Pharmacognostic studies on *Alangium salvifolium* (Linn.f.) Wang. root bark

Saraswathy A1*, Meena AK1, Shakila R1, Sunil Kumar KN1, Ariyanathan S2

1Captain Srinivasa Murthi Research Institute for Ayurveda and Siddha Drug Development (CCRAS), Anna Hospital Campus, Arumbakkam, Chennai-600 106, India.
2Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tanjore-613 402, India.
* shakilasiva@gmail.com, saraswathy20042000@yahoo.co.in.

**ABSTRACT**

Root bark of *Alangium salvifolium* (Linn.f.) Wang. (Family Alangiaceae) is a reputed drug mentioned in the ancient books of Ayurveda and Siddha for the treatment of epilepsy, jaundice, hepatitis etc. Root bark of the plant was subjected to macro-microscopic, photomicrographic, physico-chemical, fluorescence, preliminary phytochemical, TLC and HPTLC to fix quality standards for this drug. Microscopic studies have shown stratified phellem, rhytidome, cluster crystals of calcium oxalate and uni- to triseriate medullary rays in the root bark. Chloroform, ethyl acetate, ethanol extracts and alkaloid fraction revealed characteristic chromatographic patterns with presence of alkaloids in varying concentrations. This study would be useful in the identification and authentication of the raw drug.

Keywords: Alangiaceae, *Alangium salvifolium*, alkaloid fraction, HPTLC.

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*Author for Correspondence: shakilasiva@gmail.com*

**INTRODUCTION**

*Alangium salvifolium* (Linn.f.) Wang. (Family Alangiaceae) syn. *Alangium lamarckii* Thw. is a common tree growing in India. If is known as Sageleaved alangium in English. Ankola, Akoda, Dhera in Hindi, Ankota in Sanskrit and and Aliñcil in Tamil. It is a small tree growing up to 10 meter in height, with more or less spinescent branches; stem bark pale brown colored with shallow cracks; young parts pubescent; root yellow, strong with brown bark.[1,2] The tree is widely distributed throughout India, Ceylon, South China, Malaya, Philippines. The plant flowers during February to April and bears fruits during May – August.[3,4] Root bark of this tree is bitter, purgative, antihelmintic, astringent, pungent, efficacious in leprosy, has emetic properties and useful in fever, skin diseases and as a purgative, antipoissonous against rat, snake and insect bites, antipyretic, anti-inflammatory, analgesic, diuretic, antihelmintic, anti diarrhoeal, useful in insanity, epilepsy, biliousness, syphilitic and other skin diseases, antihemorrhagic, expectorant in cold and cough, rables, jaundice and hepatitis and effective remedy for blood disorders.[5,6] In the Siddha system of medicine it is used in the preparations of Pataic Cankāran and Ayapirunka Rāja Karpam.[7] Root bark of *A. salvifolium* is reported to contain alangine A, alangine B, alanginine, ankoline, la-markine, emetine, cephaeline, psychotrine, tubulosine, alangicine, desmethylpsychotrine, desmethyltubulosine, myristic, palmitic, oleic, linoleic acids, myricyl alcohol, stigmasterol and β-sitosterol.[8-13] As there is no detailed pharmacognostical data reported on the root bark of this plant, present study attempts to develop pharmacognostical data on the drug essential for its standardization and authentication.

**MATERIALS**

Plant materials were collected from Anna Hospital Campus, Chennai, identified with the help of regional floras.[3,4] The dried specimen was deposited in the crude drug museum of CSMDRIAS (J/RB3). For microscopical study properly washed plant material was cut in to desirable size and preserved in FAA.
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**METHODS**

Free hand sections were taken, stained with the reagents used in pharmacognosy studies.[14,15] Photomicrography was done using Olympus Trinocular microscope attached to Olympus digital camera, drawings for powder analysis were made with the help of camera lucida.

Powder of the dried root bark of *A. salvifolium* was used for chemical analysis. Physico-chemical studies like total ash, water soluble ash, acid insoluble ash water and alcohol solubility, loss on drying at 105°C, heavy metals and successive extractive values by Soxhlet extraction method were carried out as per the WHO guidelines.[16] Preliminary phytochemical tests were done as per the standard methods.[17] The fluorescence behaviour of the powdered plant material in the ordinary light and ultraviolet light were observed by treating the powder in different reagents and viewing under the light of required wave length in a UV chamber.[13] The alkaloid content was estimated using standard methods.[18] Four grams of powdered drug was extracted in *n*-hexane in Soxhlet apparatus for consecutively three times. Then the marc was successively extracted with chloroform, ethyl acetate and ethanol. The combined extracts were concentrated separately under reduced pressure and made upto 10 ml in standard flasks with respective solvents. These solutions were used for both TLC and HPTLC analysis. The HPTLC finger print profile of *n*-hexane, chloroform, ethyl acetate, ethanol extracts and alkaloid fraction of *A. salvifolium* root bark were performed on aluminium plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness (E. Merck) as adsorbent and employing CAMAG Linomat IV applicator. The mobile phase used was toluene: ethyl acetate: diethylamine (5:3.5:0.5 v/v). The plate after air drying was scanned using CAMAG TLC Scanner 030618 with WINCATS 4.05 version software at a wavelength of UV 254 and 366 nm using deuterium and mercury lamps respectively.[19-22]

**RESULTS**

**Macroscopy**

Root bark is 3 to 4 mm in thickness, externally rough, light brownish yellow, exfoliating into very thin papery phellem, easily peeling in layers; exfoliated bark looks light copper in colour, occasionally lenticels can be seen. Surface of the transversely cut root bark shows an outer light copper coloured phellem, pale cortex and pale brownish phloem. Fracture is fibrous, odour characteristic and taste bitter.

**Microscopy**

TS of the root bark shows outer phellem, a broad zone of irregular phellem showing development of rhytidome, a narrow band of cortex and phloem (Figure 1). Phellem is well developed and 0.1 to 0.15 mm wide. Phellem tissue is sometimes seen in 5 or more successive layers with alternating parenchymatous tissue in between them due to the formation of phellogen at different levels in the outer phloem region of the root. Some of these phellem layers have 10-20 rows of thin-walled tangentially elongated regularly arranged cells measuring 15-30-45-60 μ tangentially and 12-15 μ radially. The parenchymatous tissue in between two phellem layers is composed of slightly obliterated phloem elements and parenchyma cells. Some of the cells contain druse crystals of calcium oxalate measuring upto 18-30 μ in diameter. The phloem extends from the cambial zone up to the phellem tissue. The cells are usually uniformly thin-walled and regularly arranged except that the cells towards the phellem show radial divisions and are larger in size.

The phloem elements are arranged in narrow radial strips with alternating phloem rays. Sieve elements and companion cells are distinct. The phloem parenchyma cells contain solitary druse crystals of 9-15-27 μ in diameter. The phloem parenchyma cells are thin walled measuring 20-25-40 μ tangentially and 20-27 μ radially. Phloem rays are usually uniseriate, few biseriate rays are also seen. The cells are nearly circular to polygonal, thin-walled and become slightly larger towards the periphery. The cells near the cambial zone measure 30-36 μ tangentially and 25-30μ radially and the cells towards the phellem measure 45-60 μ tangentially and 25-30 μ radially. Most of these cells especially those of the inner half of the phloem are filled with simple round starch grains of 2-3-6 μ in diameter. Sclerenchyma cells (phloem fibres) are absent in the phloem region. Cambium is a narrow zone composed of 4-5 rows of thin-walled...
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Figure 2. Microscopy of *A. Salvifolium* root bark.  
A. Detailed TS,  
B. A portion enlarged to show dividing phellem,  
C. A portion enlarged to show irregular phellem,  
D. A portion enlarged to show cortex and phloem.  
c-t-cortex; dcr-druse crystals of calcium oxalate; ick-irregular phellem; pa-parenchyma; ph-phloem; rpa-radially dividing parenchyma; st-sieve tube.

Powder analysis

Powder drug is pale yellow with slightly bitter taste and unpleasant odour. Microscopic examination of the powder showed cluster crystals of calcium oxalate, phellem in surface view, obliquely cut phellem, elongated tubular phellem cells in sectional view, 2-3 seriate medullary rays, thin-walled phloem parenchyma embedded with druse crystals of calcium oxalate. Rows simple elongated thin-walled parenchyma each embedded with a druse crystal of calcium oxalate, fragments of radially dividing parenchyma from the rhytidome and numerous druse crystals of calcium oxalate (Figure 3).

Preliminary phytochemical study

Preliminary phytochemical results showed the presence or absence of certain phytochemicals in the root bark of

Figure 3. Powder microscopy of *A. salvifolium* root bark.  
a, phellem in surface view; b, transversely cut phellem;  
ck, obliquely cut phellem; d, parenchyma containing  
druse crystals of calcium oxalate; e, medullary ray cells; f,  
parenchyma from rhytidome; g, druse crystals of calcium  
oxalate.
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*A. salvifolium*. All the extracts showed the presence of steroid. Chloroform extract showed the presence of phenol and alkaloid. Ethyl acetate and alcohol extracts gave positive results for flavonoid, phenol, tannin and alkaloid and did not answer for quinone, coumarin, iridoid and terpenoids (Table 1). The results of fluorescence analysis have been presented in Table 2.

### Table 1. Preliminary phytochemical tests for different solvent extracts of root bark of *A. salvifolium*

<table>
<thead>
<tr>
<th>Test</th>
<th>n-Hexane Extract</th>
<th>Chloroform Extract</th>
<th>Ethyl Acetate Extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinone</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Coumarin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phenol</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycoside/Sugar</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Iridoid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 2. Fluorescence analysis of root bark of *A. Salvifolium*

<table>
<thead>
<tr>
<th>Powder</th>
<th>Daylight</th>
<th>UV 254 nm</th>
<th>UV 366 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>Pale yellow</td>
<td>Brownish yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>Powder+1N HCl</td>
<td>Yellow</td>
<td>Fluorescent yellow</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>Powder+50% H₂SO₄</td>
<td>Pale brown</td>
<td>Fluorescent yellow</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>Powder+1N NaOH</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Bluish yellow</td>
</tr>
<tr>
<td>Powder+Alcoholic 1N NaOH</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Bluish yellow</td>
</tr>
</tbody>
</table>

### Table 3. Physico-chemical parameters of root bark of *A. salvifolium*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean value (% w/w, n=3) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105°C</td>
<td>10.81 ± 0.04</td>
</tr>
<tr>
<td>Total ash</td>
<td>11.28 ± 0.69</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>3.52 ± 0.045</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>2.48 ± 0.059</td>
</tr>
<tr>
<td>Solubility values (Cold)</td>
<td></td>
</tr>
<tr>
<td>a. Water</td>
<td>24.74 ± 0.36</td>
</tr>
<tr>
<td>b. Ethanol (95%)</td>
<td>13.88 ± 0.33</td>
</tr>
<tr>
<td>Extractive (Hot successive) values</td>
<td></td>
</tr>
<tr>
<td>a. n-Hexane</td>
<td>0.73</td>
</tr>
<tr>
<td>b. Chloroform</td>
<td>3.30</td>
</tr>
<tr>
<td>c. Ethyl acetate</td>
<td>9.31</td>
</tr>
<tr>
<td>d. Ethanol</td>
<td>8.62</td>
</tr>
<tr>
<td>Alkalinity (cc of 0.1N HCl/g)</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>Alkaloid content</td>
<td>1.743</td>
</tr>
<tr>
<td>Cadmium</td>
<td>BDL (&lt; 0.2 ppm)</td>
</tr>
</tbody>
</table>

SD-Standard Deviation; BDL-Below Detection Limit

**Physio-chemical study**

Physio-chemical parameters of the root bark of *A. salvifolium* are shown in Table 3. The chloroform and ethyl acetate extractive (hot successive) values were indicative of the presence of various phyto-constituents (Table 3).
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![Figure 4. Thin Layer Chromatography of extracts of A. salvifolium root bark](image)

Track 1: Hexane extract; Track 2: Chloroform extract; Track 3: Ethyl acetate extract; Track 4: Ethanol extract; Track 5: Alkaloid fraction

**TLC/HPTLC Analysis**

TLC and HPTLC finger print profile of *n*-hexane, chloroform, ethyl acetate, ethanol extracts and alkaloid fraction are shown in Figure 4, 5 & 6.

**DISCUSSION**

Stratified phellem, development of rhytidome, druse crystals of calcium oxalate and uni- to triseriate medullary rays are salient microscopic features of the drug.

Deterioration time of the barks depend upon the amount of water present in them. If the water content is high, the bark will deteriorate due to fungal attack. The loss on drying at 105°C in root bark was found to be 10.8 %. Total ash value indicates the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value and water-soluble ash content were 11.28 % and 3.52 % respectively. The amount of acid-insoluble siliceous matter present in the plant was 2.5 %. The water-soluble extractive value (24.74%) indicated the presence of sugar, acids and inorganic compounds. The successive hot extractive value with ethanol (8.62%) was found to be less than that of ethanol soluble extractive value (13.88%) by cold extraction method. The alcohol soluble extractive values (13.88%, 8.62%) indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. *n*-Hexane (hot) extractive values indicated the non-polar secondary metabolites present in the plant.

The finger print profile of *n*-hexane extract under UV light at 254 nm showed no peaks; but under 366 nm showed 5 spots at R<sub)f</sub> value 0.05, 0.18, 0.21, 0.26 and 0.82 (all blue colour); after derivatization with Dragendorff’s reagent *n*-hexane extract showed no spot indicating the absence of alkaloid. The chloroform, ethyl acetate, ethanol extracts and alkaloid fraction under UV 254 nm showed 8 spots at R<sub>f</sub> value 0.07, 0.14 (both blue), 0.19, 0.26, 0.36 (all green), 0.53,0.72 and 0.94 (all blue); under UV 366 nm showed 8 spots at R<sub>f</sub>, 0.04, 0.09, 0.15 (all blue), 0.21 (dark blue), 0.24, 0.32, 0.39 and 0.49 (all blue); and after derivatization with Dragendorff’s reagent showed 5 spots at R<sub>f</sub>, 0.07, 0.14, 0.19, 0.21 and 0.26 (all orange). The finger print profile of chemicals in chloroform, ethyl acetate and ethanol extracts were found to be similar and hence either of the solvent can be used for extraction and also for identification of root bark.

**CONCLUSION**

Morphology as well as various pharmacognostic characters of the root bark was studied and preliminary phytochemical, physico-chemical, TLC and HPTLC finger print analyses were also done for authentication and quality control of the drug. The study showed stratified phellem, rhytidome, druse crystals of calcium oxalate and uni- to triseriate medullary rays are the characteristic microscopic features of the drug. Chloroform, ethyl acetate, ethanol extracts and alkaloid fraction revealed characteristic chromatographic pattern with varying concentrations of alkaloids. Chromatogram has shown a common spot
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5A. Chloroform Extract 10μl

5B. Ethylacetate Extract 10μl

5C. Ethanol Extract 10μl

5D. Alkaloid Fraction 10μl

6A. Chloroform Extract 10μl

6B. Ethylacetate Extract 10μl

6C. Ethanol Extract 10μl

6D. Alkaloid Fraction 10μl

(R_{f} 0.19) for ethyl acetate and ethanol extracts when visualized under UV at 254 nm which is also detected in the alkaloid fraction indicating a common constituent to all the three fractions. This study would be highly useful for identification and standardization of this raw drug.

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