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Research Article

PHARMACOGNOSTIC AND PHYSICOCHEMICAL STUDIES OF THE LEAVES AND STEM OF *ACTINODAPHNE MADRASPATANA* BEDD

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ABSTRACT

Objective**:** To study the detailed pharmacognostic profile of the leaves and stem of *Actinodaphne madraspatana* Bedd (Lauraceae), an important medicinal plant in the Indian system of medicine.
Methods**:** Leaf and stem samples of *Actinodaphne madraspatana* Bedd were studied by macroscopical, microscopical, physicochemical, phytochemical, fluorescence analysis of the powder of the plant and other methods for standardization recommended by WHO.
Results**:** Macroscopically, the leaves are 6 in a whorl, 10-30 cm long, coriaceous, lanceolate, oblanceolate or elliptic. Microscopically, the leaf showed the presence of sunken stomata, prominent midrib, dorsiventral lamina, three stranded vascular system, small xylem and phloem elements with a vertical extension of bundle sheath fibres, tannin, non glandular trichomes and small epidermal peeling of the stem. Physicochemical parameters such as extractive values, ash content and fluorescent behavior of leaf powder were also determined. Preliminary phytochemical screening showed the presence of triterpenoids, flavonoids, glycosides and steroids.
Conclusions**:** This is the first report on the pharmacognostic and physicochemical studies of *Actinodaphne madraspatana* Bedd and is helpful in the characterization of the crude drug.

Keywords: *Actinodaphne madraspatana* Bedd, Pharmacognostic, Physicochemical, Fluorescence behavior

INTRODUCTION

Actinodaphne madraspatana (*A. madraspatana*) Bedd is belongs to the family Lauraceae. It is commonly known as 'Putta Thali' in Tamil, 'Ray Laurel' in English, 'Irolimarom, Mungali' in Malayalam, 'Kovangutti' in Telugu [1-3]. It is a medium-sized evergreen tree and Shrub, widely distributed Common on the rock hill slopes at higher elevations, Aruku valley, Vishakhapatnam District, Talakona, Dharmagiri, Microwave station, on the way to Thumburu Theertham [4-6]. Leaves are 4-6 in a whorl, 10-30 cm long, coriaceous, lanceolate, oblanceolate or elliptic. Flowers are small, dioecious, yellowish, the males in clusters of about 8, the females umbellate or sub-racemose on very stout peduncles. Berry 8 mm across, ellipsoid, red when ripe. The plant flowering and fruiting during the January to July [7]. The Leaves of the plant are used traditionally to cure wounds, cure mania, fickle minded behavior and diabetic [8]. However the available literature revealed that no pharmacognostic study has been carried out on the plant; hence the present investigation was undertaken. The object of the present study is to evaluate various pharmacognostical parameters such as macroscopic, microscopy, physicochemical, fluorescence and phytochemical studies of the plant.

MATERIALS AND METHODS

Plant material

A. madraspatana plant leaves were collected from Talakona forest near to Tirupati, Andhra Pradesh, India in the month of July and were authenticated by Dr. K. Madavachetty, S. V. University, Tirupati, Andhra Pradesh. A voucher specimen (ACD) has been kept in the Herbarium of the Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh, India-524346.

Pharmacognostic study

Fresh leaves and stem were taken for morphological and histological studies. Coarse powder (60≠) was used to study microscopical characters, physicochemical parameters and phytochemical investigation. The macrosopy and microscopy of the leaf and stem were studied according to the method of Brain and Turner (1975) [9, 10].

For the microscopical studies, the plant material was fixed in a mixture of solvents containing formalin, acetic acid and ethanol (70% v/v) for histological studies. After 24 hours of fixing, the specimens were dehydrated with a graded series of tertiary butyl alcohol as per the schedule given by Sass 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax until tertiary butyl alcohol solution attained super saturation. The specimens were cast into paraffin blocks. Transverse sections (T.S.) of the different organs of the plant materials were taken using a rotary microtome and stained with different stains. Microphotographs of the sections were made by using Nikhon Labhot 2 microscopic units [11-16].

Physicochemical and phytochemical analysis

Physicochemical values such as percentage of ash values and extractive values were determined according to the well established method and procedure [17]. Preliminary phytochemical screening was carried out by using standard procedure described by Kokate and Harborn [18, 19].

Powder Microscopy, Fluorescence Analysis and Leaf constants

The leaf and stem powder of *A. madraspatana* was analyzed under the dark field and bright field microscope for powder characteristics. Fluorescence analysis of the leaves and young stem powder of *A. madraspatana* was observed in daylight and UV light (long-365 nm and short-254 nm). Leaf constants such as stomatal number, stomatal index, vein islet number and vein termination number was carried out by using standard procedures described by Kokate and Lala [20-23].

RESULTS

Macroscopic characteristics

Macroscopically, fresh leaves are 6 in a whorl, 10-30 cm long, lanceolate, coriaceous, oblanceolate or elliptic. Flowers are small, dioecious, yellowish, the males in clusters of about 8, the females umbellate or sub-racemose on very stout peduncles. Berry 8 mm across, ellipsoid, red when ripe.

Microscopic characteristics

Anatomy of the leaf

The leaf consists of thick and prominent midrib and their lamina (Figure 1). The midrib hemispherical in cross sectional view with convex adaxial side and wide and thick bowl shaped abaxial part. The midrib is 900µm thick and 1.9 mm wide. The epidermis of the midrib is very thin and consists of small squarish cells containing dark inclusions (Figure 2). The cuticle is fairly prominent. The ground tissue of the midrib is parenchymatous. The cells towards the periphery of the midrib are smaller and the size of the cells gradually increases towards the interior of the midrib. The cells are angular, thin walled and compact. These wide circular secretory cavities in the ground tissue which are surrounded by a layer of semicircular cells. These cavities are secretary structures. The secretary cavities are 50 µm in diameter.

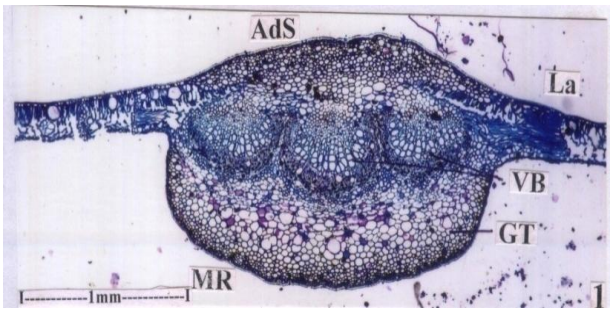

Fig. 1: T.S of leaf through midrib (Ads-Adaxial side, GT-Ground tissue, La-Lamina, MR-Midrib, Sc-Sclerenchyma, VB-Vascular bundle)

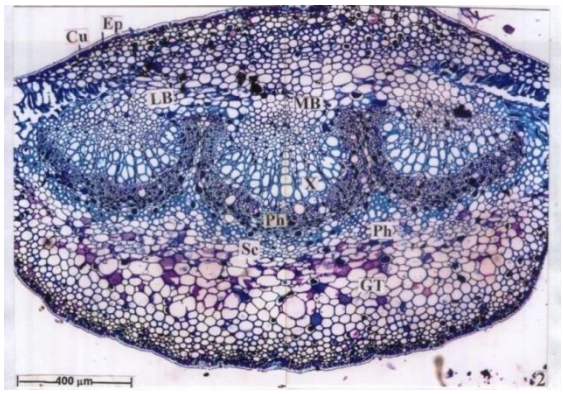

Fig. 2: T.S of midrib enlarged (Ads-Adaxial side, Cu-cuticle, Ep-epidermis, GT-Ground tissue, La-Lamina, LB-Lateral bundle, MB-Median bundle, MR-Midrib, Sc-Sclerenchyma, VB-Vascular bundle)

The vascular system is three stranded and are three large, arc shaped vascular strands of equal size, placed in a horizontal row. The strands are collateral with adaxial xylem and abaxial phloem. The xylem elements are in long, parallel rows; these are five xylem cells in the middle and two cells in the lateral ends. Phloem occurs in a thick deep arc on the lower part of the xylem. A thin continuous layer of fibers occurs enclosing the three vascular strands both on the abaxial and adaxial sides (Figure 3).

The lateral is thick and projects much below the lower part of the lamina in the form of dome shaped outline. This is a single, triangular, prominent collateral vascular strand located in the upper part of the lateral vein. The lateral vein is 470 µm thick and 300 µm wide.

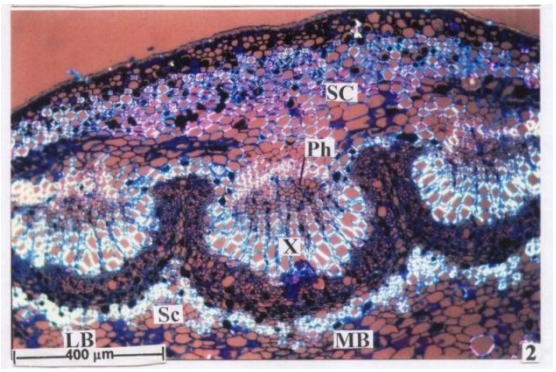

Fig. 3: Vascular strands of the midrib viewed under polarized light showing lignified sclerenchyma layer and xylem elements (SC-Secretary canal, Ph-Phloem, X-Xylem, LB-Lateral bundle, MB-Median bundle)

Lamina

The lamina is dorsiventral with smooth and even surfaces having 400 µm thick. The adaxial epidermal cells are squarish and thick walled; the cuticle is thick. The abaxial surface consists of hemispherical cells with outer tangential walls being papillate. The vascular strands of the veinlets occur in horizontal and row of vertical pillars. The vascular strand consists of a small xylem and phloem elements with a vertical extension of bundle sheath fibers (Figure 4). The mesophyll consists of adaxial layers of the cylindrical palisade cells and abaxial zone of small, lobed spongy parenchyma cell.

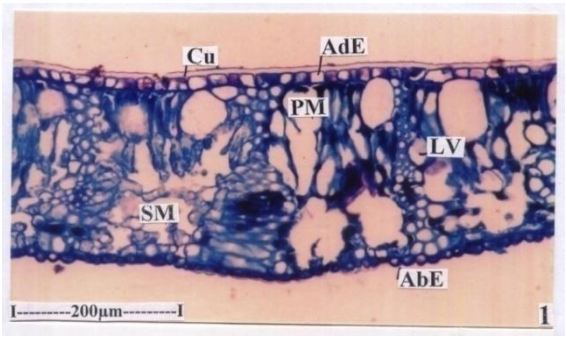

Fig. 4: T.S of lamina showing mesophyl tissues, lateral veins and secretory cavities (Cu-Cuticle, AdE-Adaxial epidermis, PM-Palisade mesophyll, LV-Lateral vein, SM-Spongy mesophyll, AbE-Abaxial epidermis)

Leaf margin

The marginal part of the lamina is blunt and 170 µm thick. It is semicircular along the margin and slightly bent down. The epidermal layer of the margin consists of small thick walled cells with prominent cuticle. The mesophyll tissue is replaced by a compact mass of small, thick walled sclerenchymatous elements (Figure 5).

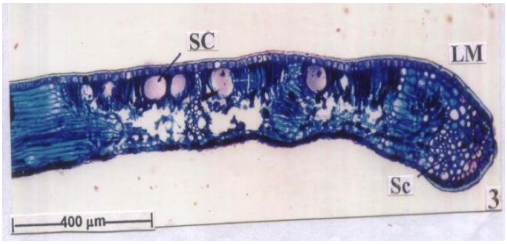

Fig. 5: T.S of lamina along the marginal part (SC-Secretary canal, LM-Leaf margin, Sc-Sclerenchyma)

Venation and leaf clearing

The venation system is reticulate and the veins are thick and straight. The vein islets are rectangular, squarish or polyhedral. The boundaries of the veins islet are thick and straight. The vein terminations are less frequent. Secretary cavities are abundant in surface view. The lamina after the clearing was examined with their surface view. The venation is densely reticulate. The vein islets are variable in shape and size and are mostly polygonal in outline. Vein terminations are present as short and thick (Figure 6).

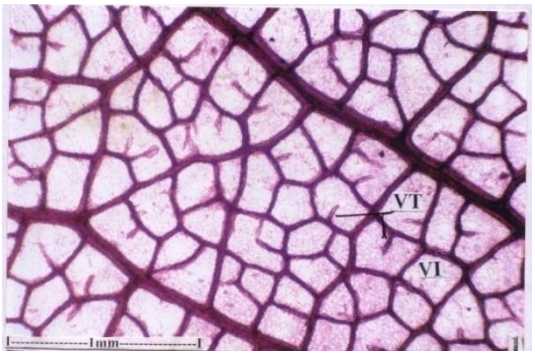

Fig. 6: Venation pattern of the lamina as seen in cleared leaf (VI-Vein islet, VT-Vein termination)

Epidermal cells and stomata

The epidermal surface view was studied from the paradermal sections of the lamina. The epidermal cells of adaxial side are apostomatic (without stomata). The epidermal cells are polygonal in outline with fairly thick and slightly wavy auticlinal walls. The walls are smooth (Figure 7). The abaxial epidermis is stomatiferous (having stomata). The stomata sunken in the intercortal cavities. The stomata are cyclocytic type, i.e, each stoma is surrounded by four or more large subsidiary cells. The guard cells are circular to elliptic, measuring 25x30 µm in size. The abaxial epidermal cells are polygonal in outline.

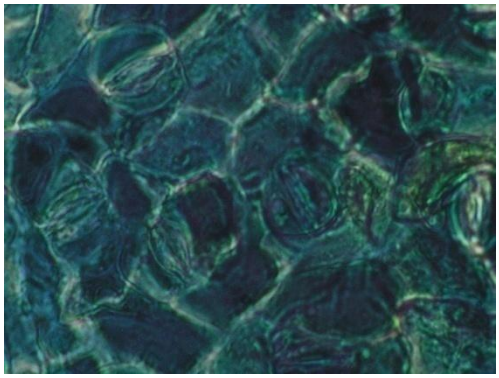

Fig. 7: Abaxial epidermis showing the sunken stomata

Petiole

The petiole is semicircular in sectional view, 1.7 mm thick and 2 mm wide. The abaxial part is convex and the adaxial part is slightly raised (Figure 8). The epidermis is thin with small circular cells bearing dense trichomes. The ground tissue consists of outer zone of smaller, compact parenchyma cells and inner zone of larger cells. Circular, wide secretary cavities are often present in the ground tissue. Tannin is abundantly present in the ground cells. The vascular system includes horizontally placed a straight row of three arc shaped vascular strands which are very close to each other. The strands have fairly long compact several rows of xylem elements and thick arc of phloem elements along the lower part of the vascular strand. Secretary cavities are found within the phloem zone.

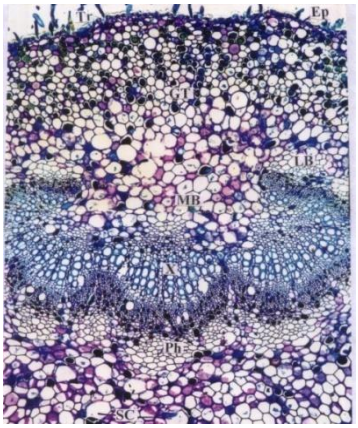

Fig. 8: T.S of petiole a sector view (LB-Lateral bundle, MB-Median Bundle, Ph-Phloem, GT-Ground tissue, Tr-Trichome, Ep-Epidermis)

Stem

The stem is 3mm thick with well developed secondary growth and is circular in outline. The epidermal ray is intact comprises small thick walled cells and small trichomes. The cortex is narrow, parenchymatous and most of the cells have dark tannin content. The inner boundary of the cortex consists of thin, more or less continuous sclerenchyma elements (Figure 9). The vascular cylinder has outer thick and continuous layer of secondary phloem which includes phloem parenchyma and small sieve elements. Phloem rays are also distinct and posse's tannin. Secondary xylem cylinder is hollow and thick consisting of dense radial multiples of vessels, narrow thick walled and lignified fibres. Xylem rays are thin and straight. The vessels are up to 50 µm in diameter and are angular or elliptical with thin walls.

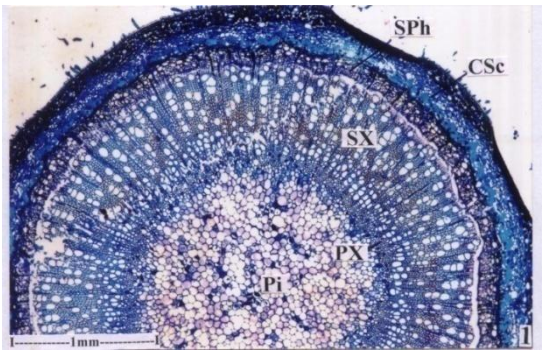

Fig. 9: T.S of stem-ground plan (CSC-Cortical sclerenchyma, Pi-Pith, PX-protoxylem, SX-Secondary xylem, SPh-Secondary phloem)

Powder microscopic characteristics

Small epidermal peeling of the stem is occasionally seen. In surface view the epidermal cells are in vertical parallel lines, the cells being rectangular or squarish. The cells have thick walls. Fibres and vessel elements are seen as isolated elements. The vessel elements are narrow, 450 µm long and possess dense circular lateral wall pits. Some of the vessel elements have horizontally elongated scalariformed pits. The end wall perforation is simple, mostly oblique and wide. Fibres are libriforem type and are very 600 µm long, narrow thick walled and gradually tapering at the ends. Some of the fibres are separate (Figure 10).

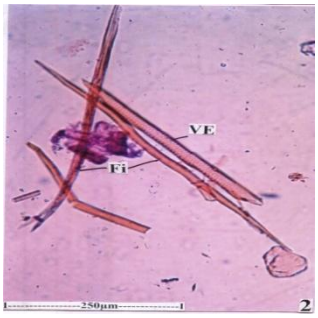

Fig. 11: Fibres and vessel elements

Preliminary phytochemical screening

Preliminary phytochemical screening of leaf mainly revealed the presence of triterpenoids, flavonoids, glycosides and steroid.

Physicochemical parameter

Physicochemical analysis of leaf and stem bark powder viz. ash value and extractive value. The results are presented in Table no.1. The fluorescence analysis of *A. madraspatana* leaf and stem bark under day light and UV (Short 254 and long 365 nm) light. The results are presented in Table no. 2.

Table 1: Ash and extractive values of the powdered leaf of *A. madraspatana* Bedd[a]

Ash values		Extractive values	
Types of ash	**% of ash**	**Types of extractive**	**% of extractive**
Total	7.48±0.026	Petroleum ether soluble	2.24±0.015
Water soluble	1.24±0.023	Chloroform soluble	4.12±0.015
Acid insoluble	0.53±0.002	Methanol soluble	6.54±0.140
Sulfated	5.90±0.182	Ethanol soluble	11.40±0.110
		Water soluble	3.84±0.168

[a]All values are means of triplicate determinations expressed on dry weight basis. ± denotes the standard error.

Table 2: Fluorescence analysis of powder of leaf and stem of *A. madraspatana* Bedd

Parts used	Treatment	Day Light	Short UV Light (254nm)	Long UV Light (365nm)
Leaves	Powder as such	Light green	Yellowish green	Green
	Powder + 1N NaOH (aqueous)	Brown	Black	Blackish green
	Powder + 1N NaOH (alcoholic)	Chocolate brown	Light green	Lower-brown Upper-green
	Powder + 1N HCl	Pale brown	Black	Dark green
	Powder + 50% H_2SO_4	Yellowish green	Black	Dark green
Stem	Powder as such	Yellow	Pale yellow	Pale green
	Powder + 1N NaOH (aqueous)	Brown	Dark green	Light green
	Powder + 1N NaOH (alcoholic)	Light brown	Light green	Bluish green
	Powder + 1N HCl	Light brown	Green	Pale green
	Powder + 50% H_2SO_4	Brown	Blackish brown	Blockish green

Leaf constants

Leaf constants such as stomatal number, stomatal index, vein islet number and vein termination number were performed. The results are presented in Table no. 3.

Table 3: Leaf constants data of the *A. madraspatana* Bedd

S. No	Leaf Surface	Data Values (per Sq.mm)
1	Stomatal Index of Lower Epidermis	16
2	Vein Islet Number	9.25
3	Vein Termination Number	12.50
4	Stomatal Number	10

DISCUSSION

The evaluation of a crude drug is an integral part of establishing the correct identification of a plant material. For this, pharmacognostic and physicochemical parameters must be determined. In this regard, the microscopic and macroscopic features of leaf have been studied. Studies revealed the presence of a sunken type of stomata, glandular trichomes, three stranded vascular system and tannin which are the characteristic features of lauraceae family. Studies of physicochemical constants can serve as a valuable source of information and are usually used in judging the purity and quality of the drug. The extractive values give an idea about the chemical constitution of the drug and from the study, the extractive value of alcohol was highest followed by water. The ash value determines the earthy matter or inorganic composition and other impurities present along with the drug. The pharmacognostic standard for the leaves of *A. madraspatana* Bedd is laid down for the first time in this study.

CONCLUSION

This study could be used as a diagnostic tool for the standardization of this medicinal plant and will helpful in the characterization of the crude drug.

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