**INTRODUCTION**

_Callicarpa macrophylla_ Vahl (Lamiaceae), commonly known as Priyangu, is an erect medicinal shrub, commonly 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 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 Rf value under UV light 366λ.

**Keywords:** _Callicarpa macrophylla_; morphoanatomical; pharmacognosy; standardization; HPTLC

**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 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 acid11,12. 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\(^2\). 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\(^2\).

**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-F254 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 sclerenchymatous 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 secretory cells are seen. Uniseriate 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, glycose, flavonoid, 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).

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**Table 1: Leaf constants of *C. macrophylla***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Value (in 1 mm² area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stomata number (Upper surface)</td>
<td>10-12</td>
</tr>
<tr>
<td>2.</td>
<td>Stomatal index (Upper surface)</td>
<td>2.63-3.14</td>
</tr>
<tr>
<td>3.</td>
<td>Vein-islet number</td>
<td>14-18</td>
</tr>
<tr>
<td>4.</td>
<td>Veinlet termination number</td>
<td>5-7</td>
</tr>
<tr>
<td>5.</td>
<td>Palisade ratio</td>
<td>4.0-6.0</td>
</tr>
</tbody>
</table>
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
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 crude drug. TLC profile also helps the identification of various phyto constituents, and presence of C. macrophylla by morpho-anatomical characters of leaf, petiole and stem. For this purpose, morpho-anatomical studies are important measure for ensuring quality, purity and authenticity of the plant materials.

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REFERENCES


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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