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Research Article

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Isolation of Phytochemical Constituent, Characterization and Pharmacognostic Studies of Stem and Leaves of *Alangium Salvifolium Wang*

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Abstract Phytochemical constituent from Ethanolic Extract of *Alangium Salvifolium* [EEAS] was isolated using column chromatography and characterized by various spectroscopic studies such as Infrared (IR) spectra, Nuclear Magnetic Resonance (NMR) spectra and Mass Spectral studies. Results of various spectroscopic studies were interpreted and the interpreted results suggested that the isolated compound may be Di-demethoxyalanginol. Further the plant parts were subjected to Pharmacognostic studies.

Keywords EEAS, Column chromatography, Spectroscopy, Interpretation, Di-demethoxyalanginol

Introduction

Alangium salvifolium wang commonly called as Stone mango, ancole fruit plant in English, Alangi in Tamil, Nallaoodaga in Telugu and Aankol in Hindi. It belongs to the family Alangiaceae. This species is globally distributed in the Paleotropics, it is a tall thorny tree native of India. It is widely distributed throughout the greater part of India over the plains and foothills. *Alangium salvifolium* is common in the plants. Flowering and fruits in the month of February to July. It is a small-moderate sized deciduous tree, sometimes straggling, thorny when young. Leaves are alternate, oblong-lanceolate, entire, base oblique, apex subacute. The plant contains amorphous alkaloid alangine A and B, alangicine, marckine and marckidine, emetin, dimethyl cephaeline, cephaeline, tubulosine and psychotrine. Leaves are used as hypoglycemic & antidiabetic and stem can be used as anti-arthritic & anti-fertility agent [1-5].

Figure 1: Alangium salvifolium wang

Materials and Methods

Plants Collection and its Authentication

The stem and leaves of *Alangium salvifolium* were collected from Sri Venkateswara University Campus, Tirumala gardens of Chittoor District of Andhra Pradesh, India. The collected species were authentified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V.University, Tirupati, AP. Voucher specimens were deposited at Department of Pharmacognosy for further reference.

Preparation of Extracts

The collected plant materials were cleaned thoroughly and dried under shade. The dried materials were powdered using a mixer grinder and used for the extraction. The powdered material was evenly packed in the Soxhlet apparatus and then extracted with various solvents from non-polar to polar successively. After each extraction, the extracts were filtered through Whatman filter paper to remove any impurities if present. Then they were concentrated by the process of distillation and transferred into a beaker. The remaining solvent was evaporated on a water bath, cooled and placed in a desiccator to remove any excessive moisture. Then the extracts were packed in air tight containers for their further use in the phytochemical screening and experimental studies. Further the extract was subjected to preliminary phytochemical screening for the identification of the presence or absence of various constituents such as phytosterols, triterpenoids, carbohydrates, glycosides, alkaloids, saponins, tannins/phenols and flavonoids [6-7].

Isolation and Characterization of Phytochemical Constituent

40gm of crude EEAS was mixed with 120gm of silica gel (60-120 mesh) to make admixture by stirring with the help of a glass rod. The mixture was dried and the dried silica gel extract was carefully packed in a 2.4 diameter glass column with hexane. The column was eluted with increasing solvent polarity from hexane to ethyl acetate.

The column was first eluted with n-hexane with 5% increments of ethyl acetate for each eluent mixture, more than 300ml fractions were collected in glass beaker. Collected fractions were concentrated using Buchi R-114 Rota vapor. The fractions were analyzed using TLC plates. Ethyl acetate and hexane were used as mobile phase with different ratios and finally selected with ethyl acetate: hexane (3:7). The TLC spots were derivatized with Dragendorff's reagent, the product appeared as orange color spot. The product obtained in the fraction of 21-28, eluted with the ratio of ethyl acetate: hexane (3:7).

Table 1: Column Elution– EEAS

S. No.	Number of fractions	Percentage of Solvents	Volume of solvent
1	1 - 8	100 % Hexane	400 ml
2	9 – 14	5% Ethyl acetate : 95% hexane	400 ml
3	15 – 20	10% Ethyl acetate : 90% hexane	600 ml
4	21 – 28	15% Ethyl acetate : 85% hexane	800 ml
5	29 - 40	15% Ethyl acetate	400 ml

Isolated phytochemical constituent from EEAS was characterized by IR, ^{H}NMR, ^{13C}NMR and Mass spectral analysis.

Pharmacognostic Studies

Stem and leaves of *Alangium salvifolium* were cut and removed from the healthy plants. The collected plant parts were fixed in 5 ml formalin, 5 ml acetic acid and 90 ml 70% ethanol (FAA). After 24 hours of fixing, graded series of tertiary butyl alcohol was used to dehydrate the specimens. Paraffin wax was gradually added to infiltrate the specimens until tertiary butyl alcohol solution attained super saturation. The specimens were casted into paraffin blocks.

Sectioning of the specimens was done with the help of Rotary microtome. Toluidine blue (polychromatic stain) was used as a staining material to stain the dewaxed sections. The dye rendered blue color to the protein bodies & lignified cells, dark green to suberin, pink color to the cellulose walls and violet to the mucilage, etc. Wherever necessary, sections were also stained with safranin and fast-green and iodine potassium iodide.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections as well as clearing of leaf with 5% NaOH or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials.

Photographs were taken with Nikon lab photo 2 microscopic units in different magnifications. For the study of crystals, lignified cells and starch grains polarized light was employed and for the normal observations bright field was used [8-15].

Results and Discussion

Spectral analysis of isolated constituent from EEAS

IR spectra (Fig. 2)

O-H (stretching)	: 3358 cm^{-1}
Aromatic ring	: 2927 cm^{-1}
Alkane	: 2860 cm^{-1}
Hydrocarbon	: 1620 cm^{-1}
C-N (stretching)	: 1196 cm^{-1}

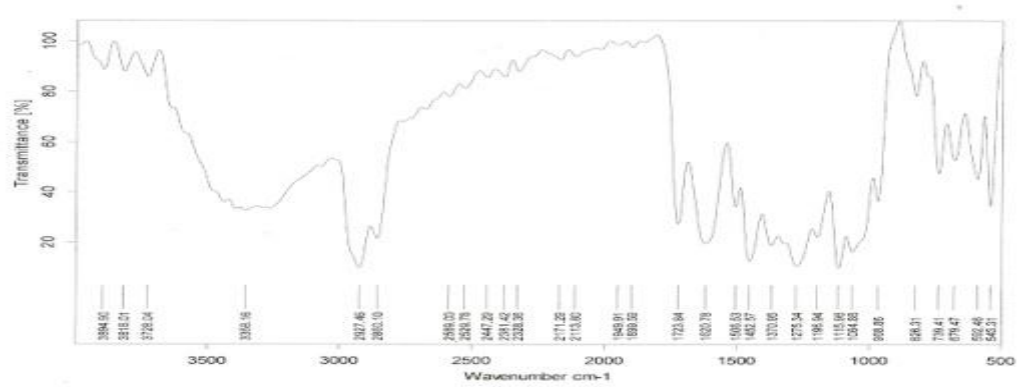


Figure 2: IR Spectrum

The IR absorption of EEAS showed the absorption peaks at 3358 cm^{-1} (OH stretching), 2927 cm^{-1} (aromatic ring), 2860 cm^{-1} (alkane) and other absorption peaks include 1620 cm^{-1}, 1196 cm^{-1} for hydrocarbon and C-N.

^{1}H NMR spectra (Fig. 3)

R-CH_3	: δ 0.9 – 1.1
-OH	: δ 3.7
Aromatic	: δ 6.5 – 8.0

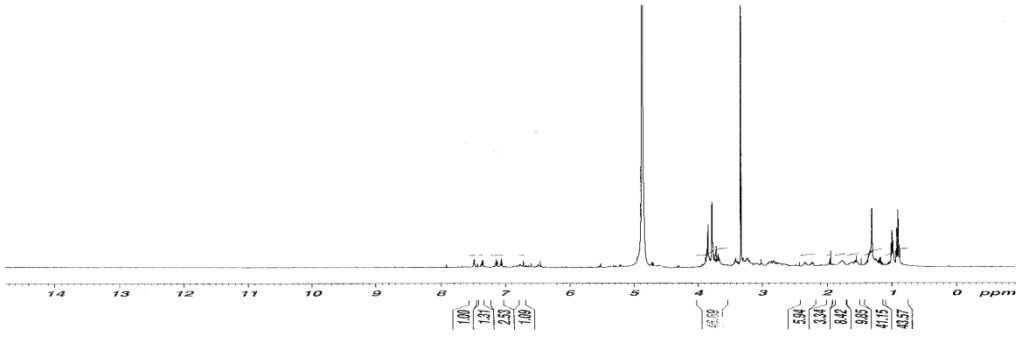


Figure 3: ^{1}H NMR Spectrum

^{13}C NMR spectra (Fig.4)

The ^{13}C NMR (MeOD, 125 MH_2) of EEAS has given the signal at 149.57, 110.96 – 126.86 (C-14,13,12,11,7,8), 10.37 – 29.43 (C-6, 3,4), 59.68 (C-9,5,15,17,18) and signal 8-30 (C-16).

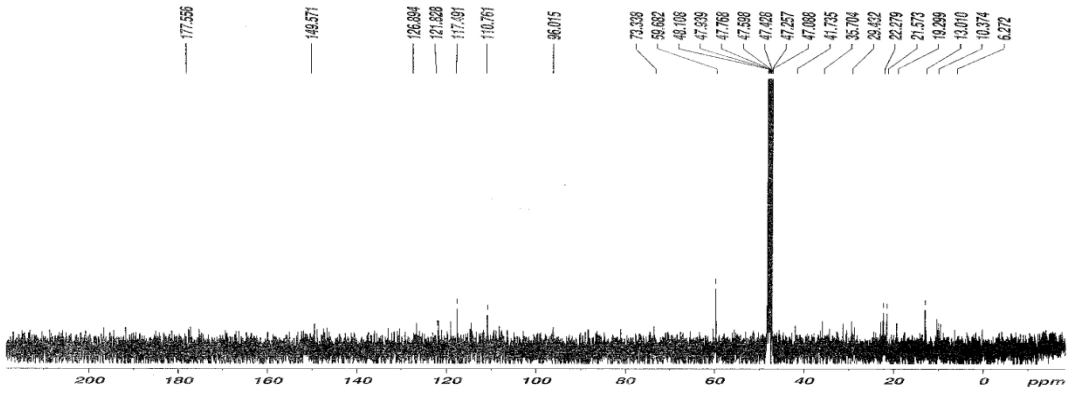


Figure 4: ^{13}C NMR Spectrum

Mass Spectra (Fig. 5)

Mass spectrum of EEAS showed the molecular ion peak at 259 corresponding to the molecular formula $C_{17}H_{25}NO$. The fragmentations of the constituent also observed at m/z 229, 133, 120, 77 and 41.

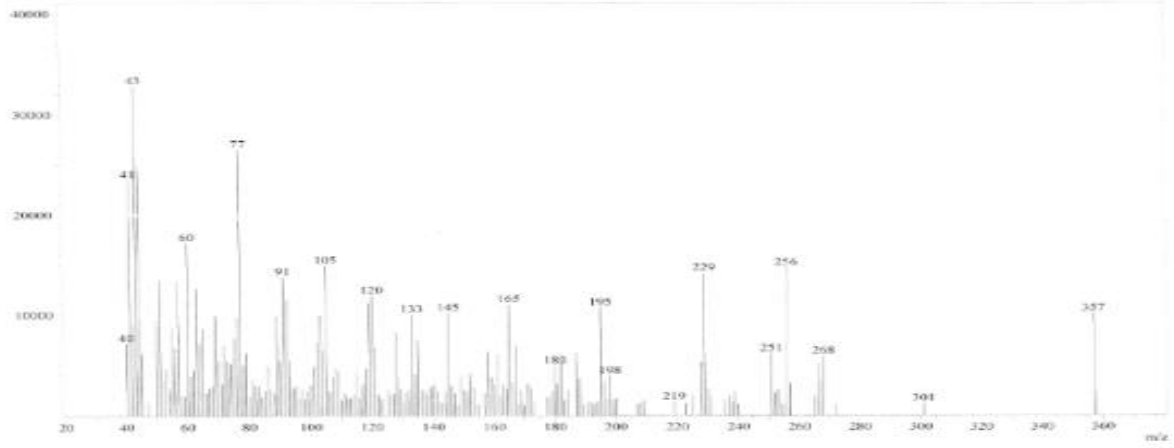
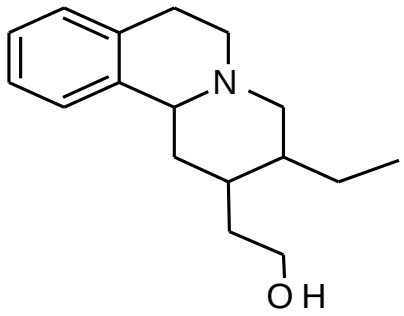


Figure 5: Mass Spectrum

The recorded spectrum's of IR, NMR and mass and its interpretation suggested the possible structure of isolated constituent from EEAS may be "Di-demothoxyalanginol".



Di-demethoxyAlanginol

Pharmacognostic Studies of Stem and Leaves of EEAS

b) Microscopic Features of Stem

Stem of *Alangium salvifolium* measuring nearly 3mm thick having circular outline. The transverse section of the stem includes epidermis, cortex, secondary phloem and secondary xylem (Fig.6).

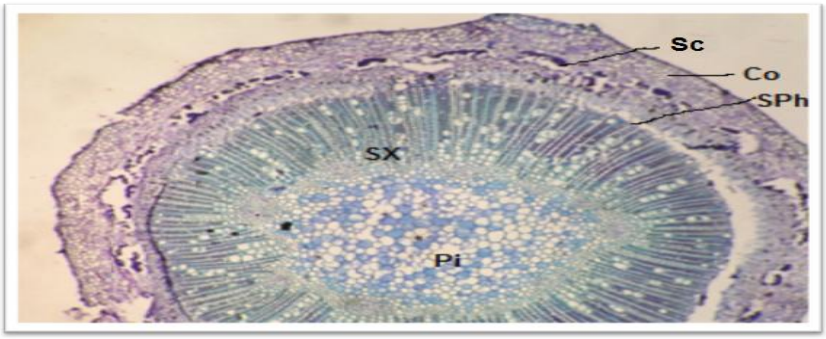


Figure 6: T.S of stem

VT-Vein termination, Sc-Scleroids, Co-Cortex, SPh-Secondary phloem, SX-Secondary xylem, Pi-Pith

Epidermal Layer

Epidermal layer is thin and continuous. It consists of intact and small, squarish thick walled cells.

Cortex

Cortex is narrow measuring 120µm thick. Inner to the cortical zone, a thin discontinuous cylinder of sclerenchyma fibres are present.

Secondary Phloem

It is fairly wide and continuous. It consists of narrow, radial compact parallel lines of phloem elements and phloem parenchyma. Phloem rays are narrow and undilated. Calcium oxalate druses are fairly abundant in the phloem parenchyma. The druses are diffuse in distribution and are located in ordinary parenchyma cells (Fig.7).

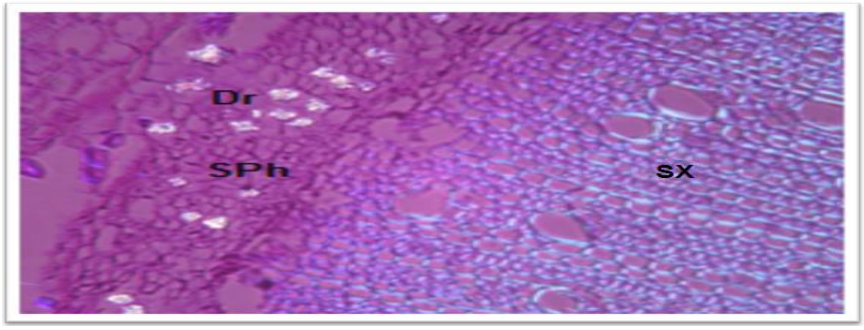
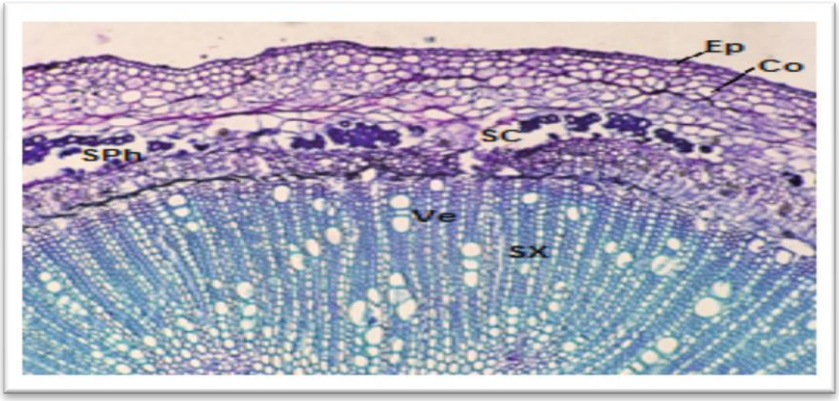


Figure 7: Druses distribution in the Secondary phloem

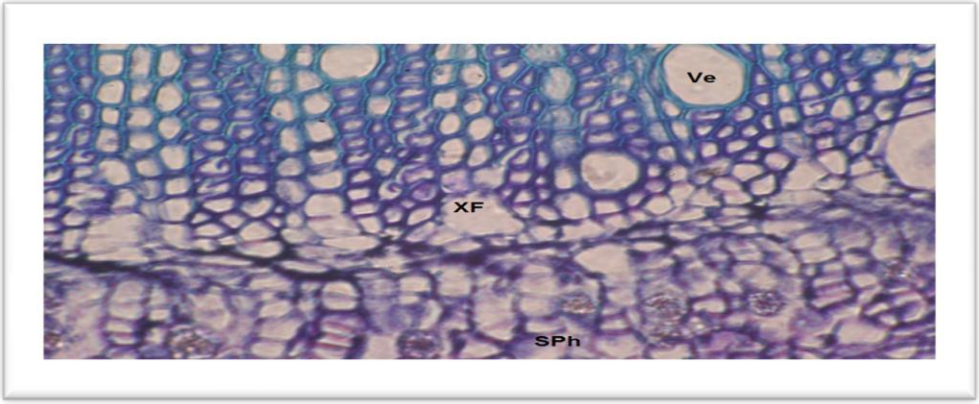
Secondary Xylem

It is thick, hollow, cylindrical and encloses wide parenchymatous pith. They are 20-40µm wide. The xylem cylinder consists of vessels, xylem fibres, rays and parenchyma cells. The vessels are less in frequency and they are either solitary or in short radial multiples.



Dr-Druses, SPh-Secondary phloem, SX-Secondary xylem, Ep-Epidermis, Co-Cortex, Ve-Vessel, Sc-Scleroids

Figure 8: T.S of stem – A sector enlarged



Secondary phloem & secondary xylem enlarged; Ve-Vessel, XF-Xylem fibre, SPh-Secondary phloem

Figure 9: T.S of Stem

The vessels are elliptical or circular and thin or thick walled. The xylem fibres are of two types, some of the fibres are thick walled and have lignified inner walls; these fibres are libri forms fibres. The other types of fibres have gelatinous secondary walls; these fibres are called g-fibres. These two types of fibres are found in alternate radial rows (Fig.8 & 9).

a) Microscopic features of leaf

The leaf of *Alangium salvifolium* is dorsi ventral with prominently projecting abaxial midrib and a thin lamina (Fig.10).

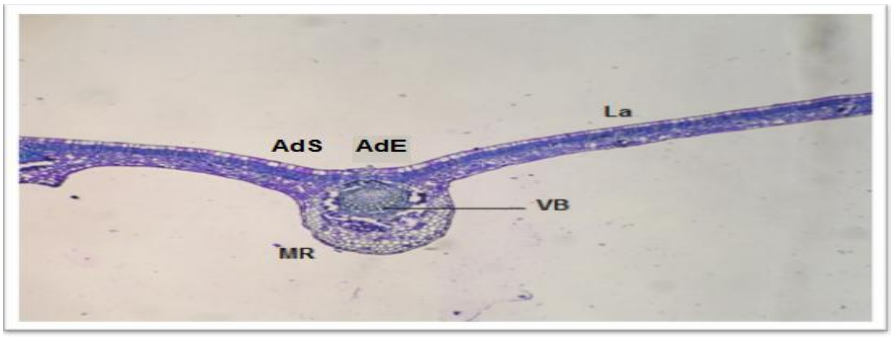
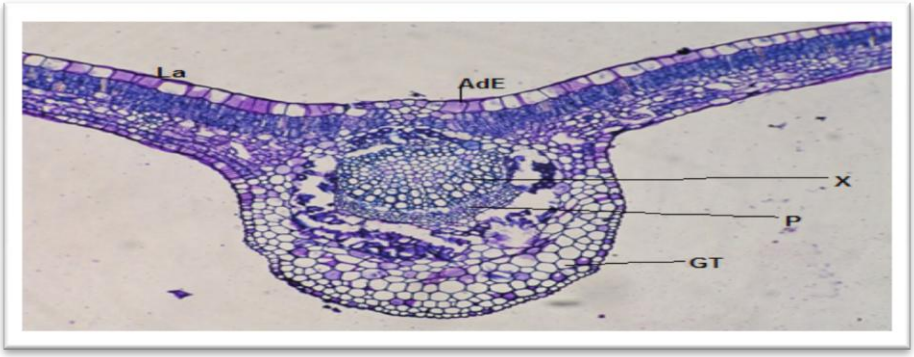


Figure 10: T.S of leaf through midrib with lamina

Midrib

Midrib of the leaf is 380µm thick and 280µm wide. It is flat on the adaxial side, thick and semicircular on the abaxial side. The epidermis on the adaxial part of the midrib consists of smaller squarish cells. On the abaxial semicircular part, the midrib epidermis is thin and the cells are circular to squarish with thin walls. The ground tissue in the midrib region consisting of parenchyma cells which are angular thin walled and compact (Fig.11).



AdE-Adaxial epidermis, La-Lamina, MR-Midrib, VB, Vascular bundle, AdS-Adaxial side, X-Xylem, P-Phloem, GT-Ground tissue

Figure 11: T.S of midrib with lamina enlarged

Vascular Bundle

The vascular bundle of the midrib is single, prominent and collateral. It is fan shaped in outline and consisting of short, continuous parallel lines of xylem elements and thick phloem located on the lower part of the xylem. The vascular bundle is surrounded by a thick sheath of sclerenchyma cells (Fig.12).

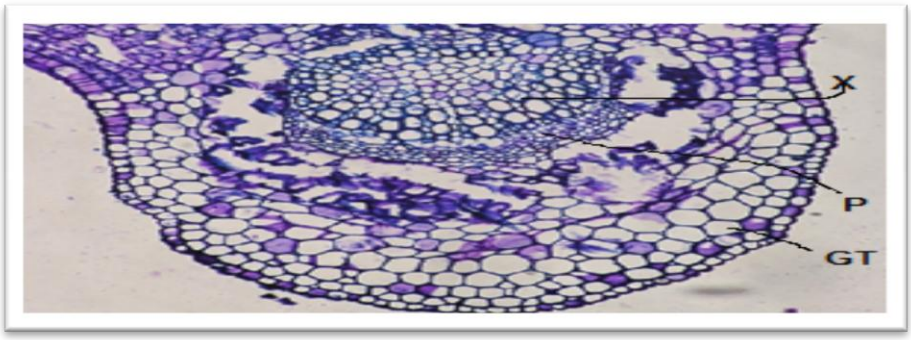
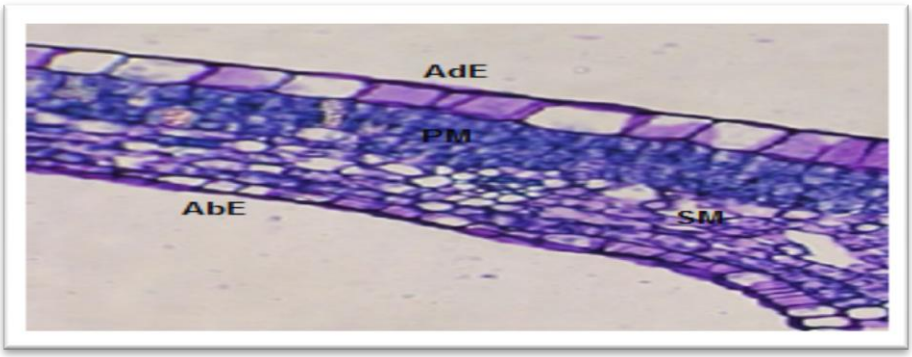


Figure 12: Microscopic structure of Vascular bundle

Lamina

The lamina is 110µm thick. It is smooth and even on both surfaces. It is hetero lateral, being differentiated into abaxial and adaxial sides. The adaxial epidermis consists of very thick, squarish or rectangular thick walled cells which are 25-30µm thick. The abaxial epidermis is thin, cylindrical and containing stomata. The mesophyll tissue is differentiated into adaxial layer of cylindrical compact palisade cells and 4-5 layers of circular or angular spongy mesophyll cells (Fig.13).



X-Xylem, P-Phloem, GT-Ground tissue, AdE-Adaxial epidermis, PM-Palisade mesophyll, SM-Spongy mesophyll, AbE-Abaxial epidermis

Figure 13: T.S of Lamina

Leaf Margin

Themargin of the lamina is blent and semicircular measuring 100µm thick. The epidermal layer of the marginal part is thinner than the epidermal layer of the midrib part of the lamina.

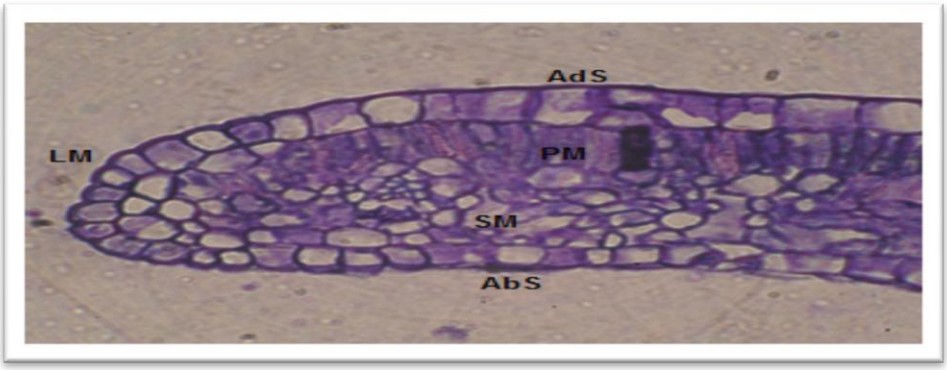
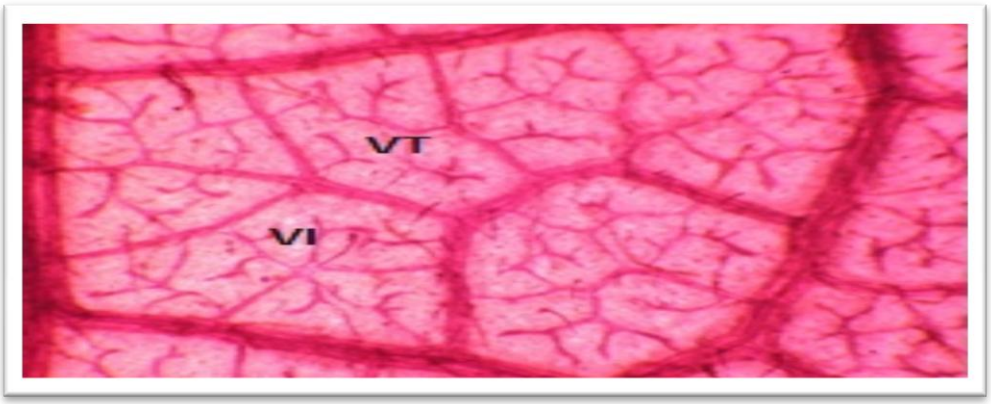


Figure 14: T.S of Leaf margin

The epidermal layer contains thick walled square shaped cells. Narrow sub-epidermal layer of cells are located inner to the marginal epidermis. The mesophyll tissue is not differentiated into palisade-spongy parenchyma cells. The cells are polygonal and compact (Fig.14)

Venation of the Lamina

The venation is densely reticulate. The primary veins are very thick, secondary and tertiary veins become gradually thinner. The vein islets are wide and polygonal in outline. The marginal veins of the islets are thick and straight. The vein terminations are complex. They range from simple to more complex type. The simple terminations are unbranched, thick and straight. The complex terminations are branched, dichotomously forming dendroid outline. The branchlets are straight or undulate (Fig.15&16).



AdS-Adaxial side, LM, Leaf margin, PM-Palisade mesophyll, SM-Spongy mesophyll, AbS-Abaxial side, VI-Vein islets, VT-Vein termination

Figure 15: Venation pattern

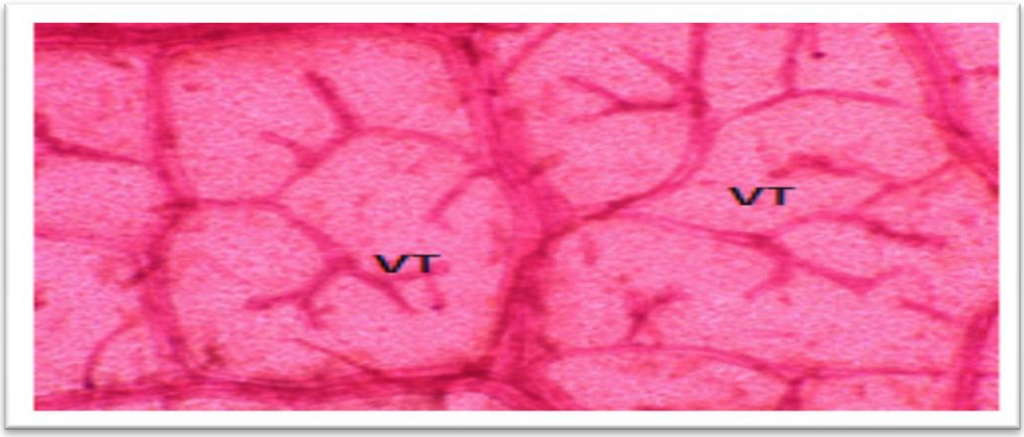


Figure 16: Vein islets and vein termination (A portion magnified)

Summary and Conclusion

Preliminary phytochemical screening confirmed the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins/phenols, amino acids, saponins and triterpenoids in various plant extracts.

Results of interpretation of spectrum obtained from various spectroscopic studies suggested the probable structure is di-demethoxyalanginol.

Pharmacognostic studies are useful in developing histological standards of plant materials and its characteristics, by which the genuine and authentic samples from the adulterated samples can be differentiated[16]. It will also help in developing herbal monographs and standardize them.

Microscopy of stem and leaves of *Alangium salvifolium wang* suggested, the stem of *Alangium salvifolium* measuring nearly 3 mm thick having circular outline. The transverse section of stem includes epidermis, cortex, secondary phloem and secondary xylem. The leaf of *Alangium salvifolium* is dorsi ventral with prominently projecting abaxial midrib and a thin lamina.

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