular hairs present. Pith consists of thin walled parenchyma cells. (Plate L)

In *A. marina* outline was wavy with thick cuticle intermittned by salt glands. Pinnate like expansion contains chlorenchyma and separate bundles present in adaxial side. Non -glandular trichomes and extra floral nectarines present in abaxial side. Trichomes were consists of small stalk cell and large terminal cell. Outer phloem with sclereids. Vascular bundles were medulatted. Pith were consists of thin walled parenchyma and large sclereids delimited with endodermis. (Plate M)

In *A. officinalis* outline was wavy with thick cuticle intermittned by salt glands. Non -glandular trichomes and extra floral nectarines present in abaxial side. Trichomes were consists of small stalk cell and large terminal cell. Outer phloem with sclereids. Pith were consists of thin walled parenchyma and large sclereids delimited with endodermis. (Plate N)

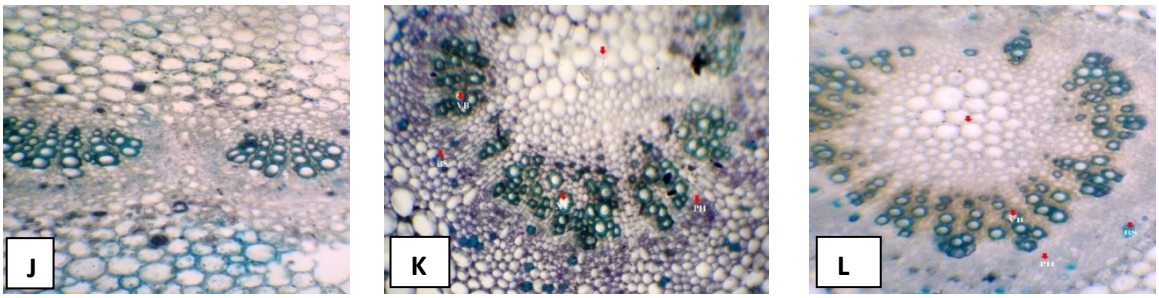


Sonneratia alba *Sonneratia caseolaris* *Bruguiera cylindrica*

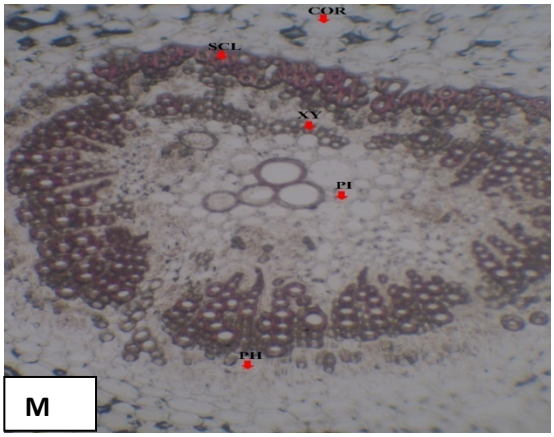
Bruguiera gymnorrhiza *Bruguiera sexangula* *Kandelia candel*

Rhizophora apiculata *Rhizophora mucronata* *Aegiceras corniculata*

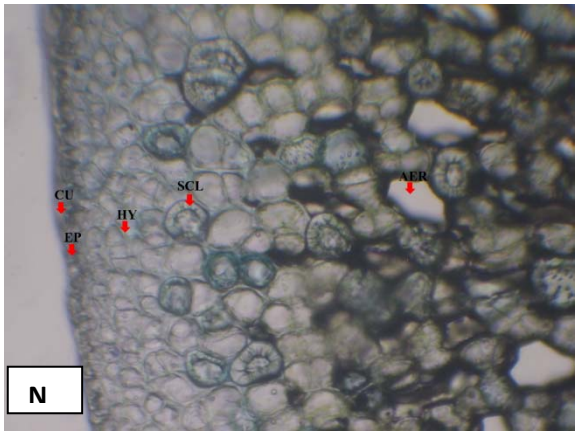
Plate A - *Sonneratia alba*, (10X) Plate B - *Sonneratia caseolaris*,(10X) Plate C- *Bruguiera cylindrica*,(10X) Plate D - *Bruguiera gymnorrhiza* (10X) Plate E- *Bruguiera sexangula* (10X) Plate F - *Kandelia candel*(10X) Plate G- *Rhizophora apiculata* (10X) Plate H- *Rhizophora mucronata* (10X) Plate I- *Aegiceras corniculata* (10X) CF-Crystalliferous cell, PAR-Parenchyma, PH-Phloem, BS- Brachy sclereids, XY-Xylem, CU-Cuticle, AS- Air space, EP-Epidermis, HP- Hypodermis, PAR-Parenchyma, BS- Brachy sclereids, SCL-Sclrenchyma, COR-Cortex,PI-Pith



Excoecaria agallocha *Acanthus ebracteatus* *Acanthus ilicifolius*



Avicennia marina



Avicennia officinalis

Plate J- *Excoecaria agallocha,* (10X) Plate K- *Acanthus ebracteatus,* (10X) Plate L- *Acanthus ilicifolius,* (10X) Plate M- *Avicennia marina,* (10X) Plate N- *Avicennia officinalis* (10X).

Conclusion

Leaf petiolar features that disclosed somewhat close relationship among mangrove taxa. Members of rhizophoraceae cortex region consists of numerous aerenchyma cells, cutinized epidermal cells and variations in the presence of parenchyma cells showed that some anatomical modification had appear in reaction to adverse climate and bare environment. In Acanthaceae both the taxa possess uniseriate unbranched trichomes. *A. marina* and *A. officinalis* have outgrowth of glandular and non-glandular trichomes; presence of extra floral nectarines were the main features.

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