Pharmacognostic Study

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

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**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*, Pharmacognosy, *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 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...
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 Thom., *Phyllanthus fraternus* Web., *Phyllanthus maderaspatensis* Linn., *Phyllanthus simplex* Retz., and *Phyllanthus urinaria* Linn. were collected from the foothills 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]

**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 1c] 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 places 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 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

**Figure 2** (A-E): Leaf margin 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

**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
Table 1: Comparative microscopic diagnostic characters of various *Phyllanthus* species

| Plant name                      | Stomatal type (Figure 1a-e) | Cell wall     | Margin of lamina (Figure 2 a-e)                          | Crystals (Figure 3a-d) | Plisade tissue in midrib |
|--------------------------------|------------------------------|---------------|---------------------------------------------------------|------------------------|--------------------------|
| *P. amarus* 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                 | Continuous               |
| *P. maderaspatensis* Linn       | Anisocytic                   | Straight      | Regular cells all along the margins                     | Clusters                | Continuous               |
| *P. simplex* Retz               | Paracytic                    | Straight      | Dentate all along the margin                            | Clusters                | Discontinuous            |
| *P. urinaria* 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.* 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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भूम्यामलकी की प्रजातियों का अध्ययन

सुरेन्द्र शर्मा, एम. ए. शीला

आज पूरे विश्व में आयुर्विदिक चिकित्सा पद्धति के लाभ एवं विभिन्न आयुर्विदिक पादपों की मांग में निरंतर वृद्धि हो रही है। परिवर्तित भौतिकीय परिस्थिति जहां ये पादप उपयोग में लाए गए हैं वहां नये उद्देश्य और उनके उद्देश्यों के प्रशांत के वर्तमान वाणिज्यक वाजार में प्रदुहर मात्रा में मिलते हैं। आयुर्विदिक पादपों की आंतरिक संरचना के अध्ययन में न केवल मिलावट बल्कि उचित पहचान करने के लिए भेषजीय गुणों का अध्ययन भी किया जाता है। फाइलेन्थ्स प्रजाति के सूक्ष्मदर्शी अध्ययन में एनीसोसाइटिक एवं पेयराइटिक रन्गों की घटना फाइलेन्थ्स अमरस में प्राप्त है, जबकि केवल एनीसोसाइटिक प्रकार के रन्ग स्टोमेटाईड फाइलेन्थ्स फ्रेंटर्स और फाइलेन्थ्स म्युरासपेंडिनिस्स में पाया जाता है। फाइलेन्थ्स अमरस एवं फाइलेन्थ्स फ्रेंटर्स की बाद्ध कोशिका भिडियाल्ड-मेषी एवं फाइलेन्थ्स म्युरासपेंडिनिस्स की बाद्ध कोशिका भिडियाल्ड सीधी पाई गई। भारत में फाइलेन्थ्स की उपस्थिति दर्शाई गई प्रजातियों को ‘भूम्यामलकी’ कहा जाता है, और ये यकृत विकारों के उपचार में प्रयोग किया जाता है। हालांकि फाइलेन्थ्स की सभी प्रजातियाँ मौलिक रूप से यकृत विकारों के उपचार के लिए उपलब्ध नहीं हैं। प्रस्तुत अध्ययन में उपयुक्त साधारण सूक्ष्मदर्शी तकनीक के द्वारा फाइलेन्थ्स की विभिन्न प्रजातियों का उचित पहचान सुनिश्चित की गई है।