ORIGINAL ARTICLE

Pharmacognostical studies on the leaves of *Plectranthus amboinicus (Lour) Spreng*

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Plectranthus amboinicus (Lour) Spreng belonging to the family *Lamiaceae* commonly known as Karpuravalli, Omavalli in Tamil, Patta ajavayin, Patharcur in Hindi, Country borage in English is a large succulent aromatic perennial herb. It is highly aromatic pubescent herb with distinctive smelling leaves. The plant is distributed throughout in India, cultivated in the gardens. The leaves of this plant have been used in malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic and convulsions. This paper deals with the micro morphological studies carried out on the leaves of *Plectranthus amboinicus* one of the WHO accepted parameter for identification of medicinal plants. For the better understanding of structural details Scanning Electron Microscopy (SEM) also employed.

Key words: *Coleus amboinicus, Coleus aromaticus,* micro morphology, *Plectranthus amboinicus*, scanning electron microscopy

INTRODUCTION

The plant *Plectranthus amboinicus* (synonym: *Coleus amboinicus, Coleus aromaticus*) commonly known as Country borage, Indian borage (Karpuravalli, Omavalli in Tamil and Patta ajavayin, Patharcur in Hindi,) is a dicotyledonous plant belonging to the family *Lamiaceae*.[1,2] It is a large succulent aromatic perennial herb. Much branched, fleshy highly aromatic pubescent herb with distinctive smelling leaves. The plant is distributed through out India, cultivated in the gardens. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsions, and epilepsy.[3-5] The phytochemical study reveals the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oil in the leaves.[6] Lack of proper standards of medicinal plants may result in the usage of improper drugs which in turn will cause damage not only to the individual using it, but also to the respect gained by the well known ancient system of medicine.

Therefore scientific method must be resorted to identify and maintain quality of plant drugs to be used in the traditional system of medicine. In this present study medicinally important drug of *P. amboinicus* is studied from micro morphological point of view. Scanning Electron Microscopy is also employed to obtain the best possible structural details to assist in the solution of taxonomic problem, to avoid misleading of diagnostic features by oversimplified descriptions and to locate presence of phytoconstituents which are not visible under light microscope.

MATERIALS AND METHODS

Plectranthus amboinicus was collected from Andipatty canal, Theni, Tamil Nadu, India. The plant was identified and authenticated by taxonomist and voucher specimen (PCG PA 16) was deposited in the herbarium of Department of Pharmacognosy, Madurai Medical College, Madurai, Tamil Nadu.

MACROSCOPIC CHARACTERS

The plant consists of hispidly villous or tomentose fleshy stem about 30-90 cm. Leaves are simple, broad, ovate and very thick; Thickly studded with hairs; on the lower surface the glandular hairs are most numerous and give rise to a frosted appearance. The taste of the leaf is pleasantly aromatic with the agreeable and refreshing odour. Flowers are shortly pedicelled, pale purplish in dense whorls at distant intervals in a long slender raceme.

HISTOLOGICAL CHARACTERS

Preparation of Specimen

The leaves were cut and removed from the plant and fixed in FAA (Formalin 5 ml + Acetic acid 5 ml+ 70% Ethanol 90 ml). After 24 hrs of fixing, the specimens were

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Received: 20-05-2008; **Accepted:** 19-06-2008

dehydrated with graded series of tertiary butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until tertiary butyl alcohol solution attained super saturation. The specimens were cased into paraffin blocks.

Sectioning

The paraffin embedded specimen was sectioned with the help of rotary microtome. The thickness of the section was 10-12 μm. After dewaxing the sections were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some phytochemical 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 (For starch).[7,8]

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections as well as clearing of leaf with 5% sodium hydroxide and epidermal peeling by partial maceration employing Jeffrey's maceration were prepared. Glycerin mounted temporary preparations were made for macerated materials.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot- 2 microscope units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells polarized light were employed. Since these structures have birefringent property, under polarized light they appear bright against dark back ground.

Scanning Electron Microscopy (SEM)[9,10]

SEM forms a three-dimensional image on a cathode ray tube by moving a beam of focused electrons across an object and reading both the electrons scattered by the object and the secondary electrons produced by it. The electro magnetic lenses are used in this microscope and focusing is done by varying the current and the image is projected in photographic plate on screen giving easily comprehensive, quasi three dimensional representation of the objects examined leading to the better understanding of the ultra structure of plant cells. In addition, it also reveals the spatial relations, unsuspected details and previously undescribed characters. In other words, the micrograph obtained by SEM, shows the best possible structural details of the specimens.

SEM Sample Preparation

Sample for SEM analysis were mounted on the specimen stub using fevicol adhesive. Small sample were mounted directly on scotsch double adhesive tape. Samples were coated with gold to a thickness of 100 AO using Hitachi Vacuum evaporator. Coated samples were analyzed in a Hitachi Scanning Electron Microscope model S-450 operated at 15 kV and photographed.

As a part of quantitative microscopy stomatal number, stomatal index, trichome length were determined by using fresh leaves of the plant. The total ash, water soluble ash, acid insoluble ash, extractive values for various solvents and loss on drying were determined. The dried powdered material of leaves was also subjected to identification tests for the detection of various phytoconstituents.[11]

RESULTS AND DISCUSSION

The leaf *Plectranthus amboinicus* is dorsiventeral with distinction of adaxial and abaxial faces. The surface of the leaf is smooth and densely clothed with glandular and non glandular trichomes. In transection view the shape of the midrib appears as Plano convex with flat adaxial side and hemispherical abaxial side. Epidermal layer of the midrib consists of rectangular to polygonal cells with thin and slightly wavy anticlinal walls. The stomata are exclusively diacytic (caryophyllaceous) with one smaller and one larger subsidiary cell. The larger cell encloses the stoma and partly the smaller subsidiary cell. Vascular bundles are small, single, top-shaped and less prominent. It consists of 4 to 6 parallel rows of xylem having narrow thin walls and a thin arc of phloem; the major lateral vein of the vascular bundle is about 550 μm. The upper and lower epidermal surfaces of the lamina have dense trichomes. Mesophyll of the lamina consists of 9-12 layers of undifferentiated compact squarish cells. It consists of both glandular and non glandular trichomes. Glandular trichomes are more abundant than the non glandular trichomes [Fig. 1].

The cross sectioned outline of petiole shows the shape of roughly rectangular with shallow adaxial concavity. The epidermal layer consists of thin small rectangular cells; inner epidermis is made up of a narrow zone of 3 to 4 layers of collenchyma cells; remaining ground tissue is parenchymatous with large, circular thin walled cells. Vascular system consists of collateral with radial rows of thin walled, squarish, wide xylem and thick arcs of phloem [Fig. 2].

In Scanning Electron Microscopy (SEM) maximum information of the structure is obtained by employing light and electron microscope jointly; SEM plays a pivotal role to identify the microstructures which was not previously recognized. When the leaf was viewed under light microscope no granules are seen. While examining under

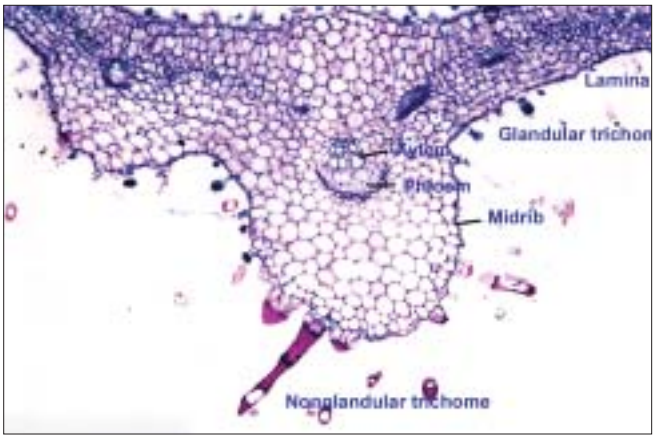

Figure 1: T.S of *Plectranthus amboinicus (Lour) Spreng* leaf through midrib

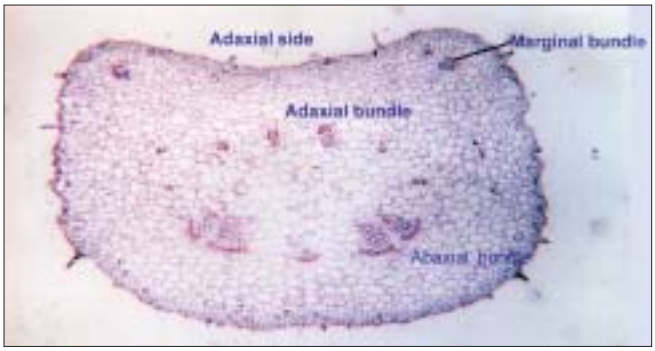

Figure 2: T.S of Petiole

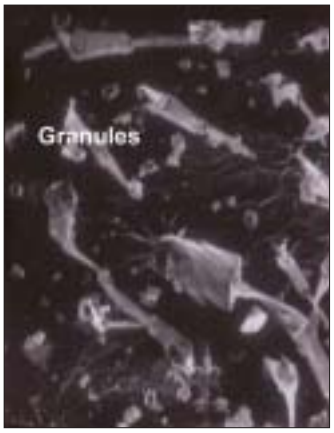

Figure 3: SEM of upper epidermis (120 x)

SEM, lot of granules are found on the upper epidermis [Fig. 3]. Scutellarin is said to occur in the epidermis of the leaf in all examined species of scutellaria and hesperidin crystals in certain members of this family. Further investigation of these granules may provide important information in relation to taxonomy and phytoconstituent which was in progress in our laboratory.

Due to the highly dense and succulent nature of the leaf determination of vein islet and vein terminal number is not possible. The stomatal values, trichome length, total ash, water soluble ash, extractive values for various solvents and loss on drying of powdered leaves are given in Table 1.

Table 1: Analytical parameters of *Plectranthus amboinicus (Lour) Spreng* leaf

Parameter	Results
Trichome length	40-90
Stomatal number	Upper epidermis: 17-22 Lower epidermis: 21-24
Stomatal Index	Upper epidermis: 17.9% Lower epidermis: 17.5%
Total ash	22.04% w/w
Water soluble ash	9.37% w/w
Acid insoluble ash	1.98 % w/w
Pet .ether soluble extractive value	3.14% w/w
Benzene soluble extractive value	5.31 % w/w
Ethyl acetate soluble extractive value	5.50% w/w
Chloroform soluble extractive value	5.54% w/w
Ethanol soluble extractive value	5.43% w/w
Water soluble extractive value	26.79% w/w
Loss on drying	8.04% w/w

The qualitative chemical test reveals the presence of flavonoids, terpenoids, saponins, steroids, tannins, proteins, carbohydrates and volatile oil in the leaf powder of *Plectranthus amboinicus*. This study on micro morphological features of *P. amboinicus*, proposed a set of anatomical parameters may enable those who handle this plant to maintain its quality control.

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Source of Support: Nil, **Conflict of Interest:** None declared.