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RESEARCH ARTICLE

PHARMACOGNOSTICAL EVALUATION OF LEAF OF *ALANGIUM SALVIFOLIUM* (L. F.) WANGERIN (ALANGIACEAE), WESTERN GHATS, SOUTHERN INDIA

***Prathyusha, P. and Modurpalayam S. Subramanian**

Department of Botany, Nirmala College for Women (Autonomous), Coimbatore-641018, Tamilnadu, India

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ABSTRACT

Alangium salvifolium (L.F.) wangerin is an important medicinal plant belonging to the family Alangiaceae commonly known as Alangi in tamil was distributed in South India. The leaves of *A. salvifolium* are used as astringent, laxative, refrigerant and used to treat rheumatism, leprosy, syphilis and asthma. This study provides taxonomical, pharmacognostical and phytochemical details helpful in laying down standardization and pharmacopoeial parameters.

INTRODUCTION

In order for natural product drug discovery to continue to be successful, new and innovative approaches are required. By applying these new approaches in a systematic manner to natural product drug discovery, it might be possible to increase the current efficiency in identifying and developing new drugs from natural products. To facilitate correct and easy identification of the drug pharmacognostical studies helps in the standardization of the drug. *A. salvifolium* is a deciduous, rambling shrub or a tree belonging to the family Alangiaceae. This family consists one genus with twenty two species, out of which *A. salvifolium* is the only species used medicinaly in Bangladesh, India, China and Phillipines. Literature survey reveals various biological activity. The leaves of *A. salvifolium* are used as astringent, laxative, refrigerant and used to treat rheumatism, leprosy, syphilis and asthma (**Kijima *et al*., 1992**). The root bark is used as purgative, astringent, anthelmintic, antipyretic, expectorant, anti-inflammatory, emetic, diaphoretic, anticancer, antimicrobial and antitumor agents (**Ali *et al*., 1983; Rao *et al*., 1997; Anonymous, 1992**). The root is used as hypotensive agent, anthelmintic and used in the treatment of biliousness, inflammation and snakebite. The bark shows antitubercular activity. The fruits are used as laxative, refrigerant, emetic and antiphlegmatic agent.

****Corresponding author: Prathyusha, P.***
Department of Botany, Nirmala College for Women (Autonomous), Coimbatore-641018, Tamilnadu, India.

As there is no detailed pharmacognostical studies of leaves of *A. salvifolium* have not been reported so far. Therefore an attempt has been made to standardize the drug on the basis of botanical and pharmaco-phytochemical parameters.

MATERIALS AND METHODS

The plant specimens for the proposed study were collected from Bolluvampatty village, of Coimbatore District, Tamilnadu, India. Flowering shoots of the plant were also collected for identification. The collected plant material was identified and their authenticity was confirmed by the voucher specimen at the Botanical Survey of India (BSI), Southern circle, Coimbatore, Tamilnadu. The voucher specimens (AS-1010-1021) were deposited in the Ethnobotany unit, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu. The collected leaves from *Alangium salvifolium* plant were thoroughly washed in tap water and shade dried for about 15-30 days, made into coarse powder in a Willey mill to 60-mesh size. The powder was used for the further study. Powdered drugs were sieved through 60 mesh and the fine powders were treated with sodium hydroxide, acetic acid, sulphuric acid, water, nitric acid, Picric acid, ethanol, ferric chloride, acetone, hexane, ethyl acetate and powder as such. The fluorescence property of each powder with these solvents and chemicals were observed under normal light and ultraviolet light (**Kokoshi *et al*., 1958**). Phytochemical analysis of the extracts of plant was carried out

and their bioactive compounds were determined (**Harborne, 1973; Kokate *et al*., 1995**) . The required samples of different organs are cut and removed from the plant and fixed in FAA (farmalin-5ml+acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol (**Sass, 1940**). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58 – 60^{o}C) until TBA solution attained super saturation. The specimens were embedded into paraffin blocks. The paraffin embedded specimens were sectioned with the help of rotary Microtome. The thickness of the sections was 10-12µm. Dewaxing of the sections was done by customary procedure (**Johansen, 1940**). The sections were stained with Toluidine blue (**Brien *et al*., 1964**). Wherever necessary, the sections were also stained with safranin, Fast-green and IKI (for starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections was taken. Sections taken parallel to the surface of leaf as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared (**Sass, 1940**). Glycerine mounted temporary preparations were made for macerated or cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in Glycerin medium after staining. Different cell component were studied and measured. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used, and for the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale – bars. Descriptive terms of the anatomical features are given in the standard Anatomy books (**Easu, 1964, 1979**).

RESULTS AND DISCUSSION

A deciduous, shrub or small tree, (Figure 1) branchlets appressed – tomentose, sometimes straggling,sometimes spinous. Bark grey, orange-yellow when young; wood olive-brown, hard and close-grained, scented. Leaves oblong- or elliptic-lanceolate, entire, attenuate or sub acute, base oblique – subacute, more or less 3-5 nerved at the base. White-scented flowers in irregular axillary cymes or clusters, buds about 75 in. long, tawny-pubescent; Calyx tube cupular, five to ten lobes. Petals five to ten lobes, linearly oblong. Stamens 10 – 30; anthers linear. Ovary inferior, unilocular; Ovule one, pendulous; style simple. Berries globose, covered by persistant calyx, stigma capitate. Seeds solitary and ovoid.

A small quantity of drug powder is mounted in different media and exposed to ultraviolet light and visible light the fluorescence emitted are recorded (Table 1). The results of qualitative phytochemical analysis of leaves of *A. salvifolium* are presented in (Table 2). The qualitative phytochemical study shows the presence of fixed oil and fat, gums and mucilage, alkaloids, saponins, glycosides, flavonoids, terpenoids, phenols, tannins and steroids in leaves.

Table 1. Fluorescence evaluation of leaves of *A. salvifolium*

S.No	Treatment	Leaves	
		Visible	UV(365nm)
1	Powder as such	Cascade green	Light green
2	Powder+ H_2SO_4	Deep green	Deep green
3	Powder+ H_2SO_4+H_2O	Teak wood	Light green
4	Powder+ HNO_3	Red sandal wood	Dawn glow
5	Powder+ Acetic acid	Brown	greenish brown
6	Powder+ Picric acid	Light grey	Olive green
7	Powder+ NaOH	coffee brown	greenish brown
8	Powder+ 5% $FeCl_2$	Deep green	Bas green
9	Powder+ Hexane	Green	Dark green
10	Powder + Ethyl acetate	golden yellow	Dark yellow
11	Powder+ Acetone	Opaline green	Light green
12	Powder + Ethanol	Cascade green	Yellowish green

Table 2. Phytochemical screening of leaves of *A. salvifolium* with various solvent extract

Test	Extract			
	PE	CH	AC	MET
	leaves			
Carbohydrates Molisch test	--	+	++	+++
Proteins Biuret test	--	--	+	++
Amino acids Ninhydrin test	+	+	++	++
Fixed oils and fats Spot test	++	+	--	--
Gums and Mucilages	+	--	--	--
Alkaloids Dragendroff's	--	+	++	+++
Wagner's	--	+	+	+++
Mayer's	--	+	++	+++
Saponins Common test	--	+++	--	++
Glycosides Keller-killani test	+	++	++	+++
Flavonoids Shinoda test	--	--	++	+++
Terpenoids Salkowski test	++	++	--	--
Phenols Lead acetate	--	++	--	++
Tannins Ferric chloride Lead acetate	--	+++	--	+++
	--	+	+	++
Steroids Libermann-Burchard's test	++	++	+++-	++

The number of + indicates the intensity of reaction and compound present. – indicate the absence of compound. PE=Petroleum Ether, CH = chloroform, AC =Acetone, MET =Methanol

The leaf exhibits mesomorphic, dorsiventral and stomatiferous structure. Surface of the leaf is even and smooth. The midrib is fairly prominent and plano convex in sectional view. It has flat on the adaxial side and broadly hemispherical in abaxial side. The ground tissue of the midrib consists of two or three layers of collenchyma beneath the epidermis and the rest of the tissue is parenchymatous (Figure 2.1, 2). The vascular strand is single, small, top shaped, collateral and surrounded by sclerenchymatous bundle sheath. Xylem elements are in dense parallel files with phloem occurring on the outer part of xylem strand. The central core is narrow and parenchymatous (Figure 2.1, 2). The vascular cylinder is 220µm vertical and 250µm horizontally. The lamina is 110µm thick. It has wide, cylindrical and thick walled adaxial epidermis.

Habit

Flower

Twig with fruit

Fruits showing persistant calyx

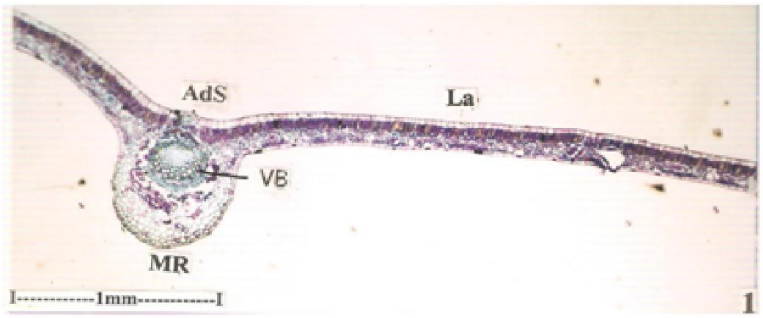
Ripe fruits

Cut view of the fruits

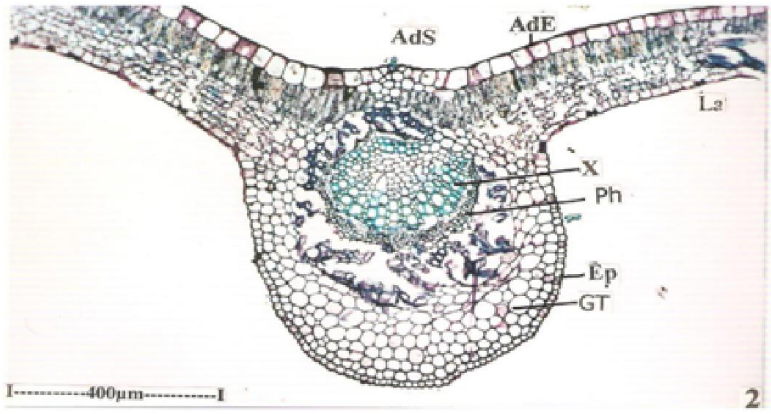
Dry Fruits

Figure 1.

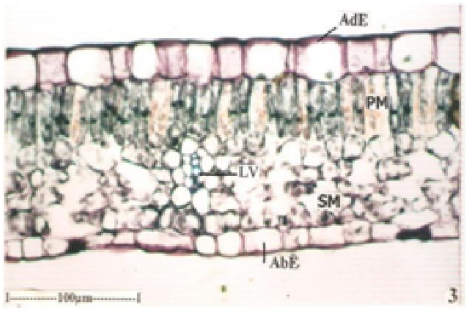
Anatomy of the leaf



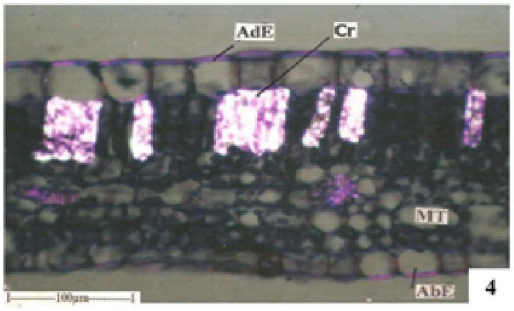
T.S of leaf through midrib with lamina



Midrib with lamina enlarged



T.S of lamina through lateral vein



T.S of lamina showing crystals

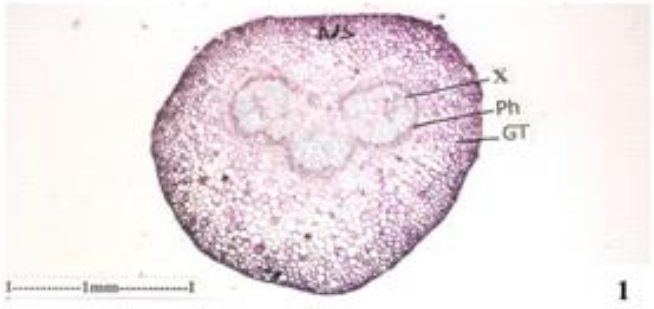
AbE – Abaxial Epidermis; AdE – Adaxial Epidermis; AdS – Adaxial side; Ep – Epidermis;GT – Ground Tissue; La – Lamina; LV – Lateral vein; MR – Midrib; Ph – Phloem; PM – Palisade mesophyll; SM – Spongy mesophyll; VB – Vascular bundle; X – Xylem; Cr – Crystals; MT – Mesophyll tissue

Figure 2.

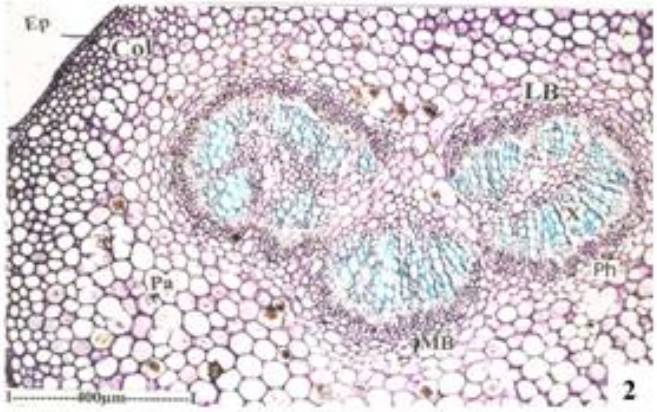
The adaxial epidermis of the lamina is 20 – 30 µm thick (Figure 2.3). The abaxial epidermis is narrow, cylindrical and thick walled cells. It is 10 – 15 µm in thick. The mesophyll tissue is differentiated into adaxial zone of compact, dense layer of palisade cells; the palisade zone is 30 µm in height. The spongy mesophyll has four or five layers of small lobed cells forming reticulate tissue; the spongy mesophyll zone is 50 µm in height.

The vascular strand of the lateral vein has small, thick walled xylem element, thin walled phloem tissue, surrounded by parenchymatous bundle sheath cell (Figure 2.3) Calcium oxalate crystals are abundant in the palisade mesophyll tissue (Figure 2.4). These crystals are styloid like, vertically oblong and rectangular. In cross sectional outline the petiole is circular. The surface is smooth and glabrous. Epidermis is single layered thin walled and cuticle is not evident.

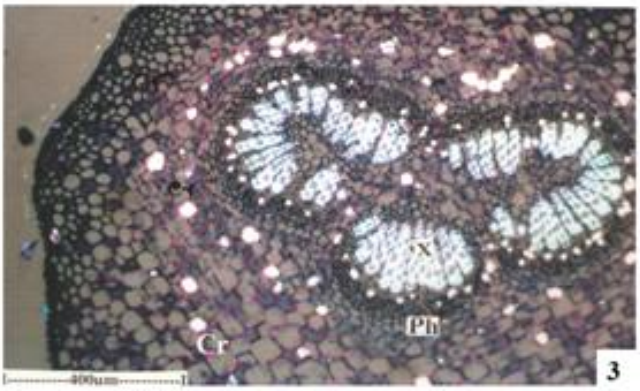
Anatomy of the petiole



T.S of petiole entire view



Vascular bundle of the petiole enlarged



Vascular bundle of the petiole showing crystal distribution under polarized light microscope

[Col – Collenchyma; Ep – Epidermis; MB – Median Bundle; LB – Lateral Bundle; Pa – Parenchyma; Ph – Phloem; X – Xylem; Cr- Crystal].

Figure 3.

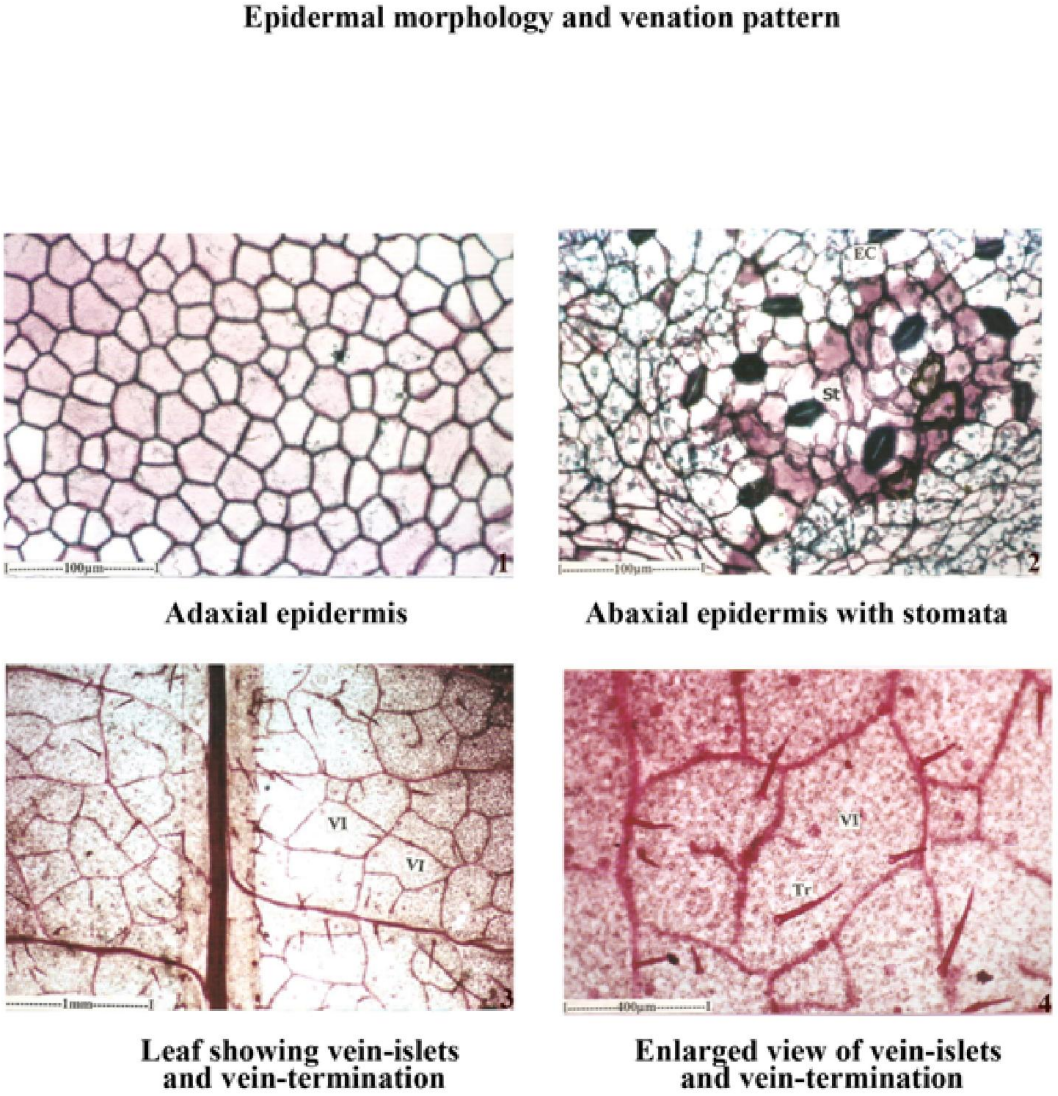


Figure 4.

Epidermal cells are barrel shaped. Ground tissue of the petiole is heterogeneous with outer six layers of collenchyma cells followed by the parenchyma cells (Figure 3. 1, 2). It is 1.5mm in vertical plane and 1.3mm in horizontal plane. The vascular strand is split up into two lateral bundles and one median bundle. Vascular strand is collateral, wider on the adaxial side and smaller on the abaxial side (Figure 3. 2). It is 850µm in horizontal and 400µm in vertical planes. The two lateral bundles are measuring about 300µm in horizontal plane and 200µm in vertical plane. Xylem elements are in parallel lines, angular, narrow and thick walled. Phloem occurs on the outer side of the xylem strand (Figure 3. 3). The petiole section was viewed under polarized light microscope and calcium oxalate crystals are observed in the ground tissue and phloem cells. Crystals are druses – type (Figure 3.3). They are 40µm wide; the druses in the phloem region are 5µm wide.

The adaxial epidermal cells are apostomatic (without stomata). In surface view, adaxial epidermal cells are angular and polyhedral. Anticlinal walls are thick and straight. The cuticular striations are less distinct and orientation of the striations is at right angle to the wall (Figure 4.1). Abaxial epidermis is stomatiferous. Stomatal morphology is actinocytic type, with a stoma surrounded by a ring of cells, elongated, radially become the guard cells. The guard cells of the stoma are 30µm in length x 20µm in breadth (Figure 4.2). The lateral veins and vein- islets vary in thickness. In the middle part of the lamina, some of the vein-islets are distinct, wide and polygonal in outline (Figure 4.3). The mid veins are thick and the lateral veins are thin. Vein terminations are simple, wavy and not well developed (Figure 4.4).

Conclusion

The pharmacognostical and phytochemical analysis carried out with a focus on bringing out diagnostic characters will be of immense help in the proper identification and standardization of this drug.

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