Pharmacognostical evaluation of *Barringtonia acutangula* leaf

Dharamaraj Padmavathi, Lakshmi Susheela, Rajkishore Vijaya Bharathi

*Department of Pharmacognosy, Madras Medical College, Chennai, Tamil Nadu, India.*

**ABSTRACT**

*Barringtonia acutangula* (L.) Gaertn. (Family: Lecythidaceae) is an evergreen tree with simple, alternate leaves, long pendulous racemes, dark scarlet flowers, and ellipsoid to ovoid berries containing one ovoid black seed. The present study deals with a detailed pharmacognostical study on the leaf of the crude drug, *B. acutangula*. Morphoanatomy of the leaf was studied using light and confocal microscopy and World Health Organization (WHO) guidelines on quality control methods for medicinal plant materials. Literature reveals that the phytoconstituents like tanginol, barrinic acid, and barringenic acid are present in the wood and fruits of this plant. Our preliminary phytochemical studies of the powdered leaves revealed the presence of terpenes, flavonoids, carbohydrates, tannins, steroids, and glycosides. The physico-chemical, morphological, histological parameters, and High Performance-Thin Layer Chromatographic (HPTLC) profile presented in this paper may be proposed as parameters to establish the authenticity of *B. acutangula* and can possibly help to differentiate the drug from its other species and the pharmacognostic profile of the leaves presented here will assist in standardization viz., quality, purity, and sample identification.

**Key words:** *Barringtonia acutangula*, high performance thin layer chromatographic profile, pharmacognosy

**INTRODUCTION**

*Barringtonia acutangula* (L.) Gaertn. (Family: Lecythidaceae) known as *Dhatriphal* in Sanskrit, is a medium-sized tree found throughout India, plentifully in the plains of Bengal.[1] *B. asiatica* and *B. racemosa* are the other species of *Barringtonia*. *B. asiatica* is a native of mangrove habitats on the tropical coasts and islands of the Indian Ocean and used to treat cysts, goiter, tumor, boils, stomach ache, and rheumatism. *B. racemosa* is found in coastal swamp forests and on the edges of estuaries in the Indian Ocean and its fruits are prescribed in the *Ayurvedic* literature for the treatment of pain, inflammation, and rheumatic conditions. *B. acutangula* is an evergreen tree with simple, alternate leaves, pendulous racemes, up to 40 cm long and 1.5 cm across, dark scarlet flowers with 4 lobed ovate calyx and 2 celled ovary. It has ellipsoid to ovoid berries, fibrous, truncate at both ends, crowned by small persistent calyx and each berry bears one ovoid black seed.[2] In Ayurveda, different formulations like *Nichuladi lepa*, *Apachihara lepa*, *Lakshmivilasa rasa*, *Taptaraja taila* are used for the treatment of various diseases such as jaundice, diseases of head, hemiplegia, joint pain, eye diseases, stomach disorders, diarrhea, cough, dyspnoea, leprosy, splenic disorders, and poisoning.[3-5] According to Ayurveda, the properties (*guna*) of *B. acutangula* are *ruksha* (dry), *laghu* (light), and *tikshna* (sharp). It has a *rasa* (taste) of *katu* (pungent), *tikta* (bitter), *sheeta* (cold), and *- virya* (potency). Despite the numerous medicinal uses attributed to this plant, there are no pharmacognostical reports on the leaf of this plant. Hence, the present investigation deals with the morphological and anatomical evaluation, determination of physicochemical constants, preliminary phytochemical screening, and HPTLC profile of the different extracts. The profile presented in this paper may be proposed as parameters to establish the authenticity of *B. acutangula* and can possibly help to differentiate the drug from its other species.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *B. acutangula* were collected from Chennai,
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Tamil Nadu, India, in the month of June 2008. After authentication by a taxonomist, a voucher specimen number, MMC/2008/07, has been deposited in the museum of the Department of Pharmacognosy, Madras Medical College, Chennai, India.

**Instruments and chemicals**

Compound microscope, stage micrometer, camera lucida, drawing sheets, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica (Dissecting Microscope Lighting System-DMLS) microscope attached with Leitz (Magnification Power System- MPS) 32 camera. Solvents viz., 95% Ethanol (EtOH), Petroleum ether (PE), Ethyl acetate (EtOAc) and reagents such as phloroglucinol, glycerin, hydrochloric acid, chloral hydrate, and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

**Macroscopic and microscopic analysis**

The macroscopy and microscopy of the leaves were studied according to the method of Brain and Turner.[6] For the microscopic studies, cross sections were prepared and stained as per the procedure of Johansen.[7] The micropowder analysis was done according to the method of Brain and Turner[8] and Kokate.[9] Leaf constants

Leaf constants such as vein islet number, veinlet termination number, palisade ratio, and stomatal index were studied according to the method of Evans.[10] Physicochemical analysis

Physicochemical analysis such as the percentage of ash values and extractive values were carried out according to the official methods prescribed in Indian Pharmacopoeia[11] and the WHO guidelines on quality control methods for medicinal plant materials.[12] Fluorescence analysis was carried out by the method of Chase and Pratt[13] and Kokoski.[14] Preliminary phytochemical screening

Preliminary screening was carried out by using standard procedures described by Kokate[9] and Harborne.[15] HPTLC profile

Qualitative densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile for PE, EtOAc, and EtOH extracts of leaves of B. acutangula. 10 μl of the sample solutions were applied and the plates were developed in PE and EtOAc (8:2) for PE extract, toluene and acetonitrile (3:1) for EtOAc and EtOH extracts. Developed plates were then scanned densitometrically at various wavelengths. Retention factor (R,) values, peak area, peak height, and spectrum of each peak were determined for these extracts.

**RESULTS**

**Macroscopic characters**

The leaves are simple, alternate, 7-14.5 cm in length and 4-8 cm in breadth, obovate to oblong, acute apex, decurrent base, minutely denticulate to crenate margin, reticulate venation with smooth, dark green upper surface and rough, faint green lower surface.

**Microscopic characters**

*Leaf*

The transverse section of the leaf of B. acutangula shows a dorsiventral nature [Figure 1]. The section is broadly divided into lamina and midrib region. The lamina of the leaf shows three distinct regions viz., adaxial epidermis, abaxial epidermis, and mesophyll [Figure 1a]. The adaxial epidermis is single layered with squarish cells covered by a distinct cuticle. The abaxial epidermis is also single layered with rectangular cells having prominent peg like outgrowths. The mesophyll is differentiated into palisade and spongy parenchyma. The palisade parenchyma has two layers of narrow, cylindrical, and compact cells. The spongy parenchyma consists of about 12 layers of small, lobed, loosely arranged cells with wide air-chambers.

The lateral veins have prominent vascular strands, but do not project onto the leaf surface. The lateral vein has a small group of xylem elements and few phloem elements. The vascular strand is en-sheathed by thick sclerenchymatous tissue which extends into adaxial and abaxial pillars. The leaf margin is thick and semicircular and has fairly prominent circular vascular strands. The epidermal layer shows anomocytic type of stomata.

The midrib has prominent adaxial hump and wide semicircular abaxial part [Figure 1b]. The epidermal layer of the midrib

![Figure 1: T. S of leaf (a) T.S. of lamina, (b) T.S. of midrib, (c) T.S. of petiole, (d) Crystal distribution (AdE- Adaxial epidermis, AbE- Abaxial epidermis, PM- Palisade mesophyll, SM- Spongy mesophyll, CP- Cuticular papillae, LV- Lateral vein, AdH- Adaxial hump, LB-Lateral bundle, MB- Main bundle, GT- Ground tissue, OB- Outer bundle, Cr- Crystal, Ve- Vein, Ep- Epidermis)](image)
is thin, comprising of small, thick walled squarish cells with outer papillate walls. The ground tissue is parenchymatous and homocellular. A wide mass of thick walled cells are located in the center of the adaxial hump. The vascular system of the midrib is complex. The vascular bundles are of three types; main bundle-xylem elements are thick walled, circular and it occurs in parallel radial rows with small sclerenchyma cells in between the rows and phloem occurs in thin sheath around the xylem, two circular lateral bundles and a row of six small circular vascular strands. The type of calcium oxalate crystals are druses and found in the vascular strands, veins of leaf, ground tissue of midrib and petiole as well as in phloem parenchyma [Figure 1d].

**Petiole**

Cross section of the petiole is circular with slightly flat adaxial side [Figure 1c]. It has a thin epidermal layer consisting of narrowly rectangular thick walled cells and some of them have peg like outgrowths on the tangential walls. The ground tissue consists of thick walled, angular, compact parenchymatous cells. The vascular system is complex, multi-stranded consisting of larger deeply curved main vascular bundle, two circular lateral bundles and eleven small vascular strands arranged in arcs. The main vascular bundle consists of several parallel rows of vessel multiples alternating with narrow intervening sclerenchyma cells. Phloem occurs in thick band all along the outer part of the xylem arc.

**Powder characteristics**

The organoleptic evaluation of the leaf powder revealed the following characteristics. The leaf powder is pale green in color, with a characteristic odor and bitter taste. On microscopic examination, the powder showed epidermal trichomes which are sparse [Figures 2a and 2b]. Starch grains are present in minute quantities and are spherical, concentric, and three lobed [Figure 2c]. Fibers of the petiole are wider and straight measuring 660-750 μm long and 20-25 μm wide [Figure 2d]. The trichomes are multicellular, uniseriate, and unbranched. The epidermal cells are small, polygonal thick walled, and straight. Fibers of the lamina are usually bent, narrow measuring 550-650 μm long and 15-20 μm wide [Figure 2e].

**Leaf constants**

The leaf constants viz., vein islet number, veinlet termination number, palisade ratio, and stomatal index are presented in Table 1.

**Physicochemical constants**

Ash values of a drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values [Table 2] of the leaf powder of *B. acutangula* revealed a high concentration of sulphated ash. The extractive values are primarily useful for the determination of exhausted or adulterated drug. The ethanol soluble extractive was high for *B. acutangula*. The results of fluorescence analysis of leaf powder are presented in the Table 3.

**Preliminary phytochemical screening**

Preliminary phytochemical screening mainly revealed the presence of carbohydrates, phytosterols, terpenoids, flavonoids, glycosides, tannins, and saponins [Table 4].

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**Table 1: Leaf constants of *B. acutangula***

| Leaf constants                           | Values       |
|------------------------------------------|--------------|
| Vein islet number                        | 8 - 11 / sq.mm|
| Veinlet termination number               | 13 - 27 / sq.mm|
| Stomatal index                           |              |
| a) Adaxial                               | 3 %          |
| b) Abaxial                               | 9 %          |
| Palisade ratio                           | 5 – 10       |

**Table 2: Physicochemical constants of leaf powder of *B. acutangula***

| S.No. | Parameters                          | Values (%w/w) |
|-------|-------------------------------------|---------------|
| I     | Ash values                           |               |
|       | Total ash                            | 6.00          |
|       | Acid insoluble ash                   | 0.92          |
|       | Water soluble ash                    | 3.92          |
|       | Sulphated ash                        | 7.48          |
| II    | Extractive values                   |               |
|       | Water soluble extractive            | 26.39         |
|       | Ethanol soluble extractive          | 36.89         |
|       | Non volatile ether soluble extractive| 5.40          |

**Table 3. Preliminary phytochemical screening of leaf powder of *B. acutangula***

| Test                | PE extract | EtOAc extract | EtOH extract |
|---------------------|------------|---------------|--------------|
| Terpenoids          | +          | +             | +            |
| Flavonoids          | -          | -             | +            |
| Steroids            | +          | -             | -            |
| Carbohydrates       | -          | -             | +            |
| Glycosides          | -          | -             | +            |
| Quinones            | -          | -             | -            |
| Alkaloids           | -          | -             | -            |
| Phenols             | -          | -             | +            |
| Tannins             | -          | +             | +            |
| Saponins            | -          | +             | +            |
| Gum                 | -          | -             | -            |
| Proteins            | -          | -             | -            |

+ Denotes the presence of respective compounds
As there is no pharmacognostic anatomical work on record for this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Macro and micro morphological standards and HPTLC profile discussed here can be considered as identifying parameters to substantiate and authenticate the drug. In Ayurveda, its preparations include powder and pastes, used in vitiated conditions of kapha and pitta, leprosy, dysmenorrhea, plumbago, skin diseases, diarrhea, inflammation, flatulence, hemorrhoids, and as an anthelmintic.

The preliminary phytochemical screening revealed the presence of terpenoids, flavonoids, glycosides, tannins, and saponins. These constituents may be possibly responsible for the above said activity.

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