Pharmacognostic evaluation of certain species of *Phyllanthus* used as botanical source of Bhumyamalaki/Tamalaki

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**Abstract**

Tamalaki/Bhumyamalaki is a herbaceous plant which is very well known for its medicinal value in Ayurveda especially in liver disorders and other common ailments. Various species names have been attributed to Bhumyamalaki by different authors due to varied geographical locations where these plants grow and varied actions exhibited by them. This leads to confusion in selection of the species and accurate identification is essential as their use in practice is becoming more popular. Hence, it is imperative to assess the genetic diversity and phylogenetic relatedness among the *Phyllanthus* species. The aim of present work is to evaluate the morphological, organoleptic characters and anatomical sections of three *Phyllanthus* species taken for study and to establish Macroscopic and Microscopic analysis of drug for future conservative strategies.

**Keywords:** Tamalaki/bhumyamalaki, pharmacognosy, *Phyllanthus*

**Introduction**

The genus *Phyllanthus* L. was first described by Linnaeus in 1753 and consists of 833 species [1] in the world which is one of the largest and most diversified genus in the family Euphorbiaceae and are chiefly distributed in moist humid tropics. In India, it is represented by 40 species [2]. Before 1985, most of the botanical, pharmacological and phytochemical work of ‘Bhumyamalaki’ was carried out on *Phyllanthus niruri* L. of Hooker [3]. Now, it is clarified that *Phyllanthus niruri* is an American species and not at all found in India [4]. The herbs known as ‘Bhumyamalaki’ in Indian literature refer to a complex group of *Phyllanthus amarus* Schum. & Thonn., *Phyllanthus fraternus* Webster, *Phyllanthus debilis* Klein ex Wild and *Phyllanthus urinaria* L. [5] Confusion exists in identification of these species mainly due to referring them all with a common vernacular name, their similarity in gross morphology, close proximity in growth habitat and lack of guidelines to check the authenticity and quality of the medicinal plant sold [6].

Tamalaki/Bhumyamalaki in India has long been used for the treatment of Jaundice, gonorrhoea, frequent menstruation, dysentery and diabetes [7, 8]. Various Studies include Antimicrobial activity of *Phyllanthus niruri* [9] and *Phyllanthus amarus* focuses on its Antiviral potential especially with Hepatitis B virus [10, 11]. A single drug remedy (Tamalaki decoction) was mentioned in the treatment of Rajayakshma associated with six symptoms [12]. Difference of opinion still exists among the various authors of literature in the selection of species. Various species of *Phyllanthus* are being sold in India under the trade name ‘Bhuiamlki’. During market surveillance of herbal drug, it was observed that almost all the commercial samples, either comprise of *Phyllanthus amarus* Schum & Thonn. or *Phyllanthus maderaspatensis* Linn. or mixture of *Phyllanthus amarus*, *Phyllanthus urinaria* and *Phyllanthus maderaspatensis* [13]. So, in this context the pharmacognostic evaluation of all the three species has been carried out with the aim to establish their identification which includes both microscopic and macroscopic study including morphological and organoleptic analysis of each species respectively.

**Materials and methods**

**Plant material:** Fresh samples of whole plant of *Phyllanthus amarus*, *Phyllanthus maderaspatensis* and *Phyllanthus urinaria* taken for the study.

**Collection of Drug:** The study area was thoroughly surveyed for the presence of *Phyllanthus* species and Samples were collected from around the campus and nearby areas of Tirumala, Andhra Pradesh, India.
Authentification of the plant materials was done by the Department of Dravyaguna, S.V. Ayurvedic Medical College, Tirupati by examining the macroscopic and microscopic characteristics of the plant materials. The species, Cross Sections (Transverse section) were obtained free hand with sectioning blades, then stained in safranine dye and mounted in glycerine. Semi-permanent slides, so prepared, were examined under electronic Digital microscope (1000x). The photography was made with the help of Camera fi2 app linked to mobile camera.

**Plan of study**

According to the W.H.O (1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity.

So, this study has been performed in following two divisions:

- Macroscopic study
- Microscopic study

**Observations**

**Macroscopic Study**

- *Phyllanthus amarus* Schum & Thonn.
  - Organoleptic characters: (Dried plant powder)
  - Colour: Dark green
  - Odour: Pungent
  - Taste: Bitter
  - Texture: Slightly smooth powder
  - Root: Hard tap root system which is nearly straight, gradually tapering, with a number of secondary and tertiary roots, external surface light brown, fracture - short.

- *Phyllanthus maderaspatensis* Linn.
  - Organoleptic characters: (Dried plant powder)
  - Colour: Light green
  - Odour: Indistinct
  - Taste: Slightly bitter
  - Texture: Slightly rough, free flowing.
  - Root: The root is taproot with numerous secondary rootlets, light brown coloured with yellow colour fracture. Woody.

- *Phyllanthus urinaria* Linn.
  - Organoleptic characters: (Dried plant powder)
  - Colour: Dark green
  - Odour: Pungent
  - Taste: Slightly astringent
  - Texture: Slightly smooth.
  - Root: Root is with cluster of small thin rootlets at the end, light reddish brown in colour.

**Stem**: Slender, Terete, light brown, 2-6 cm long branching profuse towards upper region bearing 5-10 pairs of leaves.

- *Phyllanthus maderaspatensis* Linn.
  - Organoleptic characters: (Dried plant powder)
  - Colour: Light green
  - Odour: Indistinct
  - Taste: Slightly bitter
  - Texture: Slightly smooth.

- *Phyllanthus urinaria* Linn.
  - Organoleptic characters: (Dried plant powder)
  - Colour: Dark green
  - Odour: Pungent
  - Taste: Slightly astringent
  - Texture: Slightly rough, free flowing.

**Stem**: Glabrous, woody at base, 30 to 90 cm long, erect, branches terete below, striate and flattened, particularly at nodes towards apex.

**Stem**: Stem is Reddish-green, smooth, angled (pentagonal), woody at base, acutely ridged at nodes throughout, glabrous to pubescent.

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**Table 1**: Showing the morphological variations among three species

<table>
<thead>
<tr>
<th>Morphological character</th>
<th><em>Phyllanthus amarus</em></th>
<th><em>Phyllanthus maderaspatensis</em></th>
<th><em>Phyllanthus urinaria</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>Branching annual herb</td>
<td>Erect/decumbent herb</td>
<td>Erect/procumbent herb</td>
</tr>
<tr>
<td>Habitat</td>
<td>Prefers rocky, calcareous sites in humid tropical regions.</td>
<td>Common weed of waste places, garden lawns and cultivated fields, in moist and shady places and prefers black cotton soil.</td>
<td>Grows on river or stream banks, agricultural fields, forest margins, gardens, wastelands.</td>
</tr>
<tr>
<td>Height</td>
<td>Upto 80 cm</td>
<td>Upto 1m</td>
<td>Upto 60 cm</td>
</tr>
<tr>
<td>Stem shape</td>
<td>Terete</td>
<td>Terete below, striate and flattened above</td>
<td>Terete below, flattened above</td>
</tr>
<tr>
<td>Stem colour and form</td>
<td>Greenish, smooth, rounded, glabrous and woody at base.</td>
<td>Greenish, slender, smooth and woody at base.</td>
<td>Reddish green, smooth, pentagonal, glabrous and woody at base.</td>
</tr>
<tr>
<td>Leaf shape</td>
<td>Oblong/elliptic oblong</td>
<td>Obovate / spatulate</td>
<td>Oblong/linear oblong</td>
</tr>
<tr>
<td>Leaf tip</td>
<td>Obovate and shortly mucronate.</td>
<td>Rounded mucronate</td>
<td>Obtuse with distinct mucronate</td>
</tr>
<tr>
<td>Leaf base</td>
<td>Obute / rounded</td>
<td>Cuneate</td>
<td>Unequal</td>
</tr>
<tr>
<td>Flower</td>
<td>Unisexual and bisexual cymules</td>
<td>Unisexual and bisexual cymules</td>
<td>Unisexual cymules</td>
</tr>
<tr>
<td>Female disc</td>
<td>Flat, deeply five lobed</td>
<td>Six lobed</td>
<td>Six lobed</td>
</tr>
<tr>
<td>Capsule</td>
<td>Globose and smooth</td>
<td>Depressed globose and smooth</td>
<td>Depressed globose and prominently echinate.</td>
</tr>
<tr>
<td>Seed</td>
<td>Trigonous with 6-7 parallel longitudinal ribs on back</td>
<td>Trigonous, muriculate in fine lines.</td>
<td>Triquetrous with prominent transverse ridges and crossbars.</td>
</tr>
</tbody>
</table>
• Microscopic Study

Outline is circular with fissures.
• Epidermis is broken with 2 – 4 layers of tangentially elongated and lignified cork cells, which are filled with brown content and oil globules.
• Some of the isolated 2 – 3 celled pericyclic fibres were observed in cortical region.
• Cortex ends with single layer of parenchymatous endodermis.
• Phloem: The Phloem is about 4 – 6 cells thick.
• Xylem: It includes either Solitary or multiples of thin walled circular vessels.

Image 1: Showing the Transverse section of *Phyllanthus amarus* - Root

Outline is smooth and circular covered by thick cuticle.
• Epidermis with Epidermal cells which are rectangular and thick walled.
• Hypodermis: It is 2 - 3 layers thick.
• Cortex: Cortical zone was narrow, inner border of the cortex was seen unevenly thick with discontinuous cylinder of cortical fibres.
• Sclerenchyma: It is 2 – 3 layers thick
• Larger region of the section is occupied by pith; parenchymatous cells are filled with some starch grains and rosette crystals of Calcium oxalate.

Image 2: Showing the Transverse section of *Phyllanthus amarus* - Stem

Outline is irregularly circular with shallow ridges.
• Epidermis is filled with lignified cork cells with some cells filled with brown content.
• Below epidermis is composed of tangentially elongated 2 – 3 layers of lignified cork cells, some of the cells filled with brown content.
• Cortex region is found to be moderately loosely arranged and made up of 4 – 7 layers of simple parenchymatous cells.
• Medullary rays are uni-serrate and started from central region and extended up to inner layers of the pericyclic region.

Image 3: Showing the Transverse section of *Phyllanthus maderaspatensis* – Root
- **Outline** is somewhat Pentagonal in shape.
- **Epidermis** is single layered with compactly arranged cells.
- **Hypodermis** is made up of single layered but contains 2-4 layered sclerenchyma cells.
- **Cortex** is made up of 3 – 5 layers of simple parenchymatous cells.
- **Xylem** is separated by uni-serrate medullary rays. **Phloem** is well developed situated above the xylem with sieve elements and fibres.
- Parenchymatous pith cells are compactly arranged in pith region. **Pith** cells are filled with oil globules, simple and compound starch grains and sphenoid crystals of Calcium oxalate.

**Image 4:** Showing the Transverse section of *Phyllanthus maderaspatensis* – Stem

- **Cork:** Composed of 2-4 layers of tangentially elongated lignified cork cells, some of the cells filled with brown content and oil globules.
- **Cortex** region is found to be 8 – 10 layered with loosely arranged simple parenchymatous cells which are sacrely isolated with simple starch grains and tannin content.
- **Endodermis:** The innermost layer of the cortex which is endodermis is one layer thick.
- The **Vascular bundles** which are radially arranged contained both xylem and phloem with xylem being more prominent.

**Image 5:** Showing the Transverse section of *Phyllanthus urinaria* – Root

- **Outline** is circular with angular projections/ ridges at different places.
- **Epidermis** is single layered and covered with thick cuticle.
- **Collenchyma** ranges from 2 – 5 layers. The shape of collenchyma is rectangular.
- **Cortex** forms narrow zone with polygonal shaped cells of parenchyma.
- **Endodermis:** It lies immediately outside the pericyclic sclerenchyma which is 2 – 3 layers thick.
- The **phloem** ranged from 3 – 5 layers while the no of elongated cells in xylem vessels ranged from 2 – 5 cells.
- **Pith,** parenchymatous cells are compactly arranged in pith region. Pith cells are filled with oil globules, rarely simple starch grains and some rosette crystals of Calcium oxalate.

**Image 6:** Showing the Transverse section of *Phyllanthus urinaria* - Stem
Results and discussion
The morphological, macroscopic and microscopic analysis of three species reveals the following facts
- Morphologically variation in the height of the herb, root system was noticed. Stem colour and form vary among species (Phyllanthus urinaria) in reddish brown in comparison with other two species which are greenish). Stem Fracture is Short (Phyllanthus amarus), Yellow (Phyllanthus maderaspatensis) and reddish brown (Phyllanthus urinaria).
- P. amarus can be easily distinguished from its allies by its unique characteristics of terete stem and five tepals.
- Leaf shape is specific in case of P. maderaspatensis which is spatulate with rounded mucronate tip whereas it is oblong or linear oblong in case of P. amarus and P. urinaria respectively.
- Microscopical studies indicated the presence of highly lignified with brown content in Root sections of Phyllanthus amarus and Phyllanthus urinaria than Phyllanthus maderaspatensis root.
- Stem section of P. urinaria and P. maderaspatensis possess ridges and furrows which are passed over by a single layer of epidermis. The presence of ridges and furrows were as result of the angular nature of the stem circumference. This feature is absent in P. amarus due to rounded stem circumference.
- Narrow cortical zone with unevenly thick cortical fibres was found in P. amarus (stem), whereas cortical cells are polygonal shaped in P. urinaria (stem) and with simple parenchymatous cells in case of P. maderaspatensis (stem).

The word Bhumyamalaki thus reasonable to be considered as a generic name includes different species of Phyllanthus which are having their specific action. Among various species available, three species which are most abundant and commonly sold in commercial markets were taken for the study. It is observed that P. amarus and P. maderaspatensis have five and six prominent tepals respectively, but P. urinaria got very minute six tepals. Capsule of P. urinaria is echinate, whereas rest two is smooth. Hard tap root, woody tap root, highly fibrous root system are seen in P. amarus, P. maderaspatensis and P. urinaria respectively. The Pharmacognostic study done on three different species of Phyllanthus and the data generated is helpful in determining the Quality and Purity of the Drug.

Conclusion
In Ayurveda and folklore practices Phyllanthus amarus as Bhumyamalaki, is used in various ailments especially in liver troubles, but other two species viz. Phyllanthus maderaspatensis and Phyllanthus urinaria are all mixed up and being sold in herbal drug markets of the country by the same vernacular. Considering the medicinal properties of the Phyllanthus herbs and their ethno pharmacological value, these species have been assessed for their pharmacognostic study. The macroscopic and microscopic diagnostic characters drawn from the present study by using simple techniques may eventually help to authenticate genuine samples of Phyllanthus which are being mixed up and sold in the market as Bhumyamalaki/Tamalaki.

References