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Pharmacognostic standardization and physicochemical analysis of the leaves of *Barleria montana* Wight & Nees

Sriram Sridharan^{1*}, Sasikumar Chinna Gounder²

¹Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur 613 401, Tamil Nadu, India

²PG Department of Biotechnology, Nehru Memorial College (Autonomous), Puthanampatti 621 007, Tiruchirappalli Dt, Tamil Nadu, India

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ABSTRACT

Objective: To investigate the pharmacognostic features and physicochemical properties of the leaves of *Barleria montana* Wight & Nees.

Methods: The leaf samples were subjected to organoleptic, microscopic and macroscopic analysis. Physicochemical properties and fluorescence analysis of the sample under UV and daylight were studied as per World Health Organization norms.

Results: Microscopic analysis showed that the plant possessed dorsiventral leaves, lamina, glandular trichomes, calcium carbonate cystoliths and adaxial epidermis. Physicochemical characters like ash and moisture content, extractive values, foreign matter and fluorescent characteristics of the leaf samples were determined and reported.

Conclusions: Results obtained from these studies can be used as reliable markers in the identification and standardization of this plant as a herbal remedy

1. Introduction

The process of standardization can be achieved by stepwise pharmacognostic studies that help in identification and authentication of a plant material[1]. Correct identification and quality assurance of the raw material are the important prerequisites in herbal therapy to ensure its quality, efficacy and safety[2]. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained[3]. Most of the regulatory guidelines and pharmacopeias suggest macroscopic and microscopic evaluation and chemical profiling of herbal materials for quality control and standardization[4].

Acanthaceae is a large family comprising 3175 species in 212 genera[5]. *Barleria* is a flowering herb genus in this family and is commonly found in countries like India, Malaysia, Australia,

America, Western tropical Africa, Indonesia, Philippines, Naharu, Hawaii etc. This genus comprises 300 species of herbs with a variety of medicinal activities and uses[6]. *B. montana* (*B. montana*) belongs to the family Acanthaceae and grows on exposed slopes and among rocks in the hills. It is a sub-shrub with obovate leaves. *B. montana* (synonym: *Barleria purpurea*) commonly known as Mountain *Barleria* is one of the species in the genus *Barleria*. It is an erect herb found in the mountains of Western Ghats and has been traditionally used for centuries for treating wounds, diabetes, cough, inflammation and also it is known to possess hepatoprotective activity. Phytochemical analysis of the plant leaves revealed the presence of coumarin, sterol, quinone, flavonoids, alkaloids, terpenoids and tannin. Similar studies on the methanolic extract of the plant have shown that the plant contained phytosterol, phenol, alkaloids, terpenoids, tannins and flavonoids[7].

The present study is focused on the pharmacognostic standardization parametrics such as organoleptic, microscopic, macroscopic analysis along with the determination of ash and moisture content, extractive values, foreign matter and fluorescent characteristics of the leaves of *B. montana* as described in the World Health Organization guidelines.

*Corresponding author: Sriram Sridharan, Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur 613 401, Tamil Nadu, India.

Tel: 9894091395

E-mail: sridharan.ram@gmail.com

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2. Materials and methods

2.1. Collection and authentication of plant material

Leaves of *B. montana* were collected from Kolli hills, Tamil Nadu. The plant was identified, authenticated and certified (PARC/2012/1240) by Prof. Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai.

2.2. Macroscopic analysis

Macroscopic features of the plant were analyzed by Evans method[8].

2.3. Microscopic analysis

The paraffin-embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10–12 μm . Dewaxing of the sections was carried out by customary procedure. The sections were stained with toluidine blue[9]. Photographs of different magnifications were taken with Nikon Labphoto 2 microscopic Unit.

2.4. Physiochemical analysis

The physiochemical parameters (ash and moisture contents, foreign matter, extractive value) were determined[10]. Fluorescence of the drug was observed under day and UV light (254 nm) using various solvent extracts as well as treating with acids and alkaline solutions of the drug. The powder was treated with neutral solvents like hexane, benzene, chloroform, ethyl acetate, alcohol, acetone and acids like 1 mol/L hydrochloric acid, 50% sulphuric acid and alkaline solutions like 1 mol/L aqueous and alcoholic NaOH[11].

3. Results

3.1. Macroscopic and microscopic analysis

The lamina was chartaceous, puberulous above with apiculate apex. Petiole was 2.5 cm long and the flowers were solitary, axillary or terminal. It had four equal sepals, corolla-five lobed pink or purple, four stamens, ovary two-celled with two ovules in each cell and had a capsule beaked with four and sub-orbicular seeds with hairs.

The leaf was dorsiventral with fairly prominent midrib and thin lamina (Figure 1). The midrib consisted of a short wide adaxial hump and wide and short abaxial semicircular part. The midrib possessed thick epidermal layer of thin walled epidermal layer of squarish thin walled cells. The abaxial epidermal cells had prominent outgrowth of the cuticle. The vascular strand was single, thick and bowl-shaped with 150 μm thick and 250 μm wide. It consisted of long compact rows of thick walled xylem elements and small nests of phloem elements (Figure 2). Lamina was distinctly dorsiventral with thick and wide adaxial epidermis and narrow cylindrical abaxial epidermis. Single adaxial row of palisade cells was thick, wide and spongy parenchyma cells were spherical or cylindrical and were about four layers (Figure 3). Glandular trichomes were abundant on the abaxial and adaxial epidermal layers.

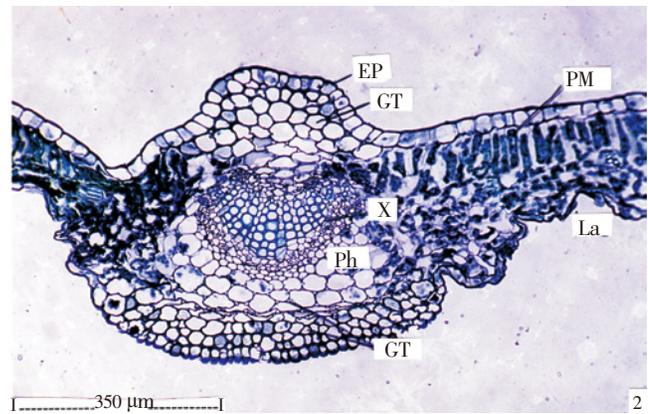


Figure 1. Transverse section of midrib entire view.

Ep: Epidermis; GT: Ground tissue; La: Lamina; PM: Palisade mesophyll; Ph: Phloem; X: Xylem.

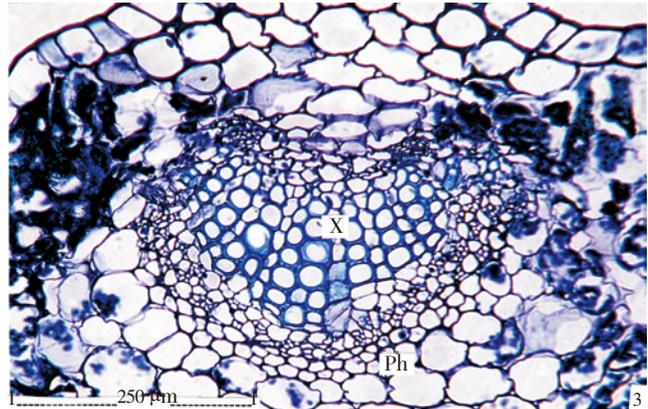


Figure 2. Vascular bundle of the enlarged leaf.

Ph: Phloem; X: Xylem.

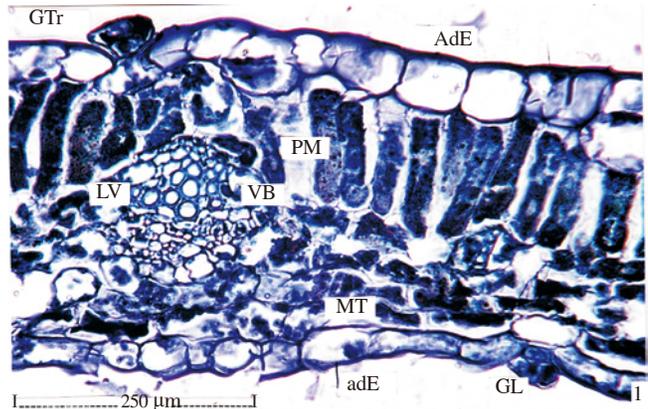


Figure 3. Transverse section of lamina showing lateral vein and glandular trichome.

AdE: Adaxial epidermis; GL: Gland; GTr: Glandular trichome; LV: Lateral vein; PM: Palisade mesophyll; VB: Vascular bundle; MT: Mesophyll tissue; abE: Abaxial epidermis.

The gland had a single, horizontally elongated stalk cell, a narrow rectangular middle cell and highly dilated rosette of four body cells with dense dark contents. Cystoliths were elongated and cylindrical and were located in the specialized, wide epidermal cells called lithocysts (Figure 4). The stomata was abundant and diacytic. Adaxial epidermal cells were apostomatic. The cells were wide and had thick, straight anticlinal walls and prominent nuclei. The cystolith was seen mostly in pairs in the surface sections. They were located in wide, rectangular lithocysts. The cells were attached end to end and the cystoliths were also seen with their ends juxtaposed (Figure 5).

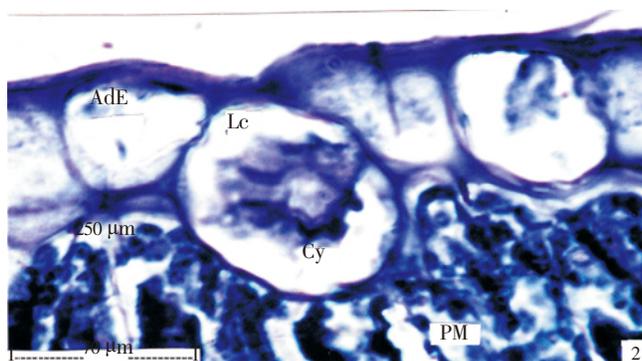


Figure 4. Lithocyst and enlarged cystolith.
AdE: Adaxial epidermis; Cy: Cystolith; Lc: Lithocyst; PM: Palisade mesophyll.

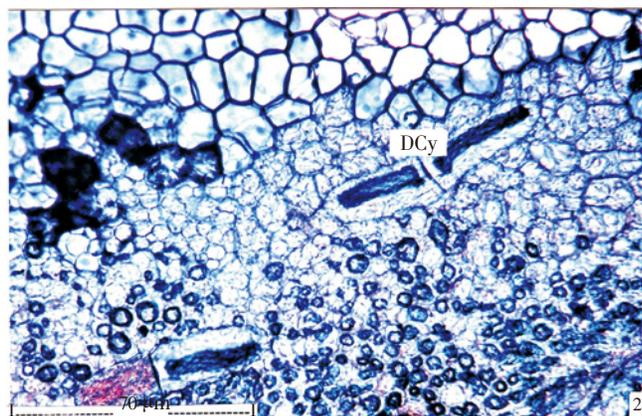


Figure 5. Double cystolith in surface view.
DCy: Double cystolith.

3.2. Powder microscopic analysis

The leaf powder revealed venation pattern as well as vein islet and vein termination. Epidermal cells, subsidiary cells and stomata were also seen.

3.3. Physicochemical analysis

Physiochemical characters like ash and moisture contents, extractive values, foreign matter were determined. The total ash content was found to be 7.5% followed by acid soluble and water-soluble ash respectively (6.3% and 4.4%). The extractive value of the plant sample in alcohol and water was determined and it was found to be 17.26% and 10.64% respectively. Fluorescence analysis of the sample under UV and daylight was studied and the results were tabulated in Table 1.

Table 1

Fluorescence analysis of *B. montana* drug powder.

Treatments	Daylight		UV light	
	24 h	48 h	24 h	48 h
Chloroform	Light green	Yellowish green	Yellowish orange	Orange
Hexane	Pale green	Pale yellow	Pale yellow	Orange
Alcohol	Green	Dark green	Light green	Green
Benzene	Yellowish green	Yellow	Orange	Orange
Acetone	Green	Green	Pale red	Light green
Ethyl acetate	Green	Green	Dark green	Orange
Water	Orange	Meganta	Greenish orange	Green

4. Discussion

The quality criteria for herbal drugs are based on a clear scientific definition of the raw material, identity, purity, content and other chemical, physical, or biological properties. The correct identity of the

crude herbal material, or the botanical quality is of prime importance in establishing the quality control of herbal drugs and can be achieved by macro- and micro-scopical examinations.

The pharmacognostic study of the plant sample revealed the presence of diacytic stomata, glandular trichomes, spongy spherical parenchyma cells, bowl-shaped vascular strand with thick walled xylem elements and small nests of phloem elements and calcium carbonate cystoliths which are predominantly found in Acanthaceae family.

The authenticity and purity of plant samples are determined by their total ash values which act as important quantitative standards and also help in detecting the presence or absence of any foreign organic matter such as metallic salts and siliceous contamination[12]. In accordance to it, the leaf samples of *B. montana* showed higher percentage of total ash. Extractive values, apart from being a useful parameter for determining the purity of a plant material, can also help to estimate specific components that are soluble in a specific solvent[11]. In the present study, the plant sample showed higher extractive value in alcohol than in water.

The present study is an attempt to evaluate the pharmacognostical and physicochemical features of the plant *B. montana*. The results of this study may serve as a useful supplement for further detailed evaluation and investigation of this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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