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PHYTOCHEMICAL AND PHARMACOGNOSTICAL STUDIES OF THE LEAVES OF *ALANGIUM LAMARKII*

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ABSTRACT

Alangium lamarkii (Alangiaceae) is commonly known as "Ankol". *In Ayurveda the leaves of the plant is* useful in treatment of inflammations, blood disorders, burning sensation, spermatorrhoea, gleets, acute fever and lumbago. The root is acrid, pungent, heating, anthelmintic and alterative and useful in treatment of biliousness and inflammations etc. The juice is emetic and alexipharmic and useful in treatment of pain, blood disorders, hydrophobia, rat-bite, lumbago, dysentery and diarrhoea whereas the seeds are cooling, aphrodisiac, indigestible and tonic. The root bark is used in piles whereas fruits are considered as purgative, expectorant and carminative. In order to ensure the use of genuine and authentic material in the preparation of herbal formulations, pharmacognostical and phytochemical methods of standardization of the plant has been carried out in the present work. Macroscopic, microscopic and physico-chemical characters of the leaves of Alangium lamarkii has been carried out. Preliminary phytochemical analysis and thin layer chromatographic studies have been performed on the various extracts of the leaves of Alangium lamarkii. All these pharmacognostical and phytochemical studies can be used as a diagnostic tool for the correct identification of the plant and also to test adulteration if any.

Keywords: *Alangium lamarkii*, Ethnobotany, Pharmacognosy, Phytochemistry

1. Introduction

Herbs are staging a comeback and herbal renaissance is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with the hope of safety and security.[1]

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unnani. This is because of the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all 'plant parts to be potential sources of medicinal substances [2]. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines [3]. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [4].

These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics [5].

Alangium lamarkii (Alangiaceae) commonly known as "Ankol" is a small genus of flowering plants.. The leaves are useful in treatment of inflammations, blood disorders, burning sensation, spermatorrhoea, gleets, acute fever and lumbago. According to Ayurveda, the root is acrid, pungent, heating, anthelmintic and alterative and useful in treatment of biliousness and inflammations etc. The juice is emetic and alexipharmic and useful in treatment of pain, blood disorders, hydrophobia, rat-bite, lumbago, dysentery and diarrhoea whereas the seeds are cooling, aphrodisiac, indigestible and tonic. The

root bark is used in piles whereas fruits are considered as purgative, expectorant and carminative. [6-7]

The present paper deals with the macroscopic and microscopic studies on the plant. Physico-chemical characters *viz*., total ash, acid-insoluble ash, water-soluble ash has been determined. Thin layer chromatography studies of the various extracts have been performed in different solvent systems and the R_f values have been determined. Preliminary phytochemical screening of the various extracts has been carried out.

2. Material and Method

2.1 Plant Material: The leaves of *Alangium lamarkii* are collected form Bankura and Asansol, West Bengal, India. A herbarium sheet was prepared and it was identified and authenticated by the Botanical Survey of India, Howrah, and West Bengal, India (CNH/35/2011/TECH.II/446). The leaves were dried in shade or under controlled condition to avoid too many chemical changes occurring and made into a coarse powder.

2.2. Macroscopy: The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, the apex, margin, base, lamina, texture, odour and taste.

2.3 Microscopy

2.3.1 Qualitative microscopy

Transverse section of leaf and young stem: Microscopic evaluation was carried out by taking transverse sections of fresh leave cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope at projection

10x. the presence/ absence of the following were observed: epidermal cells (upper and lower), covering trichoms, xylem, phloem, stomata (type and distribution) and collenchyma. The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed.[8]

A very young stem of *Alangium lamarkii* of family Alanginaceae should be selected, because secondary commences unusually early in this plant. Transverse section are taken and stained suitably for the internal structure. The stem is square in cross section. It shows the following plan of arrangement of tissues.

2.4 Quantitative investigation: Quantitative leaf microscopy was performed to determine stomata number, stomata index and palisade ratio on epidermal strips**.** [9]

2.5 Physicochemical parameters: The various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value and alcohol soluble extractive value were determined by the method reported by Sailor *et al.* [10] with slight modification.

2.6 Phytochemical Screening: The concentrated extracts were used for preliminary screening of various phytoconstituents *viz*. steroids and terpenoids, alkaloids, tannins and phenolic compounds, flavonoids, sugars and amino acids were detected by usual methods prescribed in standard tests. [11-12]

2.7 Thin layer chromatography: Thin layer chromatographic studies of the extract was carry out in various solvents at 30°C using precoated silicagel G plate as adsorbent.[13]

3. Results and Discussion

3.1 Macroscopic characters: *Alangium lamarkii* is a tree. Stem is cylindrical. Leaf is simple, alternate, margin- entire, apex- acute, laminar, venation, reticulate, petiolate. (Figure 1).

Fig 1: Macroscopic characters of *Alangium lamarkii.*

3.2 Microscopic studies

A) Internal Structure of Leaf *Alangium lamarkii*:

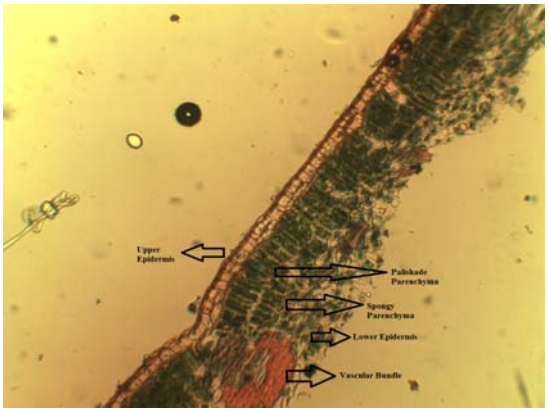

Fig 2: Microscopic characters of *Alangium lamarkii* leaf.

Thin sections shows the following structure-

i. **Epidermis:** There are two epidermal layers on adaxial and abaxial surfaces of the leaf. Each is uniseriate, composed of a row of compactly set tubular cells. The outer walls are cutinized and possess the thin cuticle, the thickness being more pronounced in the cells of upper epidermis than those of lower side. Stromata occur on the lower epidermis.

ii. **Mesophyll:** The ground tissue forming the mesophyll is differentiated into palishade and spongy cells. The palishade cells occur towards upper epidermis. They are columnar cells with scanty intercellular spaces and remain arranged more or less at right angles to the upper epidermis. Chloroplast are abundantly present, which particularly occurs along the radial walls of the cells. There are two layered of palishade cells. The spongy cells occur towards the lower epidermis. They are quite loosely arranged with conspicuous intercellular spaces. The number of chloroplast is naturally much smaller here, which explains the pale green color of the lower surface of the leaf.

iii. **Vascular Bundles:** Bundles are collateral closed. They are located in the mesophyll. The size of the bundle depends on the position one chooses to take in making a section. A bigger bundle is composed of xylem and phloem, the former occurring towards upper epidermis and later towards the lower side. The xylem is made of tracheary elements and the phloem of sieve tube and companion cells. The bundle remains surrounded by a row of colourless parenchyma cells. The band is referred to as bundle sheath or border parenchyma. Thus the bundle is not in direct contact with mesophyll cells. Parenchyma and Collenchyma cells are present on the outer and inner side of the bundle which may reach upto the two epidermal layers. These cells constitute what is known as Bundle Sheath Extension.[14]

B) **Description of Stem of *Alangium lamarkii*:**

A very young stem of *Alangium lamarkii* of family Alanginaceae should be selected, because secondary commences unusually early in this plant. Transverse section are taken and stained suitably for the internal structure. The stem is square in cross section. It shows the following plan of arrangement of tissues.

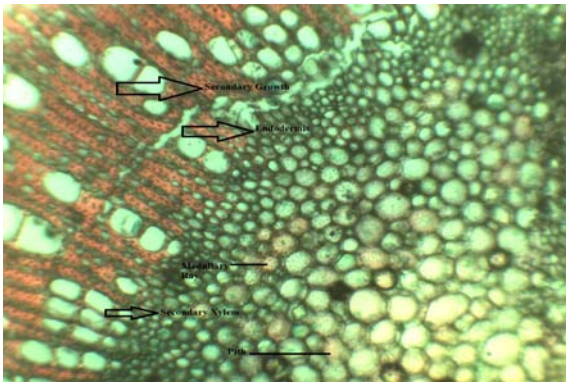

Fig 3: Microscopic characters of *Alangium lamarkii* stem.

i. **Epidemis:** It is uniseriate zone composed of tubular cells attached end on end, without intercellular spaces. The cells are living with vacuolated protoplast. The outer walls are strongly cuticularised. Many multicellular hairs develop on the epidermis. Stromata may be present here and there.

ii. **Cortex:** Like sunflower stem the cortex is differentiated into three zones, though it is less massive. Next to epidermis occurs hypodermis composed of collenchyma cells. The cells aggregate densely at the four corners of the stem so that these patches serves as diagonally placed I-girders for withstanding flexion. Collenchyma cells extend beyond the corners, but do not forms continuous bands. There are a few layer of thin walled parenchyma just internal to collenchymatous hypodermis. These cells contain abundant chloroplast (chlorenchyma) and appear as a green belt under microscope. At the portion where collenchymas are absent, chlorenchyma cells occur next to epidermis. These are usually the region where stomata are present on the epidermis. The last layer of cortex is the starch sheath, composed of barrel shaped compactly arranged cells with abundant starch grains.

iii. **Stele:** The central cylinder or stele is limited by the starch sheath and is made of vascular strands and intrastealar ground tissue. A few layer of sclerenchyma forming a continuous band occurs next to starch sheath. It may be called pericycle or perivascular tissue. The vascular bundles are disposed more towards the periphery, a large parenchymatous pith is present at the central portion. As secondary growth in thickness is initiated unusually early, the primary medullary rays very soon lose their identity.

The vascular bundles are collateral and open. The phloem is external i.e. it occurs towards the periphery and has the usual elements sieve tubes, companion cells, phloem parenchyma. Next to phloem there is a strip of cambium, made of a few layer of fusiform cells appearing more or less rectangular in outline. Cambium ring is formed rather early. Xylem occurs next. It has the usual tracheary elements- tracheids and trachea, parenchyma and fibers. Xylem is endarch- protoxylem occurring towards centre and metaxylem towards circumference.

3.3 Quantitative investigation: Quantitative leaf microscopy was performed to determine stomata number, stomata index and palisade ratio on epidermal strips**.** The estimation was given below-

Length of each stomata =15.4µ and Breadth of each stomata =7.7 µ

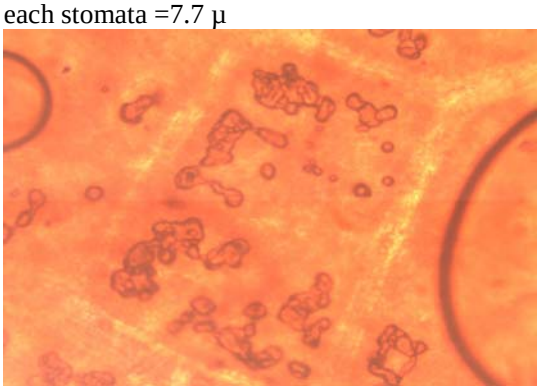

Fig 4: Picture showing Stomata in *Alangium lamarkii* Leaves.

Table 1: Quantitative investigation

Sl. No	Stromata (cm^2)	Epidermis (cm^2)
1.	10	15
2.	12	18
3.	14	21
4.	11	16.5
5.	15	22.5
6.	9	13.5
7	12	18
8.	13	19.5
9.	10	15
10.	13	19.5

Mean of stomata =11.9;
Mean of Epidermis = 17.85.
Stomata Index (S.I) = E+S/S×100
= 17.85+11.9/11.9×100 =250.

3.4 Physicochemical constants determination: The physicochemical characters are presented in Table 2. The total ash content value and water soluble ash value of powdered *Alangium lamarkii* leaves are found to be more in crude drug. Ash value is a measure of the quality and purity of the crude drug. Alcohol and water soluble extractive values were determined to find out the amount of water and alcohol soluble compounds. The leaves showed more amounts of water soluble compounds than alcohol soluble compounds.

Table 2: Physico-chemical characters of the leaf powder of *Alangium lamarkii.*

S. No.	Parameter	Values (%)
1.	Total Ash	20%
2.	Acid insoluble ash	30%
3.	Water Soluble Ash	10%
4.	Determination of Water Soluble Extractives	1.6%
5.	Determination of Alcohol Soluble Extractives	1.2%

3.5 Phytochemical Screening: In the phytochemical tests the methanolic extract revealed the presence of alkaloids, amino acids and steroids. The extractive values and results of the tests for various phytoconstituents are presented in **Table 3**.

Table 3: Preliminary phytochemical screening of the various extracts of the leaves of *Alangium lamarkii*

SL.NO.	Compound	Result
1.	Alkaloids	++
2.	Tannin	-
3.	Glycosides	-
4.	Amino acids	+
5.	Steroids	+
6.	Flavonoids	-

(+) indicates present and (-) indicates absent

3.6 Thin layer chromatography: The best separation was achieved using Toluene: Ethyl acetate: Diethyl amine (0.91) and Chlroform: Methanol (.92) as mobile phase. After developing the plates were viewed under U-V light and in iodine chamber to locate the spots. The Rf values were calculated and presented in **Table 4**.

Table 4: Thin Layer Chromatography of the leaves of various extracts of the *Alangium lamarkii.*

Solvent system (5ml)	Ratio	Solvent run	Solute run	R_f value (solute run/solvent run)
Toluene: Ethyl acetate: Diethyl amine. (For Alkaloid)	**3.5:1:0.5**	**6**	**5.5**	**0.91**
(For Isoquinoline Alkaloids) Chlroform: Methanol	**3.5:1.5**	**6.7**	**6.3**	**0.92**

Conclusion

In the present investigations, the pharmacognostical and physicochemical characteristics of *Alangium lamarkii* were studied. Various parameters established in the present study will help in controlling the standards and quality of the raw material of *Alangium lamarkii*. The preliminary phytochemical analysis showed the presence of various phytoconstituents which may contribute to the different pharmacological activity of this plant. All the pharmacognostical characters and physico-chemical parameters have been reported for the first time. Authors are actively involved in the evaluation of the different pharmacological activity of *Alangium lamarkii*.

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