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Original Article

PHARMACOGNOSTIC STUDIES AND HPTLC FINGERPRINTING OF *BLUMEA ERIANTHA* DC (ASTERACEAE) LEAVES

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ABSTRACT

Objective: To study the pharamacognostic, morphological, microscopical characters of leaves of *Blumea eriantha* DC also to carry out the chemical analysis of its leaf powder and establish the chromatographic fingerprint.

Methods: Anatomical investigations and fluorescence analysis were carried out as per the standard techniques. Various quantitative parameters like ash values, extractive values and moisture content can be used as quality control parameters for *B. eriantha* were determined. The air dried leaf powder was extracted with methanol and fingerprinting pattern was developed by using High Performance Thin Layer Chromatography (HPTLC) technique.

Results: The epidermal study of leaves revealed several stalked glandular trichomes and multicellular uniseriate trichomes on both the epidermis of the leaves. Fluorescence analysis of leaves powder of *B. eriantha* revealed a range of colours from dark green to brownish black under short Ultra Violet (UV) light. The HPTLC fingerprint of methanolic extract of *B. eriantha* leaves showed six well resolved components.

Conclusion: The pharamacognostic studies carried out for *Blumea eriantha* DC and presented here provide referential information for the identification of this crude drug and will also help in distinguishing it from its adulterants.

Keywords: *Blumea eriantha*, Phamacognostic studies, Fluorescence analysis, HPTLC, Trichome.

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda & Siddha. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plant parts to be potential sources of medicinal substances [1]. A large number of Indian medicinal plants have been screened by scientists of various disciplines viz., agriculture, botany, chemistry, pharmacology, toxicology and clinical sciences.

In the present study leaves of *Blumea eriantha* belonging to the family Asteraceae is selected for the pharmacogenetics study. It is commonly known as "Blumea camphor" and in Marathi it is known as "Nimurdi".

It is a slender, perennial herb, widely distributed in different parts of India; commonly found occurring in the states of Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh, Bihar and Orissa. It grows, up to 1 m in height often dichotomously branched, covered with white and silky hairs, Leaves 0.02-0.19 x 0.006-0.06 m, lower obovate, short-petioled, upper elliptic-ovate to oblanceolate, cordate-clasping; capitula axillary or terminal, with numerous yellow floret; achene brown, shining, with white pappus.

The leaves contain an essential oil which is minty with caraway type backnote, and is a source of camphor. The chief constituents of its oil are d-carvotanacetone, l-tetrahydrocarvone and an alcohol [2]. The essential oil shows insecticidal, antibacterial and antifungal properties and may be incorporated in dermatological medicaments. The juice of the herb is administered as a carminative.

According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken [3].

Though this plant is widely used for its multiple properties, it has not been standardized pharma cognostically and chromatographically. Therefore, the objective of the present study is to evaluate various pharmacognostic standards like macroscopy, microscopy, ash values, extractive values, microscopical characters of powdered leaf and development of HPTLC fingerprinting pattern for *B. eriantha* leaves.

MATERIALS AND METHODS

The leaves of *B. eriantha* were collected from Sawale Village of Panvel district, Maharastra, India. The plant specimen was identified from Botanical Survey of India, Western Circle, Pune, India.

Microscopic analysis

For anatomical investigations, collected material was washed and fixed in Formalin–Acetic Acid–Ethyl alcohol (FAA) and standard microtome techniques were followed [4]. Transverse sections of 10 to 15 x 10^{-6} m thicknesses were carefully taken and stained with safranin-fast green series. Photographs were taken under a Leica DM 2000 microscopes connected to a digital camera.

For micromorphological investigation i.e. for leaf constants, fresh material as well as fixed material could be used and standard peel study was followed. Stomatal index, trichome index, palisade ratio, vein-islet and vein termination numbers were calculated by using standard methods [5].

For powder study, dried plant material was finely powdered and sieved through BSS mesh no.85. The fine powder obtained was stained using 1% Safranin in water. The stained powder was mounted on a slide and observed under a microscope to locate and identify the botanical characters. The characters observed were photographed under a Leica DM 2000 microscope connected to a digital camera.

Fluorescence analysis

This study was carried out as per the standard procedures [6]. In the present study, the plant powder was treated with 1N hydrochloric

acid, 50% sulphuric acid, 40 % aqueous sodium hydroxide and 40 % ethanolic sodium hydroxide.

Proximate analysis

The various physiochemical parameters like ash values, extractive values and moisture content were determined by the standard methods [7, 8].

High performance thin layer chromatographic (HPTLC)

Accurately weighed 250 mg of leaves powder of *B. eriantha* was taken in test tube. To this 5 ml of methanol was added. The test tube was then kept on test tube shaker for 90 min. After extraction the extract was filtered through Whatman filter paper no. 41. The filtrate was then used for the development of HPTLC fingerprinting pattern. 10 µl of the sample was then accurately spotted on Silica gel 60 F_{254} pre-coated plate with a band length of 8 x 10^{-3}m by Linomat 5 sample applicator. After sample application, plate was developed up to 0.08 m in Toluene: Ethyl acetate: Formic acid (6:3:1 v/v/v) mobile phase. The plate was then air dried and scanned using densitometer at 254 nm (Camag TLC Scanner-3).

RESULTS AND DISCUSSION

Transverse section of leaf

Transverse sections of leaves showing lamina and midrib were studied (fig. 1, A-D). The leaf was dorsiventral. Midrib region was slightly rectangular to oval in shape and it projected out towards the lower epidermis. In the midrib region the cell size of epidermal cells, was almost same in both adaxial (Upper epidermis, approx. 12x10^{-6}m x 5x10^{-6}m) and abaxial (Lower epidermis); but towards the lamina portion, the epidermal cells were larger at adaxial than at the abaxial surface. Epidermis was covered with the cuticle. Trichomes were present at both the epidermal layers. Glandular trichomes with stalk and multicellular trichomes, both were present.

The mesophyll (lamina portion) consists of 2 layers of elongated, compactly arranged palisade tissues (approx. 16x10^{-6}m x 2.7x10^{-6}m) which were present towards the upper epidermis and 3-4 layers of isodimetric loosely arranged spongy chlorenchyma tissues with air cavities are present towards the lower epidermis. The midrib consists of mainly vascular tissues/vascular bundles in the center.

The vascular bundle was surrounded by the parenchymatous tissue, which makes the ground tissue. There was a single layer of collenchyma present above the lower epidermis. Vascular bundle was made up of xylem and phloem tissues. Xylem vessels were arranged in rows. The big sized vessels known as metaxylem were present towards the lower epidermis and small vessels known as protoxylem were present towards the upper epidermis. Xylem was surrounded by phloem only at lower side of xylem tissue.

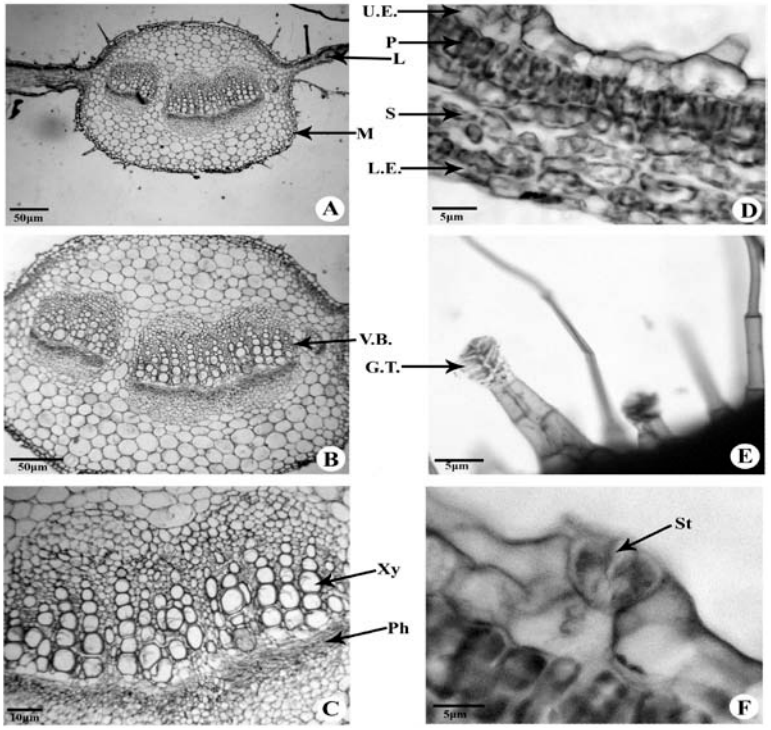

Fig. 1: Microscopic images of Transverse sections of leaves of *Blumea eriantha*

Key: A: T. S. of Leaf U. E.-Upper Epidermis; B: T. S. of Leaf showing midrib L. E.-Lower Epidermis; C: T. S. of Leaf showing vascular bundleL-Lamina of the Leaf; D: T. S. of Lamina (High Magnification) M-Midrib; E: Trichome at higher magnification P-Palisade tissue; F: Upper epidermis shows stomata S-Spongy tissue; V. B.-Vascular Bundle; G. T.-Glandular Trichome; Xy-Xylem; Ph-Phloem; S-Stomata

Determination of leaf constants

The leaf constants i.e. micro morphological study includes different parameters like type of stomata, stomatal frequency and different types of trichomes. Leaf constants such as stomatal index/mm^2, trichome index/mm^2, vein-islet number/mm^2, vein termination number and palisade ratio were calculated and data is represented in the table 1. The stomatal type was decided on the basis of the arrangement of the subsidiary cells around the stomata. In *B. eriantha*, the subsidiary cells were indistinguishable from the guard cells of the stomata, based on this the stomatal type was identified to be anomocytic (fig. 2, C). The wall of subsidiary cells was undulating. The frequency of the stomata was more on the upper epidermis than the lower epidermis; this feature is not commonly found in plants. In *B. eriantha*, two structurally different types of trichomes were observed-glandular and non glandular trichomes (fig. 2, D).

Both types of trichomes were present on both epidermises. The frequency of glandular hairs was observed to be more than that of the non-glandular trichomes. Non-glandular trichomes were unicellular & multicellular, uniseriate type with stalk and the glandular trichomes present were stalked.

Table 1: Calculated values of leaf constants of *Blumea eriantha* DC

Leaf constant	*Blumea eriantha* DC.	
Stomatal index	Upper epidermis	Lower epidermis
	9.5±0.4	3.08±1.01
Trichome index	26.6±1.4	
Palisade ratio	5.4±1.01	
Vein iselet number	11±0.8	
Vein termination number	3-4	

Note: Values in each column are the mean of three replicates±SD

Microscopy of powder

The powder of the leaves of *B. eriantha* showed interesting characters such as glandular and multicellular, uniseriate trichomes. This feature was abundantly found in the plant, and hence can be considered as an important botanical marker.

Presence of epidermal cells with undulating wall and long sized parenchymatous cells were also important characters. Other than these characters anomocytic stomata, palisade tissue, vessel with spiral thickening, pitted fibers and epidermal cells were observed. Besides these characters some calcium carbonate crystals were also found (fig. 2, F-M).

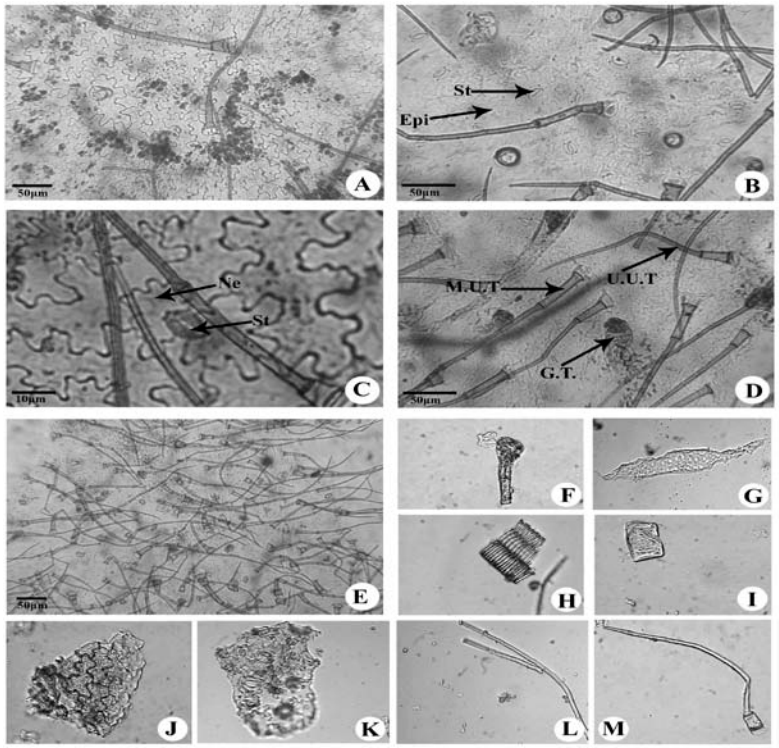

Fig. 2: Micromorphological studies and powder characteristics of the leaves of *B. eriantha*

Key: A: Peel of Upper epidermis St-Stomata; B: Peel of Lower epidermis Epi-Epidermal cell; C: Epidermis shows anomocytic stomata Ne-Neighbouring cell; D & E: Epidermis shows different types M. U. T.-Multicellular uniseriate trichome; of trichomes U. U. T.-Unicellular Uniseriate trichome; F-M: Shows different features of powder study G. T.-Glandular trichome; F: Upper epidermis shows stomata; G: Vessel member shows bordered pits; H: Vessel shoes spiral thickening; I: Vessel; J: Epidermal cells show undulating wall; K: Epidermis with stomata; L: Long parenchymatous cell; M: Unicellular uniseriate trichome

Proximate analysis

The ash values, extractive values and moisture content are given in the table 2.

Table 2: Proximate analysis of *Blumea eriantha* DC leaf powder

S. No.	Parameters	% Content
1.	Total ash	5.25
2.	Acid-insoluble ash	0.21
3.	Water soluble ash	4.90
4.	Ethanol soluble extractive	11.05
5.	Water soluble extractive	19.44
6.	Moisture content	9.65

Fluorescence analysis

Different colour ranges were obtained for the leaf powder in different reagents (table 3).

High performance thin layer chromatographic (HPTLC)

The suitable mobile phase, number of compounds, their R_f values and percentage peak area were determined by HPTLC (table no. 4). The chromatographic fingerprint of methanolic extract of *B. eriantha* showed six well resolved components (fig. 3).

This HPTLC fingerprinting pattern can be considered as analytical parameter to check identity, purity and authenticity of *B. eriantha*.

Table 3: Fluorescence analysis of *Blumea eriantha* DC. leaf powder

	Ordinary Light	UV Long	UV Short
Powder as such	Green	Green	Dark green
Powder+1 N HCl	Yellowish green	Greenish black	Dark green
Powder+50% Sulfuric acid	Yellowish green	Dark green	Brownish black
Powder+40 % NaOH	Greenish yellow	Dark green	Dark Green
Powder+40 % NaOH-ethanolic	Yellowish green	Yellowish brown	Yellowish green

Table 4: HPTLC profile of methanolic extract of *B. erinatha* leaves

Extract	Solvent system used	Number of peaks	R_f values	Percentage peak area
Methanolic	Toluene: Ethyl acetate: Formic acid (6:3:1 v/v/v)	6	0.18, 0.30, 0.35, 0.44, 0.52, 0.65	2.78, 39.58, 13.17, 20.49, 20.15, 3.83

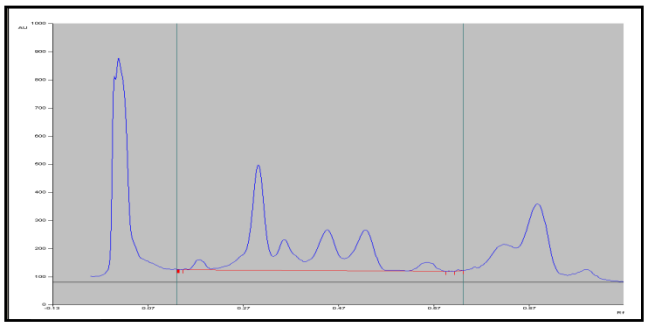

Fig. 3: This HPTLC fingerprinting pattern of methanolic extract of *B. erinatha* leaves

CONCLUSION

In the present study, we have evaluated some of the pharmacognostic standards for *Blumea eriantha DC.* Macroscopy studies for determination of leaf constants and micromorphological characteristics were performed. The ash values, extractive values, microscopical characters of powdered leaf were determined. The HPTLC fingerprinting pattern for the methanolic extracts of *B. eriantha* leaves were developed. Thus a successful attempt was made to pharmacognostically and chromatographically standardizes the leaves and extract of *B. eriantha.* The data obtained in this study can be suggested as reference information for the identification of this medicinally acclaimed crude drug and also help to discern it from its adulterants.

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CONFLICT OF INTERESTS

Declared None

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