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Pharmacognostical investigation of *Artemisia parviflora* Roxb.

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ABSTRACT

Objective: To investigate the pharmacognostic characters of important medicinal plant, *Artemisia parviflora* (*A. parviflora*) Roxb. **Methods:** The pharmacognostic studies were carried out in terms of macroscopical, microscopical characters, physicochemical parameters of *A. parviflora*. Samples of different plant parts were fixed with formalin, acetic acid and ethyl alcohol (FAA), sectioned with rotary microtome and studied under microscope. **Results:** Some of the diagnostic features of the leaves and stems are the presence of amphistomatic epidermis, cyclocytic stomata, and collateral vascular bundles. Macroscopic study showed basal and lower stem leaves cuneate, oblong-obovate to flabellate, coarsely dentate to incised-dentate at the apex; middle and upper stem leaves mostly basally auriculate, palmatifid to deeply palmatisect or irregularly incised laciniate into linear to narrow lanceolate, acute segments. Further, ash and extractive values were calculated as per WHO guidelines. The preliminary phytochemical screening showed the presence of alkaloids, sterols/terpenoids, flavonoids, tannins, phenols and coumarins. **Conclusion:** Various pharmacognostical characters were observed in study which can help in identification and standardization of *A. parviflora*.

1. Introduction

Artemisia pariviflora (*A. parviflora*) Roxb. belongs to the family Asteraceae is a member of Indian tarragon family and is of high medicinal value. This plant finds use in traditional systems of medicine *viz*., the leaves are digestive[1]. A decoction of the leaves is said to promote a plump figure, but too much is said to be deleterious and can cause hypertension. The expressed juice of the plant is used in the treatment of vaginitis[2]. It is also used to treat skin diseases. The plant is used for making antitoxifying and ant febrile drugs[3]. It is widely used by tribespeople for its wound healing properties[4]. And as an ethnoveterinary medicine[5]. This species has not been scientifically evaluated; the present study was aimed to bring this plant under a suitable pharmacognostical scheme which would be useful for the future investigations.

A. parviflora has been widely used in Indian folk medicine for the treatment of cuts and wounds. This plant is accredited with antidiabetic, antipyretic and antiviral properties. It is also considered as a good fodder. The oil posses powerful antifungal, antibacterial activity and stimulant properties.

2. Materials and methods

2.1. Chemicals

All the chemicals were of analytical grade and were obtained from Merck India limited and Loba Chemie limited, India.

2.2. Collection of plant material

Fresh samples of *A. parviflora* (Figure 1) was collected from forests of Nilgiri hills, Ooty, Tamil Nadu, India in the month of June and authenticated by Botanical Survey of Medicinal Plants and collection unit, Ootacamund. The voucher specimens were preserved in the Department of Pharmacognosy, J.S.S. College of Pharmacy, Mysore, for further reference.

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2.3. Preparation of specimen

The required fresh samples of different organs were cut and removed from the plant material and fixed in FAA (5 mL formalin + 5 mL acetic acid + 70%, 90 mL ethyl alcohol). After 24 h of fixing, the specimen was dehydrated with graded series of tertiary butyl alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 ℃) until TBA solution attained supersaturation. The specimen was cast into paraffin blocks.

2.4. Sectioning

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 done by customary procedure[6]. The sections were stained with toluidine blue[7]. Since, toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections also stained with safranin and fast-green and iodine in potassium iodide for starch[8].

For studying the stomatal morphology, venation pattern and trichome distribution, para dermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared[9]. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaoH and mounted in glycerine medium after staining. Different cell component were studied and measured.

2.5. Photomicrographs

Microscopic descriptions of tissue are supplemented with micrographs wherever necessary. Photographs of different magnification were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field 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 are indicated by the scale-bars[10,11].

2.6. Ash and extractive values

Total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble and alcohol soluble extractive values were calculated[12].

2.7. Preliminary phytochemical screening

Preliminary phytochemical screening was performed with ethanolic extract of aerial parts of *A. parviflora* to investigate the secondary metabolites present in the plant material[13].

3. Results

3.1. Macroscopic characters

Perennial, 50-90 cm tall herb with solitary or several, sulcate, branched, almost glabrous stems from woody, 1.5-2.5 cm thick, upright rootstock. Basal and lower stem leaves cuneate, oblong-obovate to flabellate, coarsely dentate to incised-dentate at the apex; middle and upper stem leaves mostly basally auriculate, palmatifid to deeply palmatisect or irregularly incised laciniate into linear to narrow lanceolate, acute segments (Figure 1).

Figure 1. Two shoots showing leaf morphology.

3.2. Microscopic characters

The leaf is uniform thick with smooth and even surfaces and fairly prominent midrib. The lamina is 200 μm thick and the midrib is 350 μm thick. The midrib is plano convex in cross sectional view with flat adaxial side slightly projecting, hemispherical abaxial part. It has centrally placed small top-shaped vascular bundle which is collateral; it is surrounded by two layers of bundle sheath parenchyma with a mass of parenchyma cells beneath the bundles (Figure 2.1). Above the vascular bundle and beneath the adaxial epidermis is the transcurrent palisade zone.

Lamina has amphistomatic epidermis and isolated mesophill tissue. In the middle part of the lamina occurs a horizontal band of parenchyma zone with two or three layers of cells.

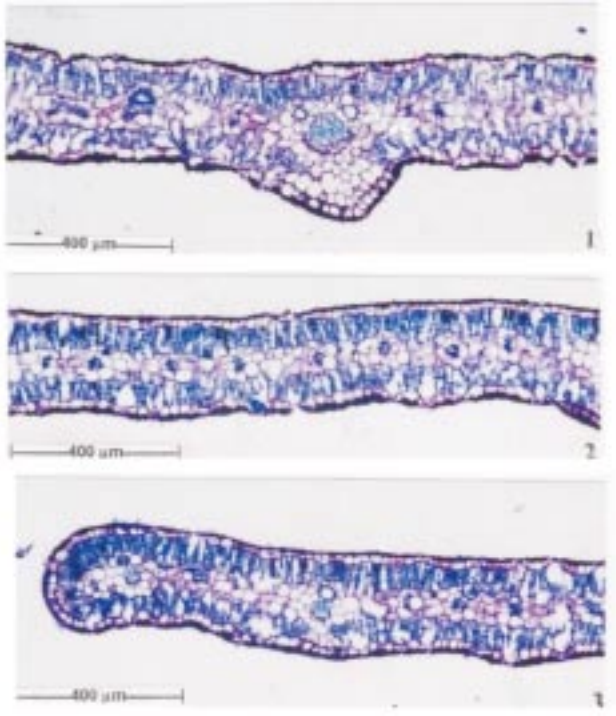

Figure 2. Anatomy of the leaf. 1: T.S. of leaf through midrib; 2: T.S. of lamina; 3: T.S. of leaf margin.

The vascular bundles of the lateral veins occur in transverse row within the median parenchymatous mesophill tissue (Figure 2.2). The vascular bundles are circular with small collateral xylem and phloem surrounded by a circle of parenchymatous bundle sheath cells. The palisade layers occur both in the adaxial and in the abaxial parts. The adaxial and abaxial palisade zones are equal in height. The epidermal layers are prominent with thick cuticle. The epidermal cells are squarish and thick walled. Stomata occur both on the adaxial and abaxial sides. The marginal part of the lamina is slightly bulged, blunt and semicircular. The palisade tissue occurs in the sub epidermal part. Vascular bundles are seen with in the palisade zone (Figure 2.3).

The lateral veins and vein lets are prominent. Distinct vein islet are lacking expect a few regions, where islets may be recognized. Vein terminations are also not distinct. But the termination is visible, long, slender, simple and undulate (Figure 3.1). Stomata are more abundant on the abaxial side than on the adaxial side. The stomata type may be cyclocytic with four or five subsidiary cells surrounding the stomataor anisocytic with three unequal subsidiary cells around stomata (Figure 3.2 and 3.3). The epidermal cells are small, polygonal, thick walled and straight.

In cross sectional outline, the stem is circular with prominent ridges and shallow, wide ridges. The stem is in primary state of growth with primary xylem and phloem.

The stem has a distinct, thin continuous epidermis, made up of small, thick squarish cells. It is followed by

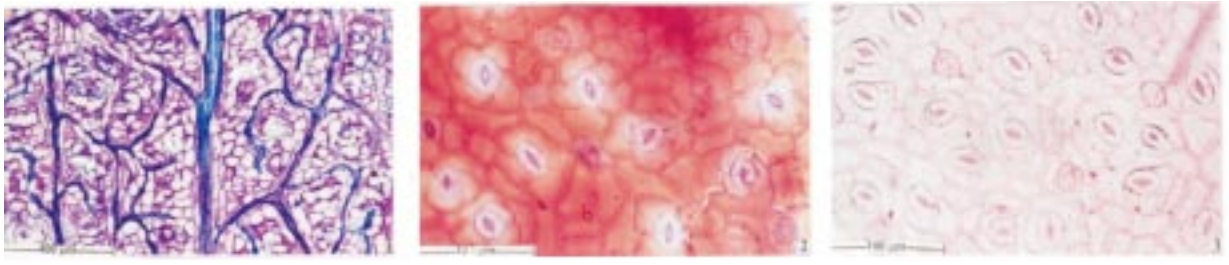

Figure 3. Venation pattern and stomatal morphology. 1: Paradermal section of the lamina showing venation; 2: Epidermal peeling showing adaxial epidermis; 3: Epidermal peeling showing abaxial epidermis.

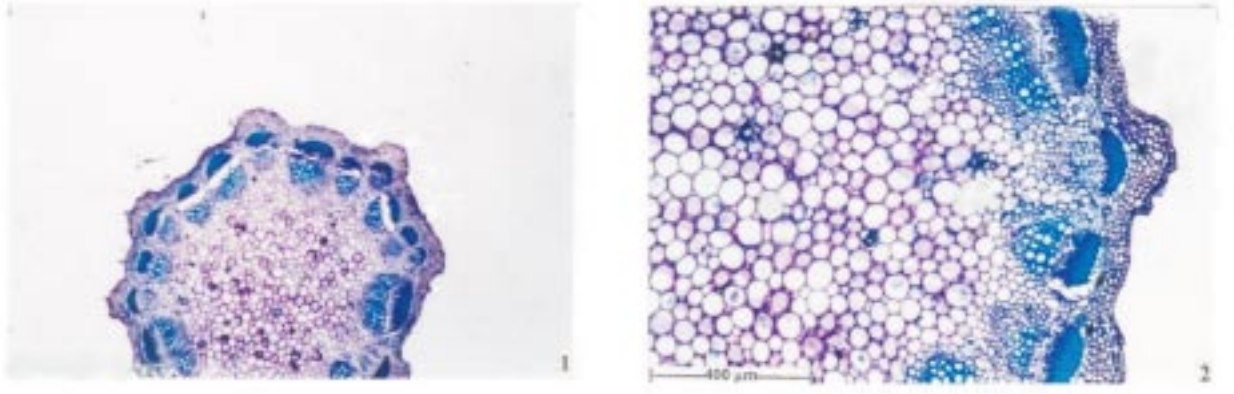

Figure 4. Anatomy of the stem. 1: T.S of stem grand-plane; 2: T.S. of stem a sector enlarged.

a narrow zone of parenchymatous cortex with four or five layers of compact cells. The stele consists of 18–20 discrete vascular bundles arranged in a wide ring (Figure 4.1). The vascular bundles are separated from each other by narrow parenchymatous medullary rays. The vascular bundles are collateral with outer thick semicircular sclernchyma cap, narrow phloem zone and a few xylem elements. The pith is wide and parenchymatous. The pith cells are circular, thick walled and compact (Figure 4.2).

3.3. Ash and extractive values

The total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble and alcohol soluble extractive values were found out to be 5.26%, 0.9%, 3.2%, 6.4%, 17.8% and 8.4% respectively.

3.4. Preliminary phytochemical screening

The ethanolic extract of aerial parts of *A. parviflora* showed the presence of alkaloids, sterols/triterpenoids, saponins, flavonoids, tannins, phenol, and coumarins.

4. Discussion

Standardization is an important for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize a drug, various microscopic, macroscopic analysis are done[14]. Microscopic method is one of the cheapest and best method to start with establishing the correct identification of the source of material[15]. Pharmacognostical studies of this plant was carried out in order to identify the correct species and also to differentiate the closely related other species of *Artemisia*.

These parameters observed may be useful for the future identification of the plant. Morphological and microscopic studies of leaves and stem bark act a reliable source for detecting adulteration. Ash values were useful in indicating extraneous matter (*e.g.* soil and sand) adhering to plant material and extractive values whether the drug purchased is fresh one and not the exhausted one. Extractive value is employed for material for which no suitable chemical or biological assay exists. Preliminary phytochemical screening also indicates the quality of drug.

In conclusion, parameters reported above are sufficient enough to identify and authenticate the plant material. The microscopic and physicochemical standards laid down can be used to distinguish the genuine drug from adulterated drug. They can be included as microscopic standards in Indian Herbal Pharmacopoeia. The plant, *A. parviflora*, entire plant is worth for further chemical isolation and pharmacological investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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