Pharmacognostic Study

Pharmacognostic evaluation of leaves of certain *Phyllanthus* species used as a botanical source of *Bhumyamalaki* in *Ayurveda*

Surendra K. Sharma, M. A. Sheela

Department of Pharmaceutical Sciences, Guru Jambeshwar University of Science and Technology, Hisar, Haryana, India

Abstract

Today, World over, there is a great deal of interest in Ayurvedic system of medicine and thus the demand for various medicinal plants in the production of Ayurvedic medicines is ever increasing. Due to varied geographical locations where these plants grow, a great deal of adulteration or substitution is encountered in the commercial markets. Histological studies of the plant drugs are not only to study the adulterants but also are indispensable in accurate identification. Microscopic observations of the *Phyllanthus* species revealed the occurrence of anisocytic and paracytic type of stomata in *Phyllanthus amarus*, while only anisocytic type of stomata is present in *P. fraternus* and *P. maderaspatensis*. Epidermal cell walls of *P. amarus* and *P. fraternus* are wavy and straight walled epidermal walls are observed in *P. maderaspatensis*. In India all the above-mentioned species of *Phyllanthus* are called "Bhumyamalaki" and they are being used in the treatment of various liver disorders. However, all the species of *Phyllanthus* doesn't have the active constituents responsible for the treatment of liver disorders. In the present investigation by using simple micro techniques accurate identification of different species of *Phyllanthus* has been established.

Key words: Bhumyamalaki, Pharamcognosy, Phyllanthus

Introduction

The World Health Assembly in resolutions has emphasized the need to ensure the quality of medicinal plant products by using current control techniques and applying appropriate standards.^[1] Typical pharmacognostical studies are normally quite adequate for quality control of herbal drugs.^[2] Pharmacognostical standardization of herbal drugs include macroscopic, microscopic, physio-chemical constants and fluorescence analysis of investigated parts, and to evolve standards for single drugs and compound preparations in order to validate genuineness of the crude drugs of plant, mineral and animal origin. The study includes to highlight the macroscopical and microscopical characters as distinctive features for authentication and identification purposes.[3,4] According to WHO (1998), the macroscopic and microscopic description of a medicinal plant is the first step toward establishing its identity and purity and should be carried out before any test are undertaken.^[5] In this direction, the plant drug "Bhumyamalaki," one of the

Address for correspondence: Dr. M. A. Sheela, W/o Dr. G. V. R. Joseph, A -5B, 126 A, Janakpuri, New Delhi, India. E-mail: sheelama@gmail.com promising herbal drugs used in Indian system of medicine for various liver disorders is attributable to Phyllanthus niruri Lin.^[6] belongs to the family Euphorbiaceae. P. niruri is endemic to West Indies and not found in India.^[7] Various Phyllanthus species like P. amarus Schum and Thonn, P. fraternus Web., P. maderaspatensis Linn., P. simplex Retz., P. urinaria Linn. are being termed as "Bhumyamalaki."^[8] Literature survey reveals that "Bhumyamalaki" has been used to treat jaundice, gonorrhea, frequent menstruation, dysentery, and diabetes.^[9-11] It has also been used as a treatment for skin ulcers, sores, swelling, etc.^[12] Phyllanthus focuses on its potential for fighting viruses specifically with hepatitis B virus.^[13,14] Studies suggest that Phyllanthus may suppress the growth and replication of the virus and may decrease the amount of hepatitis B virus found in the blood stream. It has not been reported to remove the viruses, but it is effective in suppressing the symptoms in addition fighting with hepatitis B virus. Phyllanthus may also support the overall health of the liver.^[15]

Review of literature revealed that only few handful workers studied the structural details of *P. fraternus*. Saha and Krishna Murthy (1959) studied the structural details of *P. fraternus* Web.^[16] Later Yelene *et al.*, carried out the leaf structural studies.^[17] Khatoon *et al.*, studied three species of Phyllanthus.^[18] So far there is no published report about the comparative microscopic diagnostic



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characters of all different species of *Phyllanthus* known for its hepatoprotective activity. Histological studies of the plant drugs are not only to study the adulterants but also are indispensable in the accurate identification.

The present paper deals with the distinguishing characters of different species of *Phyllanthus* by using simple microscopic techniques.^[19-21]

Materials and Methods

The fresh plant materials of Phyllanthus amarus Schum and Thonn., Phyllanthus fraternus Web., Phyllanthus maderaspatensis Linn., Phyllanthus simplex Retz., and Phyllanthus urinaria Linn. were collected from the foot hills of Western Ghats, India, and the voucher samples were deposited at Department of Pharmaceutical Sciences, Guru Jambheshwar University, Hisar, Haryana, India. All the samples were dried in shade. To study the epidermal structural studies for the dried leaf structural studies, first the epidermal imprints of the leaf lamina were prepared. A domestic adhesive (Quick fix) was applied uniformly on both the upper and lower epidermis of the dried leaves. It is then dried at room temperature. The dried transparent film of "Quick fix" is then stripped off carefully from the surface of the leaf and placed on a clean dry glass slide with the imprint surface uppermost. Cover slip is placed over it and lightly tapped to flatten the film. It is then observed under a compound microscope. Line drawings were made by using mirror-type camera lucida.^[13,22]

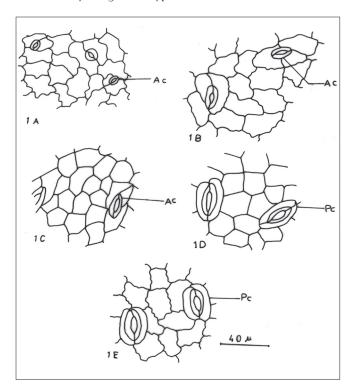


Figure 1: (A-E) Lower epidermis of the lamina in surface view 583 x - P. amarus, P. fraternus, P. maderaspatensis, P. simplex, and P. urinaria, respectively, Ac:Anisocytic type of stomata, Le: Lower epidermis, Lm: Lamina margin, Pa: Parenchyma tissue, Pc: Paracytic type of stomata, Ps: Palisade cells, Ue: Upper epidermis, Vb: Vascular bundle

Results

Epidermal cell walls of *P. amarus*, *P. fraternus*, and *P. urinaria* are wavy but *P. amarus* consist of paracytic and anisocytic type of stomata [Figure 1a], while *P. fraternus* [Figure 1b] and *P. urinaria* [Figure 1e] possess anisocytic and paracytic type of stomata, respectively. In the case of *P. maderaspatensis* [Figure 1c] and *P. simplex* [Figure 1d] the epidermal cell walls are straight and they showed anisocytic and paracytic type of stomata respectively. In *P. amarus* at some place the margin of leaf lamina possess papillae like out growths [Figure 2a]. In *P. fraternus* club-shaped cells are seen lying in an overlapping manner [Figure 2b]. The plant *P. simplex* showed dentate type cells all along the margins of leaf lamina [Figure 2d]. Trichome like two-celled out growths is observed all along the lamina margin of *P. urinaria* [Figure 2e], whereas *P. maderaspatensis* have regular cells along the lamina margin [Figure 2c].

A cluster crystals of calcium oxalate are observed in the ground tissue of *P. amarus* [Figure 3a], *P. maderaspatensis* [Figure 3b], *P. urinaria* [Figure 3d], and *P. simplex* [Figure 3c]. Rarely prismatic crystals of calcium oxalate are observed in *P. amarus*. Crystals are found to be absent in *P. fraternus*.

In *P. maderaspatensis* and *P. urinaria*, the palisade tissue is continuous even in the midrib region while it is discontinuous and restricted to only lamina blade in *P. amarus* [Figure 4], *P. fraternus*, and *P. simplex*.

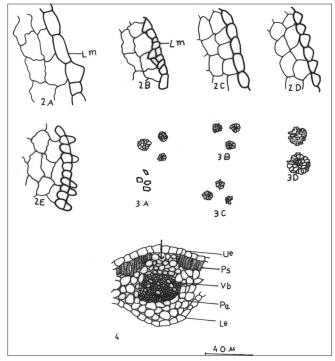


Figure 2 (A-E): Leaf margin in surface view 583 x - P. amarus., P. fraternus, P. maderaspatensis, P. simplex, and P. urinaria, respectively, [Figure 3 (A-D)]: Crystals of calcium oxalate 583 x - P. amarus, P. maderaspatensis, P. simplex, and P. urinaria, respectively, (Figure 4): Transverse section of midrib showing (arrowed) discontinuous palisade cells 583 x - P. amarus, Ac: Anisocytic type of stomata, Le: Lower epidermis, Lm: Lamina margin, Pa: Parenchyma tissue, Pc: Paracytic type of stomata, Ps: Palisade cells, Ue: Upper epidermis, Vb: Vascular bundle

Sharma and Sheela: Pharmacognostic evaluation of Bhumyamalaki

Plant name	Stomatal type (Figure 1a-e)	Cell wall	Margin of lamina (Figure 2 a-e)	Crystals (Figure 3a-d)	Plisade tissue in midrib
<i>P. amarus</i> Schum and Thonn	Paracytic and Anisocytic	Wavy	Papillae like out growths at some places	Clusters rarely prismatic	Discontinuous
P. fraternus Web	Anisocytic	Wavy	Club shaped cells are seen lying in an overlapping manner	Absent	Discontinuous
<i>P. maderaspatensis</i> Linn	Anisocytic	Straight	Regular cells all along the margins	Clusters	Continuous
P. simplex Retz	Paracytic	Straight	Dentate all along the margin	Clusters	Discontinuous
<i>P. urinaria</i> Linn	Paracytic	Wavy	Trichome like two cell out growth all along the margin	Clusters abundant	Continuous

Table 1 illustrates the distinguishing characters of various species of *Phyllanthus*.

Discussion

Phyllanthus niruri Linn. mentioned in the flora of British India (Hooker, 1887) and found on the labels of number of Indian Herbal formulations is a mixture of five distinct species, namely *P. amarus* Schum and Thonn, *P. fraternus* Web., *P. maderaspatensis* Linn., *P. simplex* Retz., *P. urinaria* Linn. In recent times based on the clinical efficacy, *P. niruri* mentioned in the flora of British India and "*Bhumyamalaki*" mentioned in the classical literature are equated with *P. amarus*.^[23] However, due to the morphological similarities, all the above-mentioned five species of Phyllanthus are mixed up and being sold in herbal drug markets of the country by the same vernacular name. From the present investigation by using very simple practical methodology diagnostic characters of all the five studied species of Phyllanthus are developed.

Conclusion

The microscopic diagnostic characters drawn from the present investigation by using simple techniques will help in authenticate genuine samples of *Phyllanthus* used in liver disorders. This is a first such report on the comparative microscopic diagnostic characteristics on "*Bhumyamalaki*."

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हिन्दी सारांश

भूम्यामलकी की प्रजातियों का अध्ययन

सुरेन्द्र शर्मा, एम. ए. शीला

आज पूरे विश्व में आयुर्वेदिक चिकित्सा पद्धति के लाभ एवं विभिन्न आयुर्वेदिक पादपों की मांग में निरन्तर वृद्धि हो रही है। परिवर्तित भूगर्भीय परिस्थिति जहां ये पादप स्वतः उगते हैं वहां मिलावट और उसके प्रतिनिधि द्रव्य वाणिज्यक बाजार में प्रचुर मात्रा में मिलते हैं। औषधीय पादपों की आंतरिक संरचना के अध्ययन में न केवल मिलावट बल्कि उचित पहचान करने के लिए भेषजीय गुणों का अध्ययन भी किया जाता है। फाइलैन्थस प्रजाति के सूक्ष्मदर्शी अध्ययन में एनीसोसाइटिक एवं पैरासाइटिक रन्धों की घटना फाइलैन्थस अमरस में घटित है, जबकि केवल एनीसोसाइटिक प्रकार के रन्ध्र स्टोमेटाद्ध फाइलैन्थस फ्रेटरन्स और फाइलैन्थस मडरासपटैनसिस में पाया जाता है। फाइलैंथस अमरस एवं फाइलैन्थस फ्रेटरन्स की बाह्य कोशिका भित्ति टेढी–मेढी एवं फाइलैन्थस मडरासपटैनसिस की बाह्य कोशिका भित्ति सीधी पाई गई। भारत में फाइलैन्थस की उपरोक्त दर्शाई गई प्रजातियों को 'भूम्यामलकी' कहा जाता है, और ये यकृत विकारों के उपचार में प्रयोग किया जाता है। हालांकि फाइलैन्थस की सभी प्रजातियों को 'भूम्यामलकी' कहा जाता है, और ये यकृत लिए उत्तरदायी नहीं हैं। प्रस्तुत अध्ययन में उपयुक्त साधारण सूक्ष्मदर्शी तकनीक के द्वारा फाइलैन्थस की विभिन्न प्रजातियों की उचित पहचान सुनिश्चित की गई है।