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Leaf and petiole anatomy of some members of Rhizophoracae (Mangroves) in Kerala

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Abstract
Leaf and petiole anatomy of *Rhizophora apiculata* Blume., *Rhizophora mucronata* Lam., *Kandelia candal* (L) Druce., were studied. All the species showed unique anatomical features adapted to salty mangrove habitat. Three species showed xerophytic characteristics like presence of thick cuticle, branched sclereids, crystalliferous cells, colourless non- assimilatory water storage tissue in hypodermis, tannin cells and terminal trachieds. Petiole anatomy helps species level identification.

Keywords: mangrove, sclereids, petiole, xerophytes, cuticle

Introduction
Mangroves in India account for about 5 per cent of the world's mangrove vegetation and are spread over an area of about 4,500 km 2along the coastal States/UTs of the country. Various studies showed that mangrove vegetation cover only 1,095 ha in Kerala. [1]According to another estimate, Kerala once supported about 700 km^2 mangroves and what we see now are only relics of a great past [2]. It can be noted that out of the total 1671 ha of mangroves in Kerala, 1470 ha are with private holders. [3]
Rhizophoraceae is a trophical and sub-trophical plant group. This family comprise of 16 genus with 120 species consists of either tress or shurbs, are distributed in the old world, as in East Africa, India, South East Asia and Australia. [4]
The genus, *Rhizophora* L. belongs to the family Rhizophoraceae. Members of the genus are referred to as red Mangrove and are the most abundant and important among the mangrove ecosystem. [5]The genus, *Rhizophora* consists of seven species worldwide. In Kerala, two species have been identified, namely, *Rhizophora apiculata* Blume., *Rhizophora mucronata* Lam.

Materials and Methods
Leaf samples were collected from the intertidal zones of Ayiramthengu (9^0 7' N: 76^0 29' E.) Kollam district in Kerala state (Figure 4) and identified by Botanical survey of India (BSI), Coimbatore. One of the healthy plants was selected and the mature leaves from fifth and sixth node were taken for anatomical studies. Sections were made at a position approximately half way between the base and apex of a sector from one side of the lamina, stained with Toluidine blue 0 and mounted in 50% glycerin. The slides analyzed by trilocular compound microscope (10093409) and imaged by using the camera Olympus E-PL3. Measurements were taken using Magnus Pro software. The Scanning Electron Microscopic images of leaf sample were takenusing Zeiss ultra 55. For the SEM studies, abaxial side of leaf was sputter-coated with gold and micrographs were taken using Zeiss ultra 55.

Result
***Rhizophora apiculata* Blume**.
Morphology of leaf is simple, leathery, lamina elliptic - oblong glabrous and coriaceous, base cuneate, apex acute, margin entire. Dark green above and pale green below, black dots present in abaxial side. Midrib prominent. Fig-1, (Plate-A&B)
Anatomy of leaf showed the lamina of the plant is studied as dorsiventral in nature. Cuticle was considerably thick and smooth in these taxa. Epidermis cutinized wholly. The outer wall of the epidermal cells is thicker than the rest of the walls and the wall is usually straight and shape of the cells is mostly rectangular. Adaxial epidermal cells are often larger than those of the abaxial cells. Hypodermis is composed of seven to eight layers of colourless, polygonal shaped cells below the epidermal cells. Colourless cells function as water storage tissue. Mangrove mesophyll is composed of thin walled chlorenchyma. These cells can be differentiated in to one or more layers of adaxial, anticlinal extended palisade cells and oval or round shaped compact or loose abaxial isodiametric cells. The spongy parenchyma cells were loosely arranged and contain large air spaces. Branched sclereids scatterly arranged in the abaxial region. Leaf hypostomatic in nature. It showed four or more subsidiary cells in close rings around the stomata; so it was cyclocytic type. Crystalliferous cells present in the cell. Fig-1 (Plate-D, E & F, Table 1)
Micro anatomy consists of outer surface showing smooth appearance. Stomata deeply sunken and cyclocytic type, unbranched hairs present. Epidermal cells polygonal in nature. Salt secreting glands found in abaxial side.Fig-1 (Plate-I)
Petiole anatomy showed arc shaped in outline. The cuticle is thick. The adaxial outline is concave; abaxial surface convex. Epidermis and hypoderm were uniseriate. Epidermal cells polygonal. Cortex is wide and made of outer collenchyma, 7-8 layers and inner parenchyma cells. Inner portion consists of numerous air cavities. Vascular bundles collateral arranged in ring 20-22. Fig-1 (Plate-G&H)

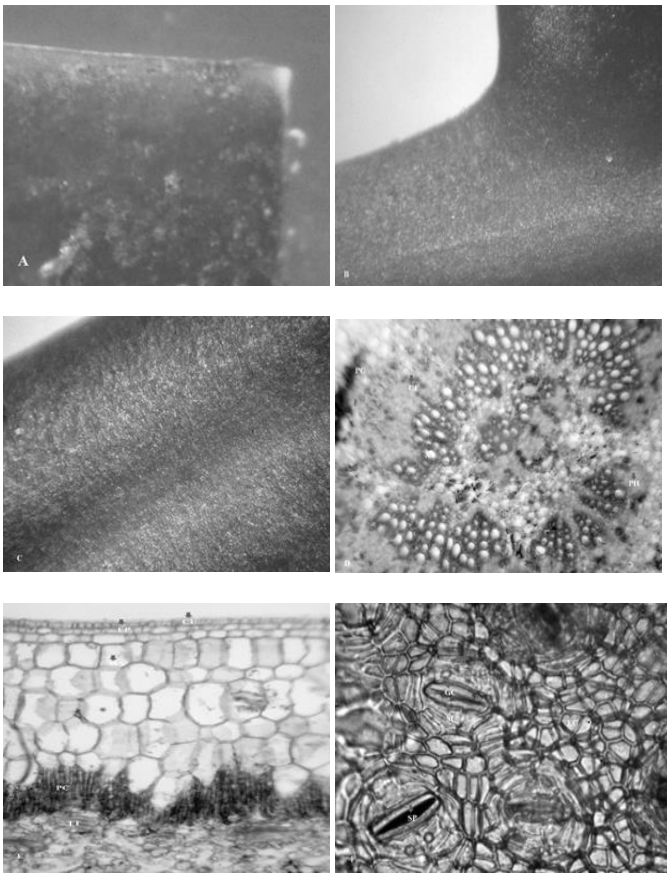
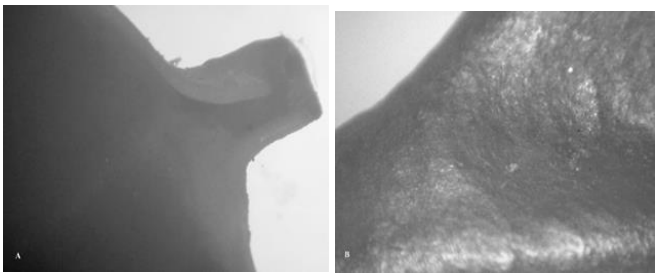


Fig 1: R.apiculata. A- Stereo microscopy of leaf apex(10X), B- Stereo microscopy of leaf base(10X), C- Stereomicroscopy of petiole (10X), D&E- Midrib and lamina portion of leaf(10X), F-Abaxial surface with cyclocytic stomata(100X).

***Rhizophora mucronata* Lam.**

Morphology of leaf has simple, leathery, lamina elliptic - oblong glabrous and coriaceous, base cuneate, apex acute, margin entire, black dots present in the lower region. Midrib prominent. Fig-2. (Plate-A&B)



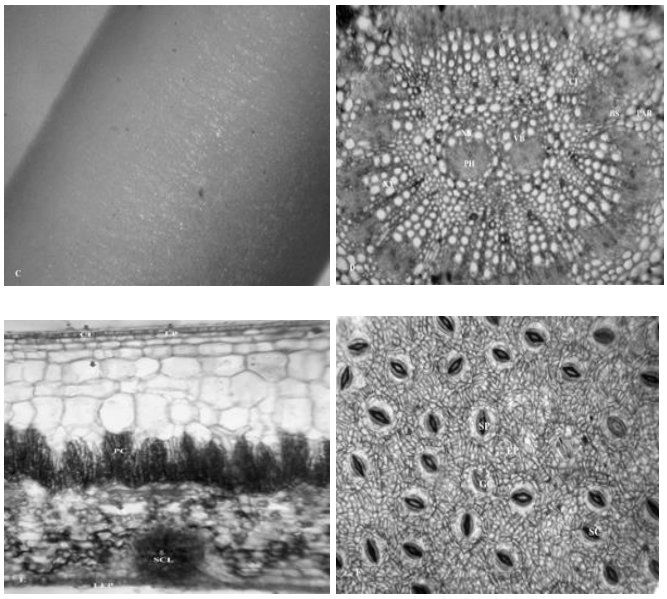


Fig 2: *R.mucronata*. A- Stereo microscopy of leaf apex(10X), B- Stereo microscopy of leaf base(10X), C- Stereomicroscopy of petiole(10X), D&E- Midrib and lamina portion of leaf (10X), F- Abaxial surface with cyclocytic stomata(10X).

Anatomy of leaf showed dorsi-ventral. Lamina with black circular cork warts in the lower surface. Cuticle was thick and waxy. Epidermis one layered in the upper epidermis and lower epidermis. Hypodermis multilayered. Stomata were sunken and present in the lower epidermis. It showed four or more subsidiary cells in close rings around the stomata; so it was cyclocytic type. Water storing tissues were multi seriate. Mesophyll with one-layered with anticlinally extended palisade cells. Lower spongy tissue consists of oval or round shaped compact or loose abaxial isodiametric cells with large intercellur spaces. Short tracheids in the vein endings was an adaptation to climate and habitat; sclereids were present in the mesophyll is the distinguishing character of this genus compared to other genus in the Rhizophoraceae family. The function of sclereid cell has to been reported to be related with the environment and mechanical protection, but the real function of the cell specifically unknown. Parenchymatous bundle sheaths present its main vascular bundle. The thickness of this tissue causes leaf succulence with increased water content. Crystalliferous cells present in the cell. Fig-2. (Plate-D,E & F, Table 1)

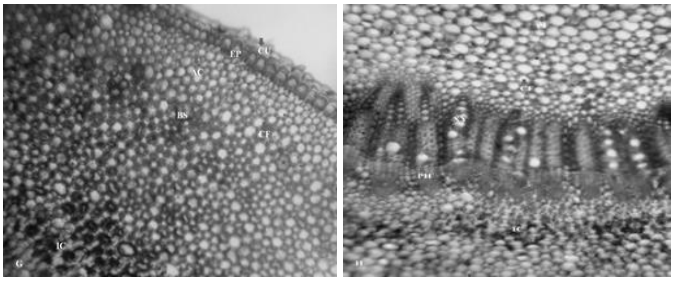
Table 1: Laminar characters of some mangroves

Species	UCT (µm)	LCT (µm)	UET (µm)	LET (µm)	UHT (µm)	LHT (µm)	PL (µm)	PW (µm)	LT (µm)	SL (µm)	SW (µm)
R. apiculata	0.52	0.44	1.36	0.91	18.18	3.90	8.42	1.83	68.26	10.55	9.43
R. mucronata	0.64	0.67	1.47	0.83	16.12	6.10	7.02	1.36	70.44	17.98	9.27
K. candel	74	0.42	1.87	1.87	12.84	11.29	9.28	1.38	80.64	15.20	2.86

UCT-Upper cuticle thickness, LCT-Lower cuticle thickness, UET-Upper epidermal thickness, LET-Lower epidermal thickness, UHT-Upper hypodermal thickness, LHT–Lower hypodermal thickness, LT-Lamina thickness, PL-Palisade length, PW-Palisade width, SL-Stomatal length, SW- Stomatal width.

Micro anatomy showed outer surface showing ridges and furrow like appearance. Stomata deeply sunken, unbranched hairs present. Epidermal cells polygonal in shape. Salt secreting glands found in abaxial side. Fig-2. (Plate-I)

Petiole anatomy consists of arc shaped in outline. The cuticle is thick. The adaxial outline is concave; abaxial surface convex. Epidermis and hypoderm are uniseriate. Epidermal cells polygonal. Cortex is wide and made of outer collenchyma, 7-8 layers and inner parenchyma cells. Inner portion consists of numerous air cavities. Vascular bundles collateral arranged in ring 24-28. Fig-2. (Plate-G & H)



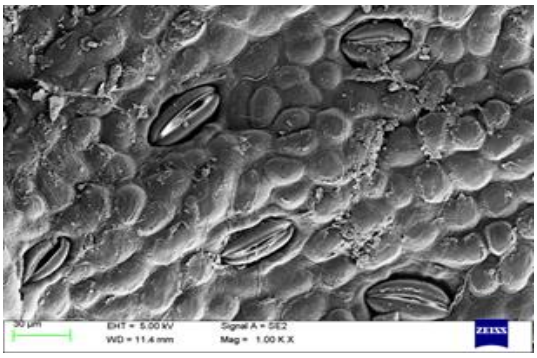
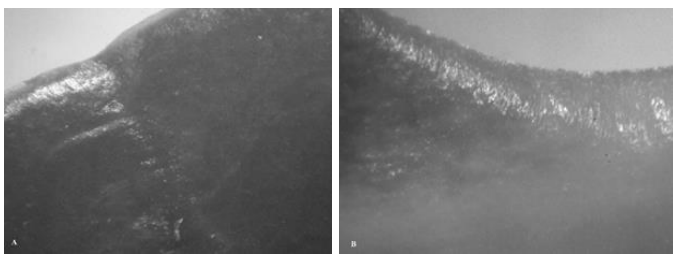


Fig 2.1: G&H-.TS of Petiole (10X), I showing SEM of abaxial side of Leaf (3000X). CU-Cuticle, AC- Angular Collenchyma, AS- Air space, EP-Epidermis, GC-Guard cells, HP- Hypodermis, PC- Palisade cell, CF-Crystalliferous cell, PAR-Parenchyma, PH-Phloem, BS-Brachy sclereids, SC- Subsidiary cells, SP- Stomatal pore, VB-Vascular bundle, TT-Terminal tracheids, WS- Water storage tissue, XY- Xylem.

***Kandelia candal* (L.) Druce.**

Morphology of Leaf was simple, leathery, lamina lanceolate or oblong glabrous and coriaceous, base cuneate, apex emarginate, margin entire. Midrib prominent. Fig-3. (Plate-A&B)

Anatomy of leaf has the lamina of *Kandelia* was studied is isobilateral. Cuticle considerably very thick and it showed two layered epidermis. Hypodermis is composed of only one layered colorless large cells below epidermis. Epidermal cells polygonal in shape and cutinized wholly. Two layers of columnar palisade cells occur beneath each surface and middle cell polygonal and colourless. Cavities present in the mesophyll region. Stomata were deeply sunken and confined to the lower epidermis. It showed four or more subsidiary cells in close rings around the stomata; so it was cyclocytic type. Crystalliferous cells present in the cell. Fig-3,(Plate-E,F&G, Table 1).



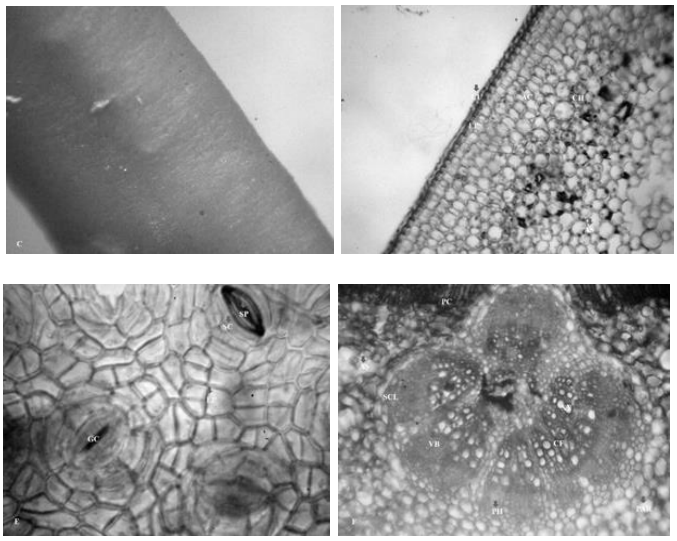
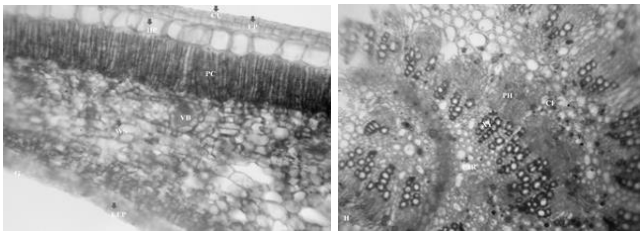


Fig 3: *K. candel,* A- Stereo microscopy of leaf apex (10X), B- Stereo microscopy of leaf base(10X), C- Stereomicroscopy of petiole(10X), D&H- TS of Petiole(10X), E-Abaxial surface with cyclocytic stomata(40X).

Micro anatomy showed outer surface showing ridges and furrow like appearance. Stomata deeply sunken, stellate hairs present. Epidermal cells polygonal. Fig-3, (Plate-I)

Petiole anatomy was arc shaped in outline. The cuticle is thick. The adaxial outline is concave; the abaxial surface convex. Epidermis and hypoderm are uniseriate. Epidermal cells polygonal. Cortex is wide and made of outer collenchyma, 9-10 layers and inner parenchyma cells. Inner portion consists of numerous air cavities. Vascular bundles collateral arranged in ring 25-30. Fig-3, (Plate-D&H)



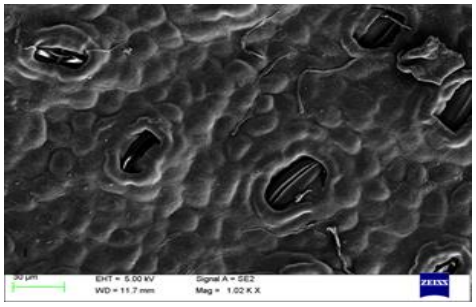


Fig 3.1: F&G- Midrib and lamina portion of leaf (10X), I showing SEM of abaxial side of Leaf (3000X). CU-Cuticle, AC- Angular Collenchyma, AS- Air space, CH- Chlorenchyma, EP-Epidermis, GC-Guard cells, HP- Hypodermis, PC- Palisade cell, CF-Crystalliferous cell, PAR-Parenchyma, PH-Phloem, LEP-Lower Epidermis, SCL- Sclereids, SC- Subsidiary cells, SP- Stomatal pore, VB- Vascular bundle, WS- Water storage tissue, XY- Xylem

Discussion

In the family Rhizophoraceae leaf and petiole anatomy of three taxa were studied. The *Rhizophora* species dorsi-ventral with extensive air cavities in nature and *Kandelia* have isobiateral leaf. [6]All of the species have thick cuticle in adaxial and abaxial side. *Rhizophora* species studied had consists of one layer of epidermal cell except *Kandelia* with two layer of epidermal cell [7] also reported. Palisade cells have one to two layered in three species. Salt secreting glands present in abaxial side except *Kandelia* [8] studied in *rhizophora* species *R. apiculata*, *R. mucronata* having black spots on the abaxial surface of the lamina known as cork warts which is the key character of the *Rhizophora* to differentiate the leaves with in the *Rhizophoracae* family. Presence of glandular and non-glandular hairs on the abaxial and adaxial leaf surfaces in some mangrove taxa are related to salt secretion of these plants. [9, 10] Stomata were deeply sunken and cyclocytic in all species. [11, 12, 13] also reported that, [8] investigated anomocytic stomata in *Rhizophora apiculata.* Hypodermal tissues one layer in *Kandelia* but multy layered in *Rhizophora* species. All species possess presence of parenchyma bundle sheath in its main vascular bundle.

All three species showed polygonal shaped epidermal cells, anticlinal walls straight and thick in both surfaces. The epidermal cells in *Rhizophora* have straight anticlinal walls. [14, 15] The epidermal cells are polygonal in outline with more or less straight anticlinal walls [8]. Presence of sclereids are theanother distinguishing feature of Rhizophoraceae. *R. mucronata* contains sclerieds, [7] presence of osteosclereids and asterosclereids were found in *Rhizophora* species [18] reported that both sclereids and tracheids are involved in capillary water storage.

All species petiole outline is boat shaped. Vascular bundles collateral and arranged in the form of ring. The number of vascular bundles in *R. apiculata* is 20-22, *R. mucronata* is 24-28 and *K. candel* is 25-30. Brachysclereids were the disticnct feature of *Rhizophora* species. Irregular sclereids present in *R. mucronata*. Branched sclereids present in *Rhizophora* [16]. petiole anatomy studies carried out in *K. candel* and *R. apiculata* [17].

Legenda

- Kollam district

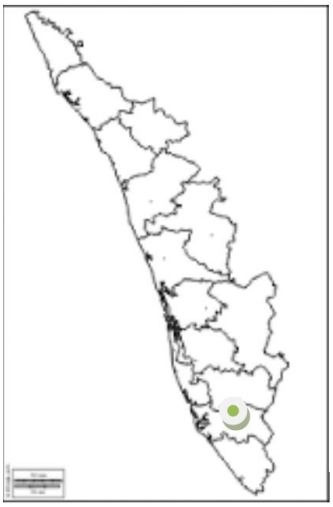


Fig 4: Aerial view of Kollam district and Kerala.

Conclusion

In the present study comprise of three species were studied coming under *Rhizophoracae* family these anatomical characters of leaf and petiole could serve useful in the identification of this plant species. From this study concluded that *Rhizophora* species were dorsi-ventral and *Kandelia* iso bilateral in nature. The three taxa showed anatomical peculiarities like presence of thick cuticle, branched sclereids, crystalliferous cells, colourless non- assimilatory water storage tissue in hypodermis, tannin cells, terminal trachieds like characters adapted to marshy environment. The number of vascular bundle is a quantitative factor for identification of species. All of this anatomical adaptations helps the plants to survive extreme salty environment.

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