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PHARMACOGNOSTIC INVESTIGATION ON *ELAEOCARPUS BLASCOI* WEIBEL LEAVES

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ABSTRACT

Pharmacognostic investigation of the fresh leaves used for anatomical sections of *Elaeocarpus blascoi* Weibel was carried out to determine its macro- and microscopical characters. Externally, the leaves possess Leaves simple, alternate, spiral, clustered at twig ends; stipules caducous; canaliculate, sparsely adpressed hairy; elliptic-ovate, apex acute to shortly acuminate with blunt tip, base rounded, margin shallowly serrate, glabrous; midrib at the base. Internally, its shows the presences of anomocytic stomata, multicellular, covering trichomes with an acute apex, prism and clustered crystals of calcium oxalate and fiber elements. The chemo-microscopy revealed the presences of lignin, starch grains and calcium oxalate crystals. Physico-chemical evaluation includes ash values, extractive values and moisture content. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

KEYWORDS

Elaeocarpus blascoi Weibel, Pharmacognostic investigation, leaves

INTRODUCTION

Elaeocarpus blascoi Weibel (Elaeocarpaceae) is a tree found in only Vattakanal shola, Western Ghats, India [1-4]. The status of the species is endemic to possibly extinct. The author Weibel (1972) described *E.blascoi* as endangered whereas it is redescribed as possibly extinct by the IUCN reports[5-6]. Traditionally the plant is used to treat various diseases. Anatomical characterization of plants used in traditional medicines has been carried out to evolve standards for genuine source plant from the spurious ones. The different treatise on medicinal plants had mentioned different uses of parts or products[7-8] .The anatomical features of the plant can be utilized to determine the identity. Microscopic quantitative characters had been attributed with

diagnostic values by the earlier pharmacognosists[9-10]. Pharmacognostic studies have not been reported for the leaves of this plant. Therefore the main aim of the present investigation is to study the macro, microscopic and some other, Pharmacognostic characters and physicochemical standards of leaves of *E.blascoi* Weibel which could be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Sample Collection and Authentication: *Elaeocarpus blascoi* Weibel plant specimens for the proposed study were positively identified the author and confirmed, certified by Botanical survey if India (Southern circle), Coimbatore. A voucher specimen (BSI/sc/5/23/09-10/tech.1405) has been deposited in the herbarium of Department of Botany, Bishop Heber College (Autonomous), Tiruchirappalli, Tamil Nadu collected from Palni hills, belongs to the Elaeocarpaceae family has been identified and recorded only in Bear shola, Pambarpuram at Kodaikanal as one of the endemic and endangered tree of the Western Ghats in Peninsular India with medicinal and timber value. Healthy plant parts were selected carefully for the purpose of study. The required samples of different parts were cut and removed from the plant and fixed in FAA (5 ml of Farmalin, 5 ml of Acetic acid and 90ml of 70% Ethyl alcohol). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary- Butyl alcohol as per the schedule given by Saas[11]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 ºC) until the TBA solution attained super saturation[12]. The specimens were embedded in paraffin blocks.

Morphological Studies: The morphological features of the plant parts were studied with the help of dissection microscope and photographs were taken with the help of Nikon D50 Digital Camera.

Anatomical Studies: The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm and Dewaxing of the sections was done by customary procedure[13]. The sections were stained with Toluidine blue as per the method published by O'Brien et al., 1964[14]. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the protein bodies. Wherever necessary sections were also the stained with Safranin and fast - green. For studying the stomatal morphology, venation pattern and trichome distribution, peradermal sections as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [11] were prepared. Glycerin mounted temporary preparations were made for macerated and cleaning materials. Powder materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell compound were studied and measured.

Photomicrographs: Microscopic descriptions of tissues were supplemented with microphotograph wherever necessary. Photographs of different magnifications were taken with Nikon Lab photo 2 Microscopic Unit. For normal observations bright field microscope was used. For the study of crystals, starch grains and lignified cells, polarized – light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures were indicated by the scale-bars. Descriptive terms of the anatomical features were as given in the standard Anatomy books[15].

Pharmacognostic investigation: Morphological studies were done by using simple microscope. The shape, apex, base, margin, taste and odor of leaves were determined. Microscopic studies were done by preparing thin rotary microtome section of leaves of *Elaeocarpus blascoi* Weibel. The section was cleared with chloral hydrate solution, stained with phloroglucinol -hydrochloric acid (1:1) and mounted in glycerin. A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder (#60) of

the dried leaves was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol-hydrochloric acid (1:1) solution, acetic acid and iodine solution to determine the presence of lignified fibres, vascular bundles, trichome, calcium oxalate crystals and starch grains[16]. As a part of quantitative microscopy, stomatal number, stomatal index, vein-islet and vein-termination number were determined by using fresh leaves of the plant[16].

Physico-chemical evaluations: Total ash, acid-insoluble ash, water-soluble ash, sulphated, ethanol ash and methanol ash were determined. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content was also been determined[17].

RESULTS AND DISCUSSION

E. blascoi were observed to be Tree; branchlets and tender parts sericeous. Leaves ovate – elliptic, to 9x4cm, coriaceous, base obtuse, margin serrate, apex acute; Petiole 2cm (Plate **1**). The *E. blascoi* leaves having characteristic odor, bitter in taste and length varying from 4 – 9 cm. In the microscopic studies, the leaf is dorsiventral, and shows all the typical characteristics of leaf, as lamina part shows presence of epidermis, upper palisade, a and middle spongy parenchyma while midrib region shows upper and lower epidermis, collenchyma and centrally vascular bundle as phloem surrounds with the xylem. The leaves showed the presence of multicellular, slightly lignified covering trichomes on both the surfaces.

Microscopic features of the leaf: The leaf has prominent abaxial conical midrib and thick leathery lamina (**Plate. 2a**). The adaxial part of the midrib is convex. The abaxial conical part has short-cylindrical, nipple like projection. The midrib is 1.1mm in vertical plane and 1.15mm in horizontal plane. The abaxial conical part is 250µm thick.

The midrib has thin layer of epidermis comprising of small, circular thick walled cells. The ground tissue around the vascular structure is parenchymatous; the cells are circular or angular with thin walls (**Plate. 2.a; 3.a).**

The vascular structure of the midrib is complex. The vascular system consists of an abaxial, thick are and wide block of adaxial of part; there may one or two lateral strands of vascular tissues (Plate. 4.a). The abaxial strand is collateral with a horizontal band of short parallel lines of elliptical, radially oblong thick walled xylem elements; phloem consists of randomly oriented, fairly wide sieve elements (**Plate. 3.a,b).**

The adaxial vascular block consists of an upper bands of xylem and phloem elements and lower, thin bands of outer xylem and inner phloem. The xylem forms a circle which is surrounded by phloem (**Plate. 3.a).** The lateral strands seem to be fragmented masses of the many strands. They also have collateral xylem and phloem elements. The entire vascular system is ensheathed by a prominent cylinder of sclerenchyma elements.

Lamina (**Plate. 4.b,c**): The lamina is 200 µm thick. The surface is smooth and even. It is distuietly dorsiventral. The adaxial epidermis has vertically oblong cells with thick cuticle. The abaxial epidermis is 20 µm thick, while the abaxial epidermis is less than 10 µm thick.

The mesophyll tissue the tissue is differentiated into adaxial zone of two layers of palisade cells and abaxial zone of spongy mesophyll tissue of about five layers of large, loosely arrangered cells. The palisade cells are narrowly cylindrical, less compact and 70 µm in height. The lateral veins circular and prominent they are

collateral with adaxial cluster of xylem, abaxial phloem, subdivided by a pad of sclerenchyma cells (**Plate. 4.c).**

The leaf margin is conical and element. It has thick epidermal layer with prominent cuticle. The mesophyll prominent tissue is undifferentiated and compact parenchyma cells **(**Plate. 2.b). The marginal part is 110 µm thick.

Epidermal cells and Stomata: The adaxial epidermis is apostomatic (without stomata). The epidermal cells are small, polygonial in outline with fairly thick, straight walls **(**Plate. 4.a).

The abaxial epidermis is stomatiferous. The stomata are cyclocytic; a stoma is surrounded by 5 or 6 subsidiary cells. The guard cells by elliptic, wide and possess slit like aperture. The epidermal cells are wider, polygonal and have thick, straight or slightly curved walls **(**Plate 4.b).

Venation of the lamina: The lamina exhalents dense reticulate venation. The vein is lets are wide and distinct. They are squarish or polyhedral in outline. The vein terminations are well developed and most of them are branched repeatedly forming a dendroid (free-like) outline (Plate **5.c).**

Crystals in the lamina (Plate 4.d): The calcium oxalate crystals are aboundant in the lamina. The crystals are prismatic type; they are rhomboidal, cuboidal or polyhedral in shape. Structure of the distal parts (upper end). The structure of the petiole in these two regions is different.

Microscopic study of powder revealed the presence of anomocytic stomata, oval, rounded starch grains, pitted xylem vessels, which are present in bundles and also clusters type of calcium oxalate crystals. Chemo-microscopy revealed the presence of starch grains, lignin and calcium oxalate crystals. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The stomatal number, stomatal index, vein-islet number, vein-termination number relatively constant for plants and can be used to differentiate closely related species. The results are depicted in Table 1.

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14%w/w.10 The ash values, extractive values and moisture content of leaves were determined. The results are depicted in Table 2.

Pharmacognostic analysis including physico-chemical evaluation is meant for identification, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs. Since the plant, *E. blascoi* is useful in traditional medicine for the treatment of some ailments, it is important to standardize it for use as a drug. The pharmacognostic constants for the leaves of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

Table 1.

Quantitative Microscopy of leaves of Elaeocarpus blascoi Weibel – an endangered tree

S.No	Parameters	Range	Mean*
1	Stomatal number		
	Adaxial Epidermis	8 -10	11

	Abaxial Epidermis		
2	Stomatal index	10 -14	
	Adaxial Epidermis		15
	Abaxial Epidermis		
3	Vein islet number	15 - 20	19
4	Vein termination number	40- 45	42

*Mean value of three counts

Table 2.

Physicochemical analysis of leaves of Elaeocarpus blascoi Weibel – an endangered tree

S. No	Physicochemical constants	Percentage (%)
1	Total Ash	7.23
2	Acid – insoluble Ash	4.20
3	Water – soluble Ash	5.44
4	Sulphated Ash	0.70
5	Ethanol soluble Ash	8.30
6	Methanol soluble Ash	7.40
7	Water soluble extractive	6.45
8	Moisture content	3.10

FIGURES

Plate 1: Morphological view of Elaeocarpus blascoi Weibel. Leaves

Plate 1

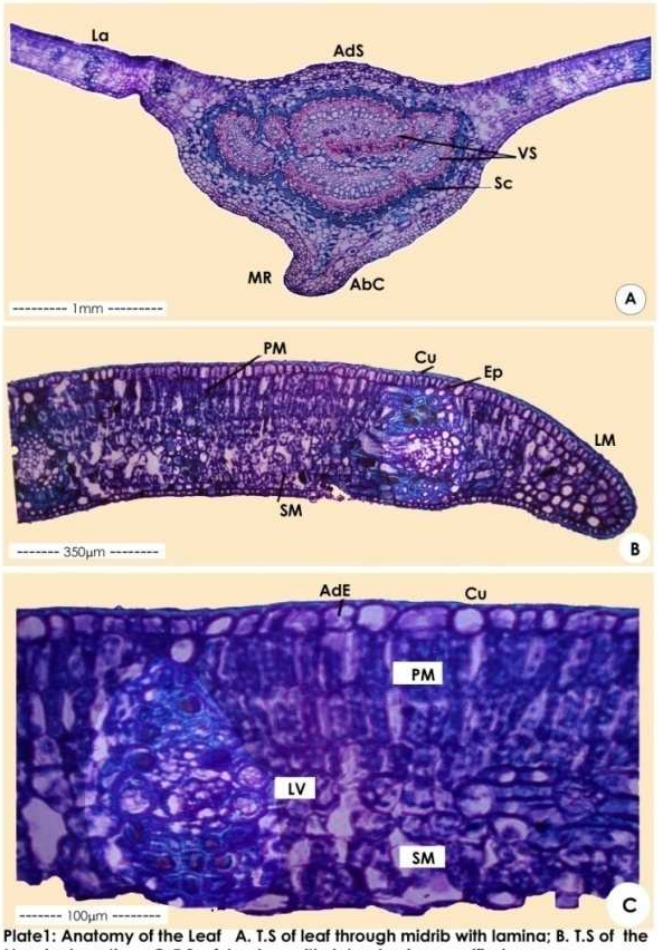

Plate1: Anatomy of the Leaf A. T.S of leaf through midrib with lamina; B. T.S of the Marginal portion; C. T.S of lamina with lateral vein magnified. (**AbC** - Abaxial Cone; **AdE** - Adaxial epidermis; **AdS** - Adaxial Side; **Cu** - Cuticle; **Ep** - Epidermis; **La** - Lamina; **LV** - Lateral Vein; **LM** - Leaf Margin; **MR** - Midrib; **PM** - Palisade Mesophyll; **Sc** - Sclerenchyma; **SM** - Spongy Mesophyll; **VS** - Vascular Strand)

PLATE 2

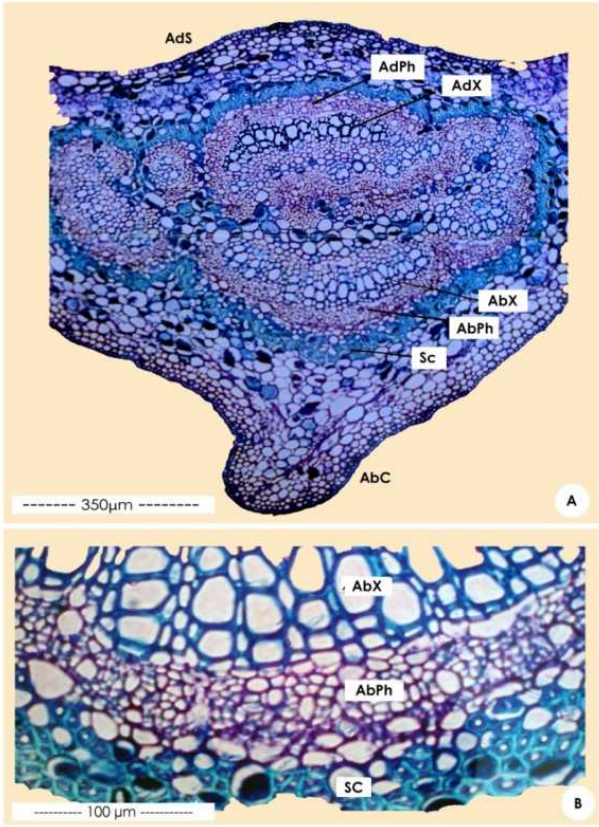

Plate 2: Structure of the Midrib (a).T.S of midrib-Magnified (b). Midrib vascular bundle Portion enlarged (AbP -Abaxial Phloem; AbC-Abaxial Cone; AbX -Abaxial Xylem; SC- Sclerenchyma; AdS -Adaxial Side; AdX -Adaxial Xylem; Adph -Adaxial Phloem)

PLATE 3

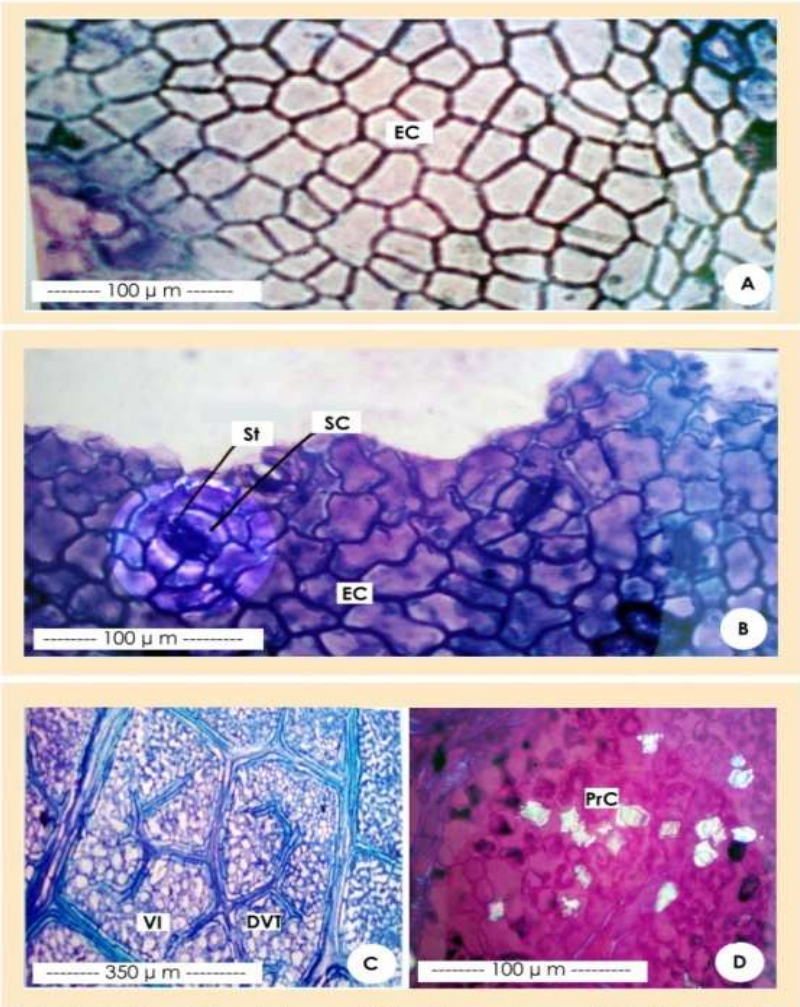

Plate 3 : Epidermal Morphology and Venation pattern and Crystal distribution (A). Adaxial Epidermis (B). Abaxial epidermis with Stomata (C). Vein - islets and termination (D). Primatic crystals in the mesophyll tissue (as seen under Polarized light microscope) (**EC-** Epidermal Cells; **SC-**Subsidiary cell; **DVT-** Dendroid vein termination; **St- Stomata; VI-** V ein-Islets; **PrC-** Primatic Crystals)

PLATE 4

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