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Anatomical, Morphological, Palynological, Phytochemical And Molecular Profiling of Medicinal Mangrove *Avicennia Marina* (Forssk.)Vierh. (Avicenniaceae)

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ABSTRACT

Avicennia marina, commonly known as grey mangrove or white mangrove, is a species of mangrove tree classified in the plant family Avicenniaceae. Medicinal mangrove plant used to antimicrobial, antioxidant, anticandidal properties. Specimens were examined by morphological, anatomical, palynological, HPTLC and DNA fingerprinting. In anatomical studies glandular trichomes occur in both adaxial and abaxial surfaces of the leaf; on the adaxial side glandular trichomes are sunken dispersed in crypts and non-glandular trichomes distributed in abaxial side; nectariferous gland present in petiole. Toluene: Ethyl Acetate: Formic acid: Methanol: 7:5:1:0.5 as good solvent system for measuring the establishment of phytochemicals in HPTLC analysis. It showed R_f values ranged in between 0.07 to 0.80 in 254 nm and 0.07 to 0.81 in 366 nm. The concatenated *rbcL* + *matK* loci were able to adequately discriminate *A. marina* genera in Avicenniaceae. Our study provides the endorsement of the species resolution among mangroves using plastid genes. The nucleotide sequence was also deposited in NCBI.

Keywords : Barcoding, HPTLC, mangrove, genomic DNA.

I. INTRODUCTION

Although early workers regarded mangrove forests as unimportant, transitional communities with a low productivity, most ecologists today view them as highly productive, ecologically important systems. Mangrove habitats are one of the most hostile environments with its fluctuating tidal and saline regime. Only limited plant species can survive under such condition. Nevertheless, these plants are a valuable resource and provide economical and ecological benefits to the coastal people. Mangrove forests have been utilized for many functions including wood production, fire wood and charcoal [1]. However, wood related activities or industries are very destructive and the rates of mangrove renewal do not match this at all [2]. Recently, it has been strongly recommended that mangroves should be considered as a valuable source for chemical constituents with potential medicinal use. Although the chemical constituents of most mangrove plants have not been studied extensively, investigations so far have led to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents [3].

Avicennia marina commonly called as *Tivar* is a cosmopolitan small mangrove tree widely distributed along tropical and subtropical coastlines. The barks, leaves, and fruits of this species have been used as traditional medicine in Egypt to treat skin diseases [4]. *A.marina* contains abundant chemical components. Leaves of *A. marina* have been reported to possess antimicrobial, antioxidant, anticandidal and cytotoxic activities [5].

II. METHODS AND MATERIAL

Plant samples were collected from the intertidal zones of Ayiramthengu (9^0 $7^{'}$ N: 76^0 $29^{'}$ E) of Kollam district in Kerala. The plants were identified with BSI, Coimbatore. One of the healthy plants was selected and the mature leaves from fifth and sixth node and stem (T.S, T.L.S, R. L.S.) 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% glycerine. The slides analysed by trilocular compound microscope model number 10093409 and imaged by using the camera Olympus E-PL3. The Scanning

Electron Microscopic images of leaf sample were taken Zeiss ultra 55.

HPTLC analysis carried out using methanol leaf extract. CAMAG HPTLC used for analysis.

The DNA extraction carried out using Nucleospin Plant II Kit (Macherey-Nagel). PCR amplification was performed using forward and reverse primers (Table 1&2). Sequencing of PCR product was carried out in Gene Amp PCR system 9700. Obtained DNA sequence was subjected to NCBI.

Phylogenetic analysis done by gene sequence of *rbc*L and *mat*K regions of DNA were obtained from GenBank (NCBI BLAST). Parameters of Clustal X 2.012 software in Bio edit [6] used for alignment. Phylogenetic trees of both *rbc*L and *mat*K constructed separately. The Maximum Likelihood (ML) method using MEGA5 [7] with using the Kimura 2-Parameter model.

III. RESULTS AND DISCUSSION

Habit showed much branched evergreen trees, pneumatophores, straight, pencil like, brown; bark grey, yellowish – grey or brown, smooth, occasionally flaky; branchlets more or less 4-angled and swollen at nodes (Plate-2).

Leaf morphology showed simple; petiolate; ovate to elliptic- oblong; green in adaxial and silvery white tomentose present in abaxial side; leathery, apex rounded and base cuneate and entire margin. Midrib was prominent. The petiole with an average length.03 cm and yellowish green in colour contains black marginal hairs. The average leaf area showed with 1087mm^2(Plate-2).

Morphology of stem yellowish gray or brown in colour. Branchlets 4-angled swollen at nodes. Average diameter of stem is 1 cm. Average diameter of stem is 1.2 cm. (Plate-2).

Morphology of fruit one seeded, leathery and capsule. Greyish green, more or less rounded, apex acute, coriaceous outer surface with silvery tomentose with persistent stylar beak. The average length and width of the fruit were 1.3 cm and.08 cm. (Plate-2).

Anatomy of leaves was dorsiventral, the epidermal cells were barrel shaped on adaxial and abaxial surface with thick cuticle and uniseriate epidermal hairs were present on the adaxial surface. Salt glands were frequently present on the upper side placed along with the epidermal cells. Each gland having stalk cells with variable number and with a single head cell. The hypodermis was six to seven layers of colourless, polygonal cells. The stomata were confined on abaxial side only. Presence of non-glandular uniseriate hairs, with bi seriate stalk cell and terminal awl shaped cell .Stomata were intermingled with non-glandular hairs. The vascular bundle is medullated and collateral. Lamina showing multi seriate palisade tissue and highly redused spongy parenchyma. (Plate-2).

Micro anatomy showed abaxial side having awl shaped non-glandular trichomes. Due to the presence of non-glandular trichomes difficult to identify stomata. (Plate-2).

Petiole outline was wavy with thick cuticle intermitted by salt glands. Pinnate like expansion contains chlorenchyma and separate bundles present in adaxial side. Non -glandular trichomes and extra floral nectaries 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-2).

The primary structure of stem showing epidermis cells were small and thin walled with thick cuticle. Non-glandular trichomes were present in the outer region of epidermis. The cortex was broad and consists of polygonal thin walled parenchyma cells. Brachy sclereids present in cortex. The vascular cylinder consists of xylem and phloem in collateral position; the segments are separated from each other by minute parenchymatous medullary rays. The xylem elements moderately large radially stretched. The vessels were circular, thick walled and wide lumened. Xylem fibres are thick walled and highly lignified. Phloem were wider and with a continuous layers. Phloem was demarked clearly in to early formed and late formed cells with a

layer of bast fibers. Pith composed of thin walled parenchyma with varying size and shape (Plate-2).
The secondary structure of stem showing unilayered epidrmis with thin film of cuticle. The mature stem showing well developed cork with lenticels and demarcated with cork warts. The phelloderm comprises two to four layers of radially-flattened cells. The cortex is heterogenous with chlorenchyma, aerenchyma and parenchyma. The thin walled parenchyma cells were deposited with sclereids. Vessels were large and distinct, evenly distributed in short radial multiples of 2 to 3.The growth rings were indistinct. Phloem developed in abnormal level. The island of phloem was distributed between the secondary xylem elements. In addition to the early formed phloem, outer to the secondary xylem there are rings of phloem were developed transverse with patches of bast fibers. Pith composed of thin walled parenchyma with varying size and shape (Plate-2).

In TLS vessel cylindrical in nature with alternate inter-vessel pitting alternate; it is small and minute. Perforation plate simple. Medullary rays multiseriate. Fibers non-septate (Plate-2).

In RLS Xylem showed spiral thickening. Rays were extremely fine, closely spaced and uniformly distributed uni or biseriate and heterogeneous (Plate-2).

Fruit anatomy showed outer epidermis covered by non-glandular trichomes, followed by cortex consists of compactly arranged parenchyma cells. Brachy sclereid present in cortex. Vascular region consists of several segments of xylem and phloem in collateral position. Pith wide and parenchymatous (Plate-2).

Pollen grains were 3-zono colporate, oblate to spheroidal. The grain size ranges from 4.00-4.42 µm X 3.58-4.00 µm. The exine surface was reticulate. The colpus margins were smooth and acute with tapering ends (Plate-2).

The best results were shown using Toluene: Ethyl Acetate: Formic acid: Methanol: 7:5:1:0.5 as solvent system. TLC plate of *A. marina* .methanol (leaf) extract scanned at 254 nm wavelength signified the existence of nine phytoconstituents whose R_f values ranged from 0.07 to 0.80. Peak one showing with an R_f value of 0.07 with area 10.34%. Peak two with an R_f value of 0.11, area of 1.95 %. Peak three with an R_f value of 0.14 and area 6.89%. Peak four showing R_f value of 0.24 with area 4.09%. Peak five showing an R_f value of 0.44 with an area of 2.83%. Peak six showing R_f value of 0.51 with 15.43 % area. Peak seven showing R_f value of 0.58 with area 11.16%. Peak eight showing R_f value of 0.76 with area 11.16%. Peak nine showed R_f value of 0.80 with area of 9.35%. The total peaks present in HPTLC profile of of *A. marina* is nine with an area of 9781.2(AU). (Figure 1, Plate 1)

The methanol (leaf) extract scanned at 366 nm wavelength signified the existence of six phytoconstituents whose R_f values ranged from 0.07 to 0.81. Peak one showing with an R_f value of 0.07 with area of 11.58%. Peak two with an R_f value of 0.14 with area of 1.55 %. Peak three with an R_f value of 0.44 and area 6.02%. Peak four showing R_f value of 0.51 with area 21.36%. Peak five showing an R_f value of 0.58 with an area of 13.79%. Peak six showing R_f value of 0.81 with area of 45.70% . The total peaks present in HPTLC profile of *A. marina* is six with an area of 17767 (AU). (Figure 2, Plate 1)

The phylogram constructed with the help of MEGA 5 helps to identify its genetic similarity and differences. From the phylogram two major clusters were identified. The major cluster includes largest mangrove family Rhizophoracean members and *Sonneratia, Lumnitzera and Excoecaria.* But *Aegiceras, Avicennia and Acanthus* found in the miner cluster. All the species and members of Rhizophoraceae share the same minor cluster showing their similarity. The two species of *Sonneratia* placed slightly away but near to *Rhizophora* showed their minor difference and major similarity. *Excoecaria agallocha* was placed in the same major cluster showing more genetic relatedness to *Rhizophora* and *Lumnitzera.* The two species of *Sonneratia* placed near to *Lumnitzera* showing more similarity.

Aegiceras, Acanthus, and Avicennia found in second cluster. *Aegiceras* showing more genetic relatedness in *Acanthus* than *Avicennia.*

Although the Rhizophoracean members having a common ancestor, all the three genus formed distinct clades. From the result, it is clear that more genetic relatedness is shown between different genus of largest

mangrove family with *Sonneratia, Lumnitzera and Excoecaria* and least genetic relatedness is shown with *Aegiceras Acanthus*, and *Avicennia.* (Figure-1)

Consensus sequence
AGAGGACAATTTTTCACATTTAAATTTTGTGT
TAGATGTACTAATACCCCACCCTGTCCATGTA
GAAATCTTGGTTCAAACTCTTCGCTATTGGTT
AAAAGATGCCTCTTCTTTGCATTTATTACGAT
TCTTTCTCAACGAGTATTGTAATTGGAATAGT
TTTATTTTGCCAAAGAAAGACGGTTCCTCTTT
TTCAAAAAGAAATCAAAGATTATTCTTATTCT
TATATAATTCTCATGTATGGGAATATGAATCC
ATTTTCGTCTTTCTACGTAATCAATCTGCTCAT
TTACGATCAACATCTTCTGGAGTTCTTCTTGA
ACGAATCTATTTCTATGGAAAAATGGAACGTC
TTGTGAACGTCTTTGTTAAGGTTAAGGATTTT
CGGTCGAACCCCAGGTTGATCAAGGAACCTT
GCATGCATTATATTAGGTATCAAAGAAAATCC
ATTCTGGCTTCAAAAGGGATGTCGCTTTTCAT
GAATAAATGGAAATGTTACCTTGTCACTCTTT
GGCAATGGCATTTTTCGCTGTGGTTTCAGCCA
AGAAGGATTTATATAAACCAATTAGCCAATC
ATTCCTTTGAATTCTTGGGCTATCTTTCAAGTG
TGCGGATGAACCCTTCAGTGATACGGAGTCA
AATTCTCGAAAATGCATTTCTAATCAATAATG
CTATTAAGAAGTTTGATACTCTTGTTCCAATT
ATTCCTCTGATTATGTCATTGGCTAAAGCAAA
ATTTTGTAACGTATTAGGCCATCCTATTAGTA
AGCCGGTTTGGGCTGATTTATCAGATTCTAAT
ATTATTGACCGATTTCGACGTATATGCAGAAA
TTTTTCTCATTATCATAGCGGATCTTCCACAA
AAAGGAGTTTGTAT

Partial sequencing of*matK*gene

Consensus sequence
AAGTGTTGGATTCAAAGCGGGTGTTAAAGAG
TACAAATTGACTTATTATACTCCTGAATACGA
AACCAAAGATACTGATATCTTGGCAGCATTCC
GAGTAACTCCTCAACCTGGAGTTCCGCCTGAA
GAAGCAGGGGCCGCGGTAGCTGCCGAATCTT
CTACTGGTACATGGACAAGCGTGTGGACCGA
TGGACTTACCAGCCTTGATCGTTACAAAGGGC
GATGCTACAACATCGAGCCCGTTCCTGGCGAA
ACAGATCAATATATCTGTTATGTAGCTTACCC
TTTAGACCTTTTTGAAGAAGGTTCTGTTACTA
ACATGTTTACTTCCATTGTAGGAAATGTATTT
GGATTCAAAGCCCTGCGTGCTCTACGTCTGGA
AGATCTGCGAATCCCTCCTGCTTATATTAAAA
CTTTCCAAGGCCCACCTCATGGGATCCAAGTT
GAGAGAGATAAATTGAACAAGTATGGTCGTC
CCCTGCTGGGATGTACTATTAAACCTAAATTG
GGGTTATCTGCTAAAAACTATGGTAGAGCATG
TTATGAATGTCTTCGCGGGGGACTTGATTTTA
CCAAAGATGATGAGAACGTGAACTCCCAACC
ATTTATGCGTTGGAGAGATCGTTTCTTATTTTG
TGCCGAAGCAATTTATAAAGCACAGGCGGAA
ACAGGTGAAATCAAAGGGCATTACTTGAAT

Partial sequencing of*rbcL* gene

IV. CONCLUSION

This study provides first scientific information on morphology, chemical and DNA fingerprinting of medicinal mangrove *A. maina* for pharmacophore application.

V. Acknowledgement

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VI. REFERENCES

[1]. Tomlinson PB. The Botany of Mangroves. Cambridge University Press. New York, USA. 1994. 163-170.

[2]. Kairo JG, Dahdouh-Guebas F, Bosire J, Koedam N. Restoration and management of mangrove systems - a lesson for and from the East African region. South African J. Bot. 2001. 67: 383-389.

[3]. Khafagi I, Ali G, Salama W, Fouda M. Biological activities and phytochemical constituents of the gray mangrove Avicennia marina (Forssk.) Vierh. Egyptian Journal of Biology 2003, 5, 62-69.

[4]. Fauvel MT, Taoubi K, Gleye J, Fouraste I. Phenylpropanoid glycosides from Avicennia marina. Planta Med 1993.59, 387- 387.

[5]. Zhu F, Chen X, Yuan Y, Huan M, Sun H, Xiang W. The Chemical Investigations of the mangrove plant Avicennia marina and its endophytes. The Open Natural Products Journal. 2009. 2, 24-32.

[6]. Hall, T A. 1999. "Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT", Nucleic Acids Symposium series, 41, 95-98 pp.

[7]. Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2011." MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0",

Molecular Biology and Evolution, 24, Issue 8, Pages 1596-1599, doi: 10.1093/molbev/msm092.

Table 1: Primers Used

Target	**Primer Name**	**Direction**	**Sequence (5' → 3')**	**Reference**
matK	390	Forward	CGATCTATTCATTCAATATTTC	CBOL Plant working group (http://www.barcoding.si.edu.
	1326	Reverse	TCTAGCACACGAAAGTCGAAGT	
*rbc*L	rbcLa_	Forward	ATGTCACCACAAACAGAGACTAAAGC	
	rbcL724	Reverse	GTAAAATCAAGTCCACCRCG	

Table -2:PCR Amplification Profile

step	Tem.(^{0}C)		Time(sec)		Cycles	
	mat K	*rbc* L	*mat* K	*rbc* L	*mat* K	*rbc* L
Initial denature	98	98	30	30	1	1
Denature	98	98	5	5	40	40
Annealing	50	58	10	10	40	40
Extention	72	72	15	15	40	40
Final Extention	72	72	60	60	1	1
Hold	4	4	∞	∞	_	_

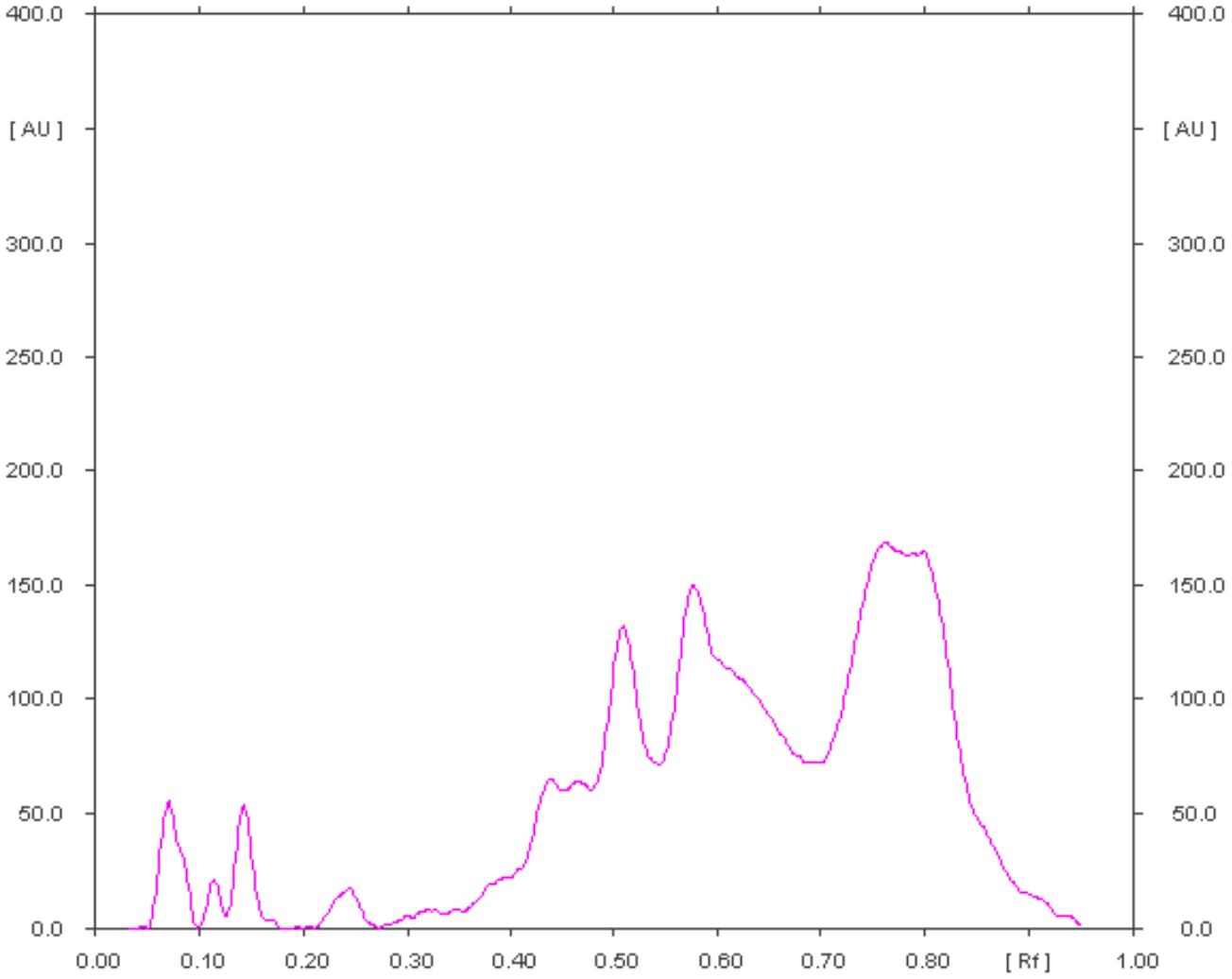


Figure 1. An overview of *Avicennia marina* (Forssk.) Vierh.,
sample at 254 nm before derivatization

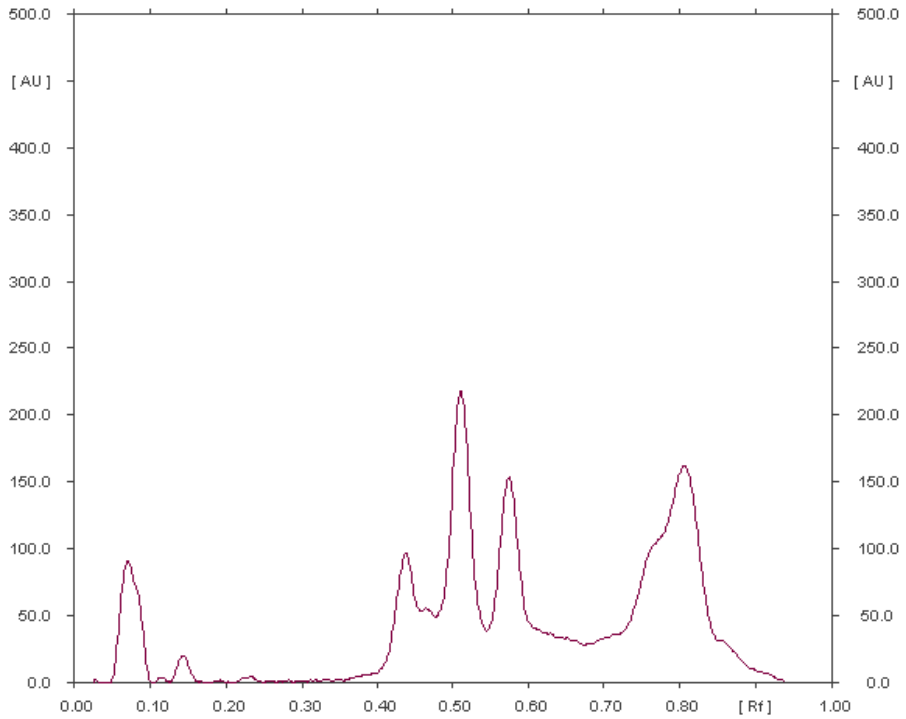
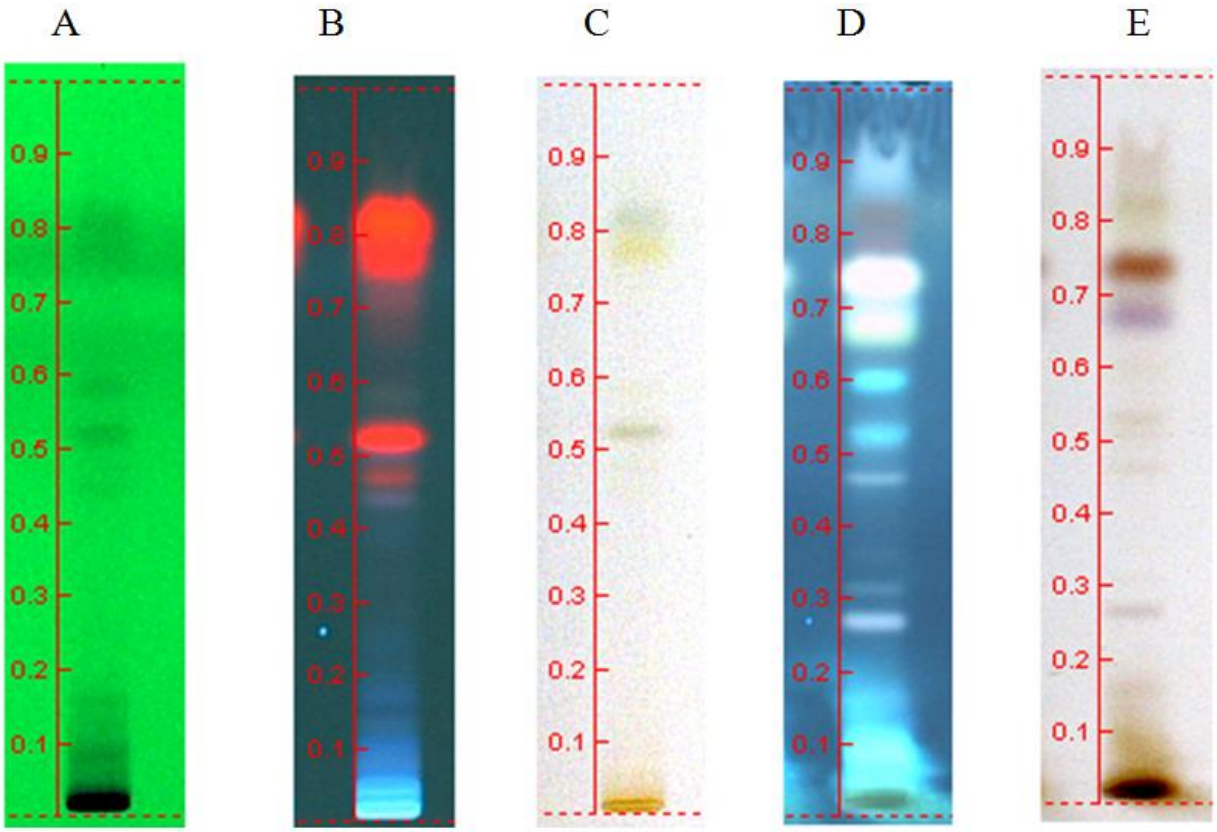


Figure 2. An overview of *Avicennia marina* (Forssk.) Vierh., sample at 366 nm after derivatization



Plates-1. Phytochemical Profile of *Avicennia marina* (Forssk.) Vierh., before (A,B & C) and after(D&E) derivatization

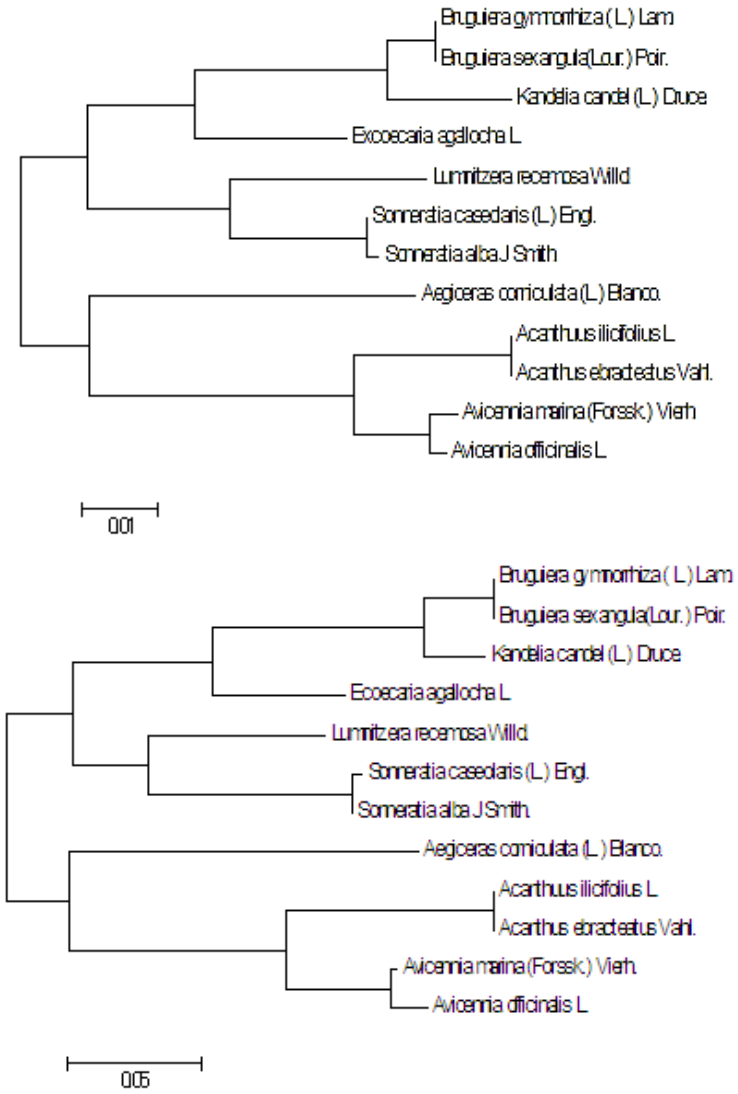
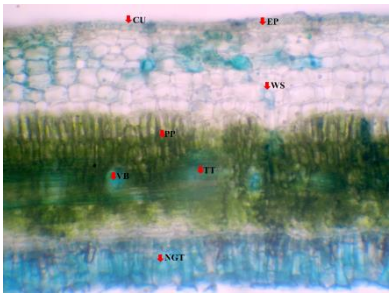
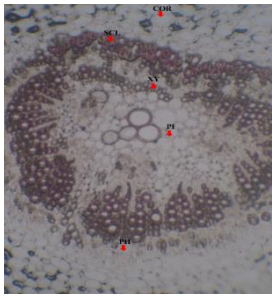


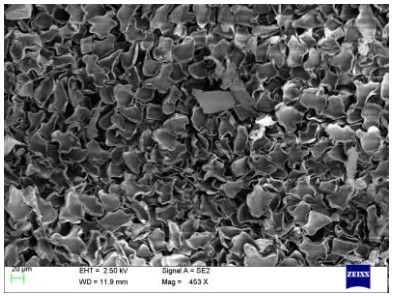
Figure: 3 ML tree obtained from phylogenetic analysis based on *rbc*L and *mat*K sequences of *A. marina* with true mangrove species in Kerala



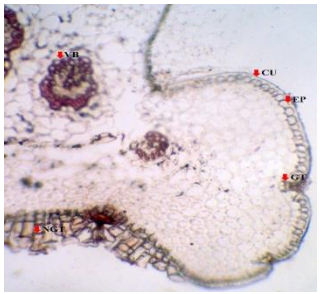
Leaf lamina



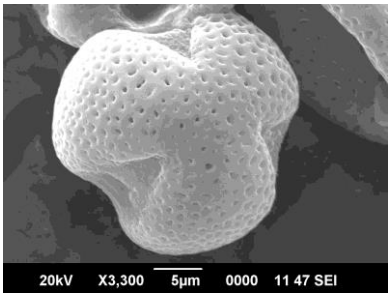
Leaf midrib



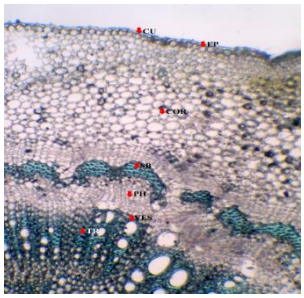
SEM-Abaxial side of leaf



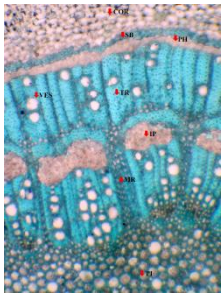
T.S. of petole



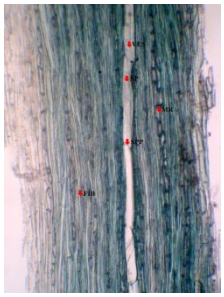
Pollem SEM



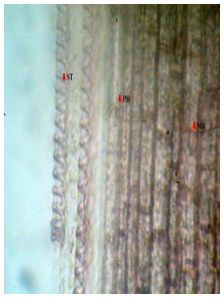
T. S. of primary stem



T. S. of secondary stem



T.L.S. of stem



R.L.S. of stem

Plate-2. Anatomical characters of *A.marina.* CU-Cuticle, EP- Epidermis, TT- Terminal tracheids, SP- Spongy parenchyma, VB- Vascular bundle, CF-Crystalliferous cell, XY- Xylem, PH- Phloem, WS- Water storage tissue, SCL- Sclereids, COR- Cortex, GT-Glandular trichomes, NGT- Non glandular trichomes, SB- Sclerenchyma bands, TR-Tracheids, SPP-Simple perforation plate, SP-Simple pits, PI-Pith, FIB- Fibers, VES-Vessel, MR-Medullary ray, ST-Spiral thickening.