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Research Article

MORPHO-ANATOMICAL STUDY OF *CALLICARPA MACROPHYLLA* VAHL: AN IMPORTANT MEDICINAL SHRUB

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ABSTRACT

Callicarpa macrophylla Vahl (Lamiaceae) is an erect medicinal shrub, commonly known as Priyangu. It is widely used in Indian and Chinese system of medicine for the treatment of various disorders like diarrhea, diabetes, dysentery, tumor and fever. The plant is also used by various ethnic communities of North-East India to treat bone dislocation, rheumatism, headache and stomach disorder. The present study deals with morpho-anatomical evaluation and establishment of its quality parameters, including chromatographic profile of *C. macrophylla*. Macroscopically, the leaf is simple, petiolate, asymmetrical, ovate-lanceolate in shape with serrate margin, acuminate apex and cuneate base. The presence of anomocytic stomata, abundant candelabra trichomes, calcium oxalate crystals, ring shaped vascular bundle surrounded by pericyclic fibers in leaf and presence of open collateral vascular bundle in stem are the some of the diagnostic features noted from anatomical study of plant. Powder microscopy of the leaf revealed the presence of epidermal cells with stomata, candelabra trichomes, palisade cells with crystals and vessels with spiral thickenings. Phytochemical screening revealed the presence of flavonoid, terpenoid, glycoside, and saponins. HPTLC finger printing of the plant with solvent system chloroform: methanol (8:2) confirmed the presence of 07 spots with different R_f value under UV light 366λ.

Keywords: *Callicarpa macrophylla;* morphoanatomical; pharmacognosy; standardization; HPTLC

INTRODUCTION

Callicarpa macrophylla Vahl (synonym: *Callicarpa incana* Roxb.) belonging to the family Lamiaceae, commonly known as *Priyangu* in Hindi and Mas-koita in Assamese is an erect shrub. The plant is used extensively in Indian and Chinese systems of traditional medicine. In India, the plant is used in treatment of various disorders like tumor, diarrhea, diabetes, fever etc[1,2]. The paste of leaves is used by tribal people of Mizoram to treat bone dislocation[3]. The plant leaves are used in headache by the Apatani tribe of Arunachal Pradesh[4]. Seeds and roots are used for digestion and abdominal troubles by the Natives of Gori Valley, district Pithoragarh and leaves are used in rheumatism in Garhwal Himalayan[5-7]. The stem bark of plant is used in gonorrhea and rheumatism in Sikkim[8]. The root powder is used against hydrophobia in Assam. The root yields aromatic oil used in the treatment of stomach disorders[9]. In traditional Chinese medicine, *C. macrophylla* is used in combination with other herbs to treat diarrhea, dysentery, intestinal worms, skin disorders, to purify the blood, and eliminate toxins[10]. The plant is reported to possess various biological activities like analgesic, anti-inflammatory, antipyretic, antifungal, anti-arthritic and antidiabetic[2]. Owing to its ethnopharmacological importance, the present investigation has been undertaken with an objective to establish morpho-anatomical, powder microscopic characters and chromatographic profile of *C. macrophylla* so that authentic plant material could be explored for its therapeutic claim.

MATERIALS AND METHODS

Plant Material

C. macrophylla plants were collected from forest patches of Kamarkuchi village, Kamrup Metropolitan district, Assam in the month of July 2017. The specimen was identified by Taxonomist, TERI-Northeastern Regional Centre, Guwahati and voucher specimen of plant (No. Coll. Kar A/43/17) was deposited in herbarium section of TERI-Guwahati for future reference.

Morpho-Anatomical Evaluation

Fresh plant of *C. macrophylla* (Figure 1) was taken for morphological and anatomical study. Various organoleptic and morphological characters of *C. macrophylla* leaves like colour, shape, size, apex, margin etc. were studied. For the anatomical studies, free hand transverse sections (T.S.) of the leaf, petiole and stem were prepared using razor blade. Lignified, cellulosic and other identifying features were studied by staining the sections with 0.1% w/v phloroglucinol followed by concentrated hydrochloric acid[11,12]. The stained sections were observed under microscope. Photomicrographs of all the sections at different magnifications were taken with Olympus digital microscope assisted with 1/3" CCD Sony camera.

Powder Microscopy

The dried aerial part of *C. macrophylla* was powdered and studied under the microscope. The powder was macerated in chloral hydrate reagent. The macerated powder was then stained with phloroglucinol and iodine reagent separately. Small quantities of

the stained powders were mounted on a glass slide with glycerin and examined under microscope[12]. Photomicrographs of the different cellular structures and inclusions were taken.

Preliminary Phytochemical Screening

Ethanol extract of *C. macrophylla* were subjected to various chemical test as per standard method to determine the nature of chemical constituents present in the plant[12].

HPTLC Profile

For proper meaningful utilization, it is important to have quality standards of materials and for this quality standardization, high performance thin layer chromatography (HPTLC) finger print profile of methanol extract of *C. macrophylla* (10 µl of 1.0 mg/ml) was developed. The HPTLC analysis was carried out on percolated silica gel on 60-F_{254} plate (Merck, India) with the help of Camag Linomat -IV applicator. The plate was eluted with Chloroform: Methanol (80:20) as mobile phase. After development, the plate was dried and densitometrically scanned on a TLC scanner III at 366 nm using Wincat software (CAMAG, Switzerland) and peak area was recorded.

RESULTS

Macroscopic Characters

Macroscopically, the fresh leaf of *C. macrophylla* is simple, petiolate, color of upper surface green and lower surface light green; texture of upper surface smooth and lower surface velvety; venation is reticulate; cuneate and asymmetrical base, ovate-lanceolate shape; acuminate apex; serrated margin; leaf length: 10 to 25 cm; width: 5 to 12 cm and petiole 1.5 to 2.5 cm long (Figure 2).

Microscopical Characteristics

Leaf Microscopy

Transverse section (T.S.) of the leaf passing through midrib region shows bifacial structure. The laminar region consists of upper epidermis which is single layered with rectangular cells, with a cuticle on outer wall. The characteristic feature of T.S. of leaf is presence of abundant candelabra trichomes in lower midrib region. Mesophyll is not clearly differentiated into palisade and spongy parenchyma. Lower epidermis is also single layered covered with thin cuticle. The epidermis continues in the midrib region also. Thick walled collenchymatous cells are seen below the upper epidermis is 5-6 layered and above the lower epidermis is 4-5 layered, which is followed by thin walled parenchymatous cells. The centre of T.S is occupied by vascular bundle which is arranged in a ring like fashion and clearly shows xylem, phloem. Xylem is surrounded by phloem and consists of vessels, tracheids, fibers and xylem parenchyma. Upper leaf surface of *C. macrophylla* shows few anomocytic stomata surrounded by abundant epidermal cells (Figure 3). The leaf surface also shows the presence of veins, vein islets, vein terminations and palisade cell. The leaf constant such as stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio were measured. The results are shown in Table 1.

Petiole Microscopy

The transverse section of petiole comprises of epidermis which is single layered with spherical cells covered with thick cuticle and abundant candelabra trichomes. The cortex is wide, comprises of 6-7 layers of collenchyma followed by 5-6 layers of parenchymatous cells. Collateral vascular bundle is arranged in form of continuous ring and pith in the centre. Xylem vessels are aligned in radial rows of 3-5. Crystals of calcium oxalate are present and distributed in the parenchymatous cells of the ground tissue (Figure 4).

Stem Microscopy

The T.S of stem comprises of epidermis covered with thick cuticle. The cells of epidermis are elongated. The hypodermis comprises of collenchymatous cells, which are 3-4 layered provides additional protection and support followed by endodermis which is distinct. Just beneath the endodermis schlerenchymatous pericyclic fibers are present in groups or as bands. Open collateral vascular bundle is present. Xylem is present in the continuous ring, consisting of vessels, tracheids, fibers and xylem parenchyma. Phloem consists of phloem fibers, sieve cells, companion cells and phloem parenchyma followed by 3 to 4 layers of cambium. Few secretary cells are seen. Uni-biseriate medullary rays are present. Centre portion is occupied by parenchymatous cells (Figure 5).

Powder Microscopic Characters

The powdered plant material is light greenish in color shows fragments of epidermal cells with stomata (Figure 6A), palisade cells with calcium oxalate crystals (Figure 6B), fibres (Figure 6C), candelabra trichomes (Figure 6D), xylem vessels with spiral thickening (Figure 6E) and mesophyll tissue (Figure 6F).

Preliminary Phytochemical Screening

Preliminary phytochemical screening revealed mainly the presence of carbohydrate, glycoside, favonoid, triterpenoid and saponins.

HPTLC Profile

A densitometric HPTLC analysis was performed for the development of specific finger print profile which may be used as a marker for quality evaluation and standardization of the drug. The preliminary HPTLC studies revealed that the solvent system Chloroform: Methanol (8:2) is ideal for the methanol extract and gave well resolved peaks of crude extract of *C. macrophylla* leaves (Figure 7).

Table 1: Leaf constants of *C. macrophylla*

S. No.	Parameters	Value (in 1 mm^2 area)
1.	Stomata number (Upper surface)	10-12
2.	Stomatal index (Upper surface)	2.63-3.14
3.	Vein-islet number	14-18
4.	Veinlet termination number	5-7
5.	Palisade ratio	4.0-6.0



Figure 1: *C. macrophylla* plant



Figure 2: Macroscopic characteristics of *C. macrophylla* Vahl. Leaf

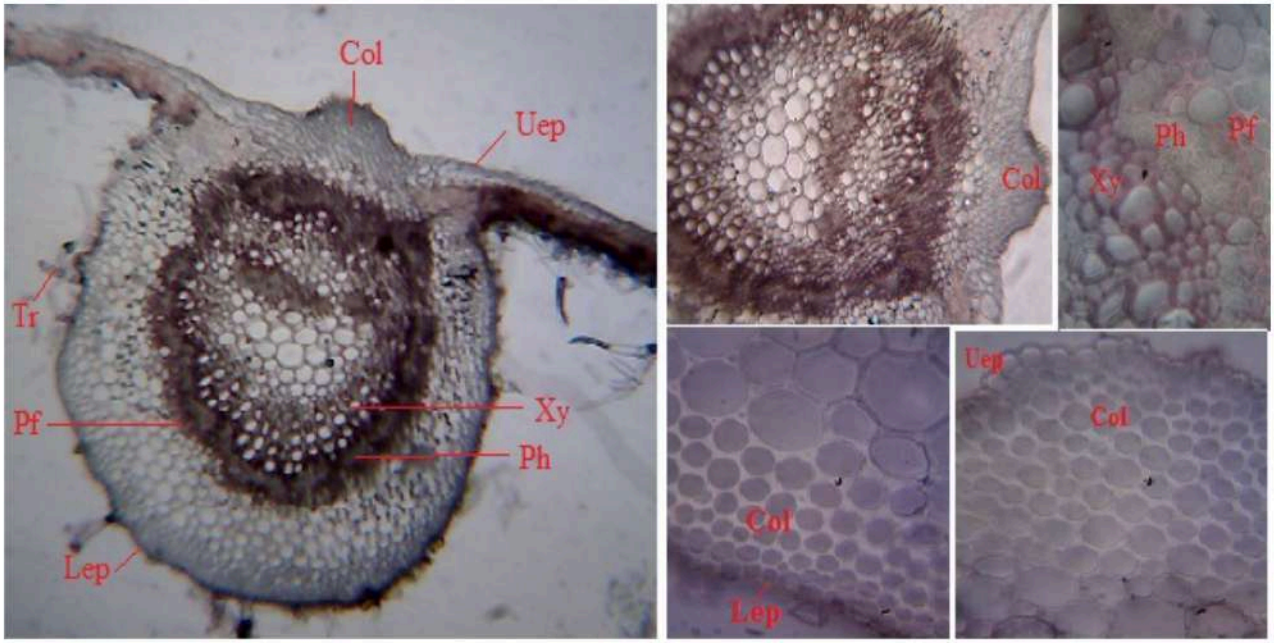


Figure 3: T.S. of *C. macrophylla* leaf
(Uep: Upper epidermis; Lep: Lower epidermis; Col: Collenchymas; Pf: Pericyclic fibres; Tr: Trichome; Xy: Xylem; Ph: Phloem)

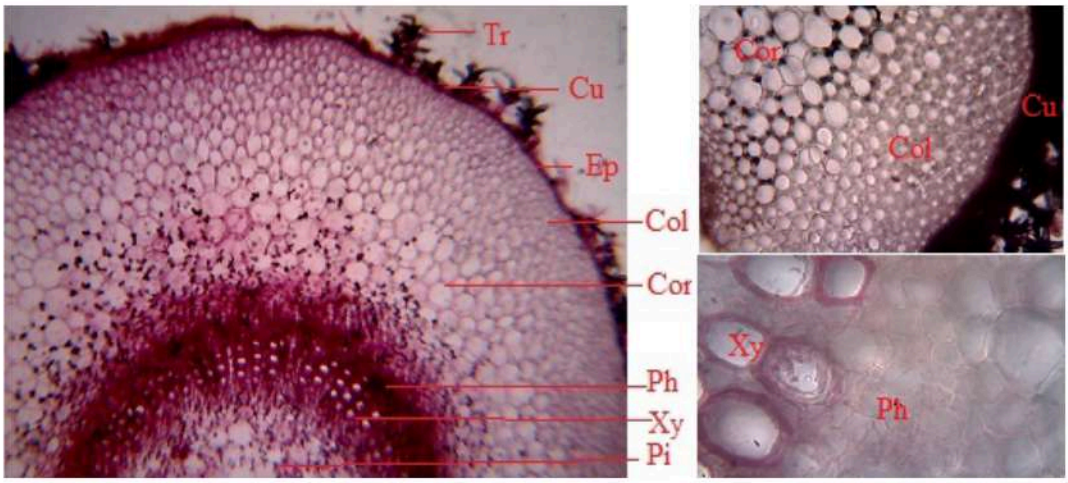


Figure 4: T.S. of *C. macrophylla* petiole
(Cu: Cuticle; Ep: Epidermis; Tr: Trichome; Col: Collenchymas; Cor: Cortex; Pi: Pith; Xy: Xylem; Ph: Phloem)

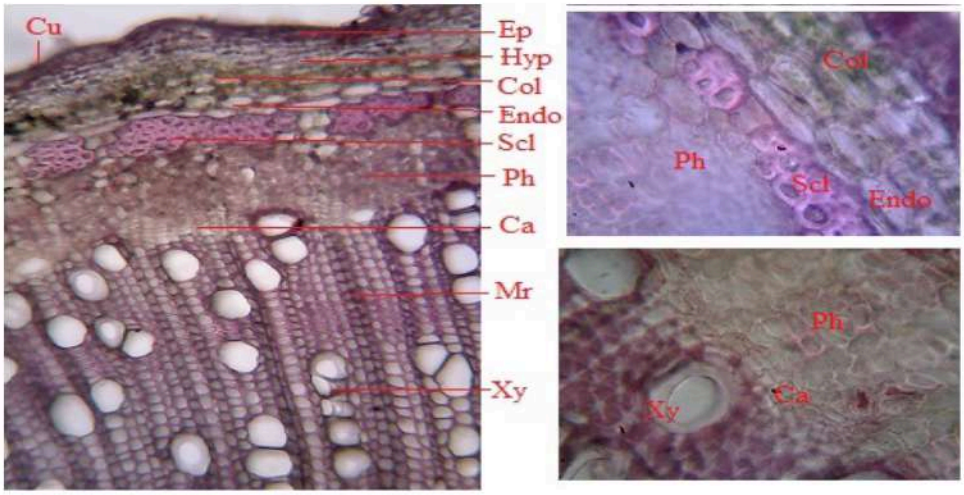


Figure: 5: T.S. of *C. macrophylla* stem
(Cu: Cuticle; Ep: Epidermis; Hyp: Hypodermis; Col: Collenchymas; Endo: Endodermis; Scl: Sclerenchyma fibres; Ca: Cambium; Mr: Medullary rays; Xy: Xylem; Mr: Medullary rays)

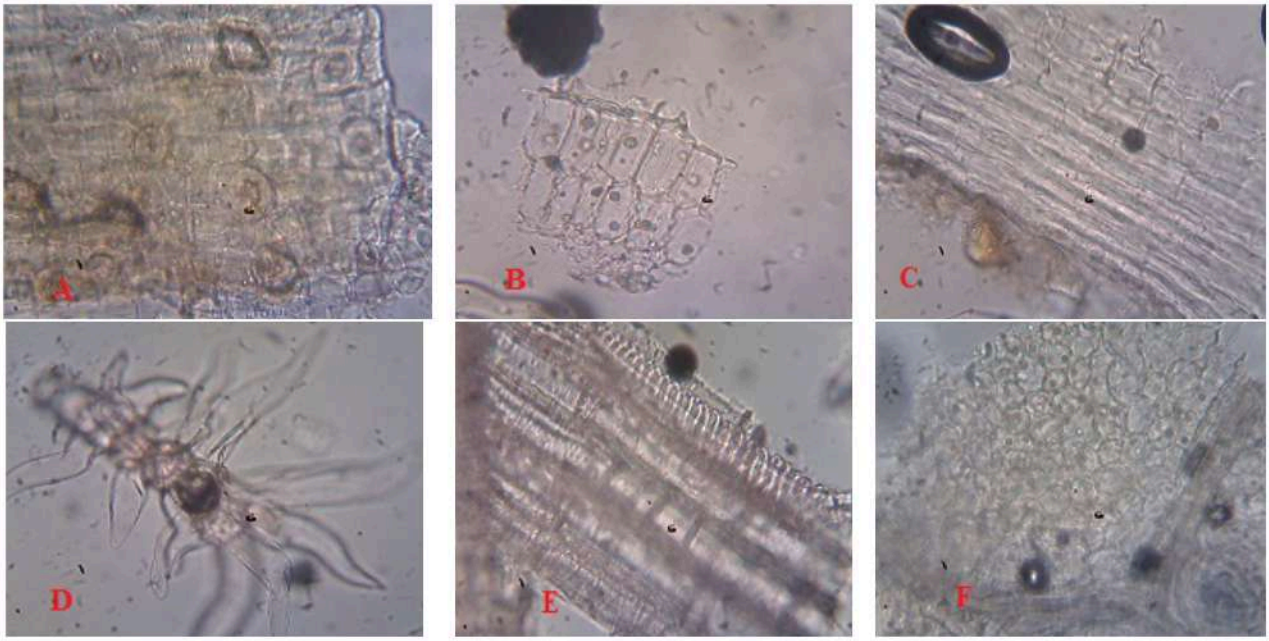
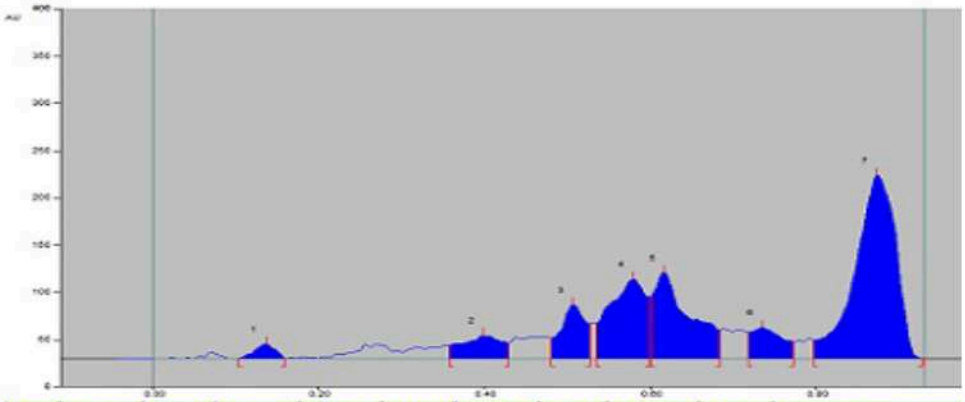


Figure 6: Powder characteristics of *C. macrophylla* leaf



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.12 Rf	0.2 AU	0.16 Rf	15.3 AU	3.07 %	0.19 Rf	0.2 AU	323.9 AU	1.78 %	unknown *
2	0.41 Rf	14.2 AU	0.46 Rf	24.4 AU	4.89 %	0.50 Rf	6.6 AU	005.5 AU	5.53 %	unknown *
3	0.56 Rf	21.8 AU	0.59 Rf	57.3 AU	11.47 %	0.61 Rf	7.1 AU	414.8 AU	7.78 %	unknown *
4	0.62 Rf	17.3 AU	0.67 Rf	84.1 AU	16.85 %	0.70 Rf	5.6 AU	075.8 AU	6.92 %	unknown *
5	0.70 Rf	15.6 AU	0.72 Rf	91.3 AU	18.29 %	0.79 Rf	0.5 AU	360.8 AU	8.49 %	unknown *
6	0.83 Rf	28.2 AU	0.85 Rf	32.9 AU	6.58 %	0.90 Rf	8.2 AU	057.4 AU	5.82 %	unknown *
7	0.93 Rf	19.5 AU	1.01 Rf	94.0 AU	18.86 %	1.08 Rf	0.2 AU	936.8 AU	3.67 %	unknown *

Figure 7: HPTLC profile and densitometric scanning of methanolic extract of *C. macrophylla* Solvent system: Chloroform: Methanol (8:2), Detection: Under UV light λ 366 nm

DISCUSSION

Owing to the wide use of *C. macrophylla* plant by various tribes and ethnic communities of Northeast and other regions of India for the treatment of various diseases, standardization becomes an important measure for ensuring quality, purity and authenticity of the plant materials. First step in this regard is the authentication of plant species. For this purpose, morpho-anatomical study is one of the simplest steps for establishing the correct identification of the plant materials[13,14]. As there is no detailed anatomical work reported on this medicinally potent plant, hence the present study reports the morpho-anatomical characters of leaf, petiole and stem of *C. macrophylla* plant. The presence of anomocytic stomata, abundant candelabra trichomes, calcium oxalate crystals, ring shaped vascular bundle capped with pericyclic fibers in leaf and presence of open collateral vascular bundle in stem are the some of the diagnostic features noted from anatomical study of plant. Morphologically the leaves are simple, petiolate, ovate-lanceolate in shape with acuminate apex, serrate margin and velvety texture of lower surface are some of the identifying macroscopic characters of the leaf. The micromorphological features of *C. macrophylla* leaf, which are the inherent diagnostic features of a plant, create a confirmed discrepancy from other members of genera. HPTLC fingerprint profile along with R_f values were recorded, which would help in the identification of various phyto-constituents present in the plant thereby authentication of crude drug. TLC profile also helps to identify and isolate important phyto-constituents.

CONCLUSION

C. macrophylla is traditionally used by ethnic and rural people to treat various diseases and the plant has wide spectrum of therapeutic properties. Therefore, morpho-anatomical studies are reported which could be used as a tool for authentication of this medicinally useful plant. The work would serve as information for further phytochemical and pharmacological study.

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