



QUALITY EVALUATION OF BHUMYAMALAKI (PHYLLANTHUS NIRURI LINN.) PANCHANGA THROUGH PHARMACOGNOSTICAL, PHYSICOCHEMICAL AND HPLC FINGERPRINT ANALYSIS

Dr. Bhumishree G.*¹, Dr. D. N. Dhari², Dr. Kasinath Hadimur³

¹Postgraduate Scholar, Department of PG Studies in Dravyaguna Vijnana, BLDEA's AVS Ayurveda Mahavidyalaya, Hospital and Research Centre, Vijayapura-586103, Karnataka, India.

²Professor, Department of PG Studies in Dravyaguna Vijnana, BLDEA's AVS Ayurveda Mahavidyalaya, Hospital and Research Centre, Vijayapura-586103, Karnataka, India.

³Associate Professor, Department of PG and PhD Studies Rasashastra and Bhaishajya Kalpana, BLDEA's AVS Ayurveda Mahavidyalaya, Hospital and Research Centre, Vijayapura-586103, Karnataka, India.

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*Corresponding author:

Dr. Bhumishree G.

Postgraduate Scholar, Department of PG Studies in Dravyaguna Vijnana, BLDEA's AVS Ayurveda Mahavidyalaya, Hospital and Research Centre, Vijayapura-586103, Karnataka, India.

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ABSTRACT

Background: *Bhumyamalaki Phyllanthus niruri* Linn. is an important medicinal plant widely used in Ayurveda for the management of various diseases. Establishing pharmacognostical and physicochemical standards is essential to ensure its authenticity, quality, and purity. **Objective:** The present study aimed to establish pharmacognostical, physicochemical, phytochemical, and High-Performance Liquid Chromatography (HPLC) fingerprint standards for *Phyllanthus niruri* Linn. Panchanga. **Materials and Methods:** Fresh Panchanga was collected, authenticated and subjected to organoleptic, macroscopic and microscopic evaluation. Physicochemical parameters including loss on drying, total ash, pH, specific gravity, and solubility profile were determined using standard procedures. An aqueous extract was prepared by Soxhlet extraction and subjected to preliminary phytochemical screening and HPLC fingerprint analysis. **Results:** The study established characteristic organoleptic, morphological, and anatomical features useful for authentication of the crude drug. Physicochemical evaluation showed a loss on drying of 10%, total ash of 16%, pH of 6.85, and specific gravity of 1.012. The extract was soluble in water and hydrochloric acid. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, triterpenoids, saponins, and proteins. HPLC fingerprint analysis produced seven well-resolved peaks, with the major peak observed at a retention time of 10.20 min. **Conclusion:** The findings provide comprehensive reference standards for the authentication, quality assessment, and standardization of *Phyllanthus niruri* Linn. Panchanga, supporting its use as a quality-assured raw material in herbal and Ayurvedic formulations.

KEYWORDS: *Phyllanthus niruri* Linn.; Bhumyamalaki; Pharmacognostical Evaluation; Physicochemical Standardization; Phytochemical Screening; HPLC Fingerprint Analysis.

INTRODUCTION

Medicinal plants form the foundation of traditional systems of medicine and remain an important component

of primary healthcare across the world. The growing global acceptance of herbal medicines has increased the need for authenticated, safe, and standardized crude

drugs.^[1,2] However, the quality, safety, and therapeutic efficacy of medicinal plants may vary considerably due to differences in geographical origin, environmental conditions, harvesting practices, processing methods, storage, and transportation.^[1,3] In addition, adulteration, substitution, and misidentification of herbal raw materials continue to pose significant challenges to the quality and reliability of herbal formulations.^[2,4] Therefore, the establishment of pharmacognostical and physicochemical standards is essential for confirming the identity, purity, and quality of medicinal plant materials.

Pharmacognostical evaluation is considered the primary step in the authentication and standardisation of crude drugs. Organoleptic, macroscopic, and microscopic characteristics provide reliable diagnostic parameters for distinguishing authentic plant materials from substitutes and adulterants.^[3,4] Similarly, physicochemical parameters such as loss on drying, total ash, pH, specific gravity, and solubility profile are important quality control indices recommended for herbal drugs.^[1,3] Preliminary phytochemical screening provides qualitative information regarding the major classes of phytoconstituents present in plant materials and serves as an important tool for quality assessment. High Performance Liquid Chromatography (HPLC) has emerged as one of the most reliable analytical techniques for herbal drug standardization because of its accuracy, sensitivity, reproducibility, and ability to generate characteristic chromatographic fingerprints, thereby facilitating authentication and batch-to-batch quality assessment.^[5,6]

Phyllanthus niruri Linn. (Family: Euphorbiaceae), commonly known as *Bhumyamalaki*, described as *Tamalaki* in classical Ayurvedic literature, is an important medicinal herb with a long history of therapeutic use. *Tamalaki* has been described in the *Charaka Samhita* under *Kāśahara* and *Śvāsahara Daśhemanī Mahākāśāya* and is included in formulations such as *Cyavanaprāśa*, *Pippalyādi Ghrta*, *Balādi Ghrta*, and *Amṛta Taila*.^[7] *Suśruta Samhitā*, *Aṣṭāṅga Hrdaya*, and *Aṣṭāṅga Saṅgraha* also describe its therapeutic utility in various *Ghrta*, *Taila*, and *Cūrṇa* formulations.^[8] In the *Nighaṇṭu* period, the drug is elaborately described in *Dhanvantari Nighaṇṭu*, *Kaiyadeva Nighaṇṭu*, *Bhāvaprakāśa Nighaṇṭu*, *Rāja Nighaṇṭu*, and *Madanapāla Nighaṇṭu*, where its synonyms, *Rasapañcaka*, *Doṣaghnaṭā*, *Karma*, and therapeutic indications are comprehensively documented, establishing its identity and importance in Ayurvedic therapeutics.^[9]

Owing to its extensive classical use and increasing medicinal importance, establishment of reliable pharmacognostical and physicochemical standards is essential to ensure the authenticity and quality of *Bhumyamalaki* before its therapeutic utilization. Although several pharmacological investigations have been carried out on *Phyllanthus niruri*, comprehensive

evaluation integrating pharmacognostical characteristics, physicochemical parameters, preliminary phytochemical screening, and HPLC fingerprint profiling is essential for developing reliable quality control standards.^[5,6,10] Therefore, the present study was undertaken to evaluate the pharmacognostical characteristics, physicochemical parameters, preliminary phytochemical profile, and HPLC fingerprint of *Phyllanthus niruri* Linn. *Panchanga* to establish reference standards for its identification and quality control.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh *Bhumyamalaki* (*Phyllanthus niruri* Linn.) *Panchanga* was collected from its natural habitat in and around Vijayapura district, Karnataka, India, during the flowering and fruiting season. The collected plant material was cleaned thoroughly to remove adhering soil and extraneous matter, shade-dried at room temperature, and coarsely powdered using a mechanical grinder. The plant specimen was botanically identified and authenticated by experts in the Department of Dravyaguna Vijnana, BLDEA's AVS Ayurveda Mahavidyalaya Hospital and Research centre, Vijayapura, Karnataka.

Standardization of the Study Drug

Pharmacognostical Evaluation

The authenticated *Panchanga* of *Phyllanthus niruri* Linn. was subjected to pharmacognostical evaluation, including organoleptic, macroscopic, and microscopic examinations, according to the standard procedures described in the *Ayurvedic Pharmacopoeia of India* (API) for authentication and quality standardization.^[11] Organoleptic characters such as colour, odour, taste, texture, and appearance were recorded. Macroscopic evaluation included the examination of the morphological characteristics of the root, stem, leaves, flowers, fruits, and seeds, while microscopic evaluation was carried out to identify the characteristic anatomical features of the plant.

Physicochemical Evaluation

The physicochemical evaluation of the powdered *Panchanga* was carried out according to the standard procedures prescribed in the *Ayurvedic Pharmacopoeia of India* (API) and the *WHO Quality Control Methods for Herbal Materials*.^[2,11] The parameters evaluated included loss on drying at 105°C, Total ash, pH, Specific gravity and qualitative solubility profile.

Solubility Analysis

The powdered *Panchanga* of *Phyllanthus niruri* Linn. was subjected to qualitative solubility analysis using Distilled water, Hydrochloric acid, Ethanol, Methanol, Chloroform, and Petroleum ether according to standard laboratory procedures to determine its solubility characteristics.

Preparation of Aqueous Extract

The aqueous extract of *Panchanga* was prepared by Soxhlet extraction. Briefly, 50 gm of the shade-dried and coarsely powdered drug was extracted with distilled water until complete exhaustion. The extract was concentrated by evaporating the solvent under reduced pressure using a rotary vacuum evaporator to obtain a semisolid extract. The concentrated extract was preserved in an airtight container until further use for preliminary phytochemical screening and HPLC fingerprint analysis.

Preliminary Phytochemical Screening

The aqueous extract was subjected to preliminary qualitative phytochemical screening using standard laboratory procedures to detect the presence of major phytochemical constituents, including alkaloids, flavonoids, tannins, saponins, glycosides, steroids, terpenoids, phenolic compounds, carbohydrates, proteins, amino acids, and fixed oils.

High-Performance Liquid Chromatographic Analysis

HPLC fingerprint analysis of the aqueous extract was carried out at the Department of Pharmaceutical Quality Assurance, Maratha Mandal's College of Pharmacy, Belagavi, Karnataka, India. Chromatographic separation was performed using a High-Performance Liquid Chromatography system equipped with a reverse-phase C18 analytical column under optimized chromatographic conditions. The chromatographic profile was recorded, and the retention times and peak areas of the resolved peaks were documented to establish the characteristic HPLC fingerprint profile of the aqueous extract of *Phyllanthus niruri* Linn. *Panchanga*.^[5,6]

RESULTS

Organoleptic Evaluation

The organoleptic evaluation of *Phyllanthus niruri* Linn. *Panchanga* revealed characteristic features useful for the identification of the crude drug. The observations are summarized in **Table 1**.

Table 1: Organoleptic characters of *Phyllanthus niruri* Linn. *Panchanga*.

Sl no	Parameter	Observation
1	Colour	Pale Green to dark green
2	Odour	Characteristic odour
3	Taste	Bitter, slightly astringent
4	Texture	Fresh: Soft, smooth, succulent, and herbaceous. On drying: Rough, fibrous, brittle, and easily breakable.

Macroscopic Evaluation

Macroscopic examination of the fresh *Phyllanthus niruri* Linn. *Panchanga* revealed characteristic morphological features that facilitated its identification. *Phyllanthus niruri* is a small erect annual herb (Figure 1). The root was thin, cylindrical, light brown, and slightly branched (Figure 2). The stem was slender, green, smooth,

herbaceous, and profusely branched (Figure 3). Leaves were small, simple, alternate, oblong, and light green in colour (Figure 4). Flowers were small, greenish, axillary, and unisexual. Fruits were globose capsules, green when immature and brown on maturation, enclosing small, hard, triangular, brownish seeds (Figure 5)



Figure 1



Figure 2



Figure 3



Figure 4



Figure 5

Microscopic Evaluation

Microscopic examination of the transverse sections of different plant parts revealed characteristic anatomical features useful for authentication of the crude drug. The transverse section of the root of *Phyllanthus niruri* reveals a typical dicotyledonous structure. The outermost layer is a single-layered epidermis composed of thin-walled cells. Beneath the epidermis lies a wide cortex consisting of several layers of parenchymatous cells, which serve as storage tissue. A distinct endodermis is present, characterized by compact barrel-shaped cells.

The pericycle is single-layered and located just inside the endodermis. The vascular system exhibits a radial arrangement, with xylem and phloem alternating with each other. The xylem is exarch in nature, consisting of vessels, tracheids, and fibers, while the phloem is composed of sieve elements and parenchyma. The pith is either small or inconspicuous. The root also shows the presence of starch grains and occasional calcium oxalate crystals as diagnostic features. These features are depicted in **Figure 6** and **Figure 7**.



Figure 6

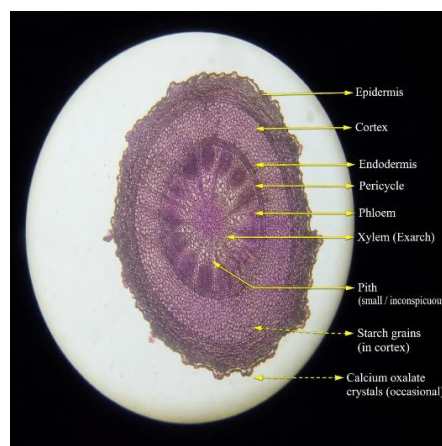


FIGURE 7

The transverse section of the stem shows a circular outline with a well-organized internal structure. The epidermis is single-layered and covered with a thin cuticle. The cortex is differentiated into an outer collenchymatous region providing mechanical support and an inner parenchymatous region. A distinct endodermis is present, often forming a starch sheath. The pericycle consists of sclerenchymatous fibers that provide additional strength. The vascular bundles are

collateral and open, arranged in a ring, which is a characteristic feature of dicot stems. The xylem is oriented towards the inner side and the phloem towards the outer side. Medullary rays are present between the vascular bundles, facilitating lateral conduction. The central region is occupied by a large pith composed of parenchymatous cells. Calcium oxalate crystals and starch grains are commonly observed. These features are depicted in **Figure 8 & 9**.



Figure 8

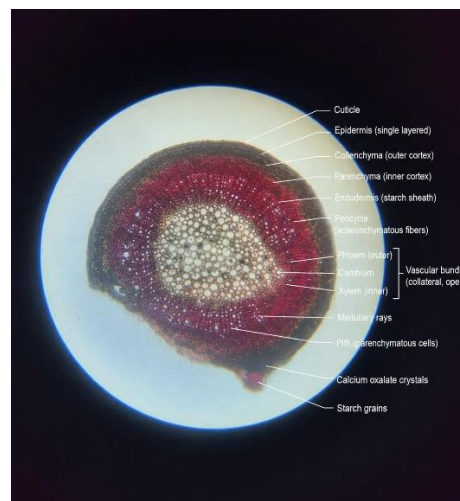


Figure 9

Physicochemical Evaluation

The physicochemical parameters of *Phyllanthus niruri* Linn. *Panchanga* were evaluated according to the standards prescribed in the *Ayurvedic Pharmacopoeia of India*. The results are presented in **Table 2**. Figures are

shown respectively in **FIGURE 10** (Loss on drying), **FIGURE 11** (Crude drug before incineration), **FIGURE 12** (Crude drug during incineration), **FIGURE 13** (Ash value), **FIGURE 14** (Ph) **FIGURE 15**(Specific gravity)

Table 2: Physicochemical parameters of *Phyllanthus niruri* Linn. *Panchanga*.

Sl no	Parameter	Result
1	Loss on drying (105°C)	10%
2	Total ash	16%
3	Specific gravity	1.012
4	pH	6.85



FIGURE 10



FIGURE 11



FIGURE 12



FIGURE 13



FIGURE 14



FIGURE 15

Solubility Analysis

The powdered *Panchanga* was subjected to qualitative solubility analysis using different solvents.^[13,14] The observations are presented in Table 3 and Figure 16.

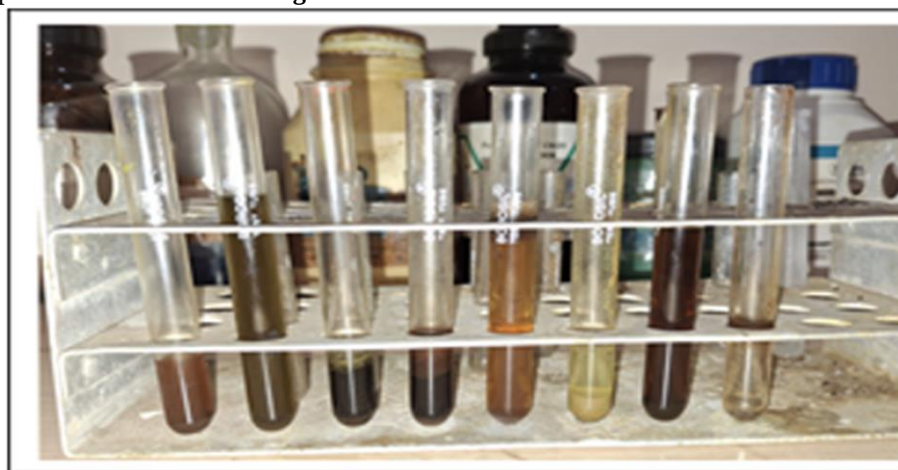


FIGURE 16

Table 3: Solubility profile of *Phyllanthus niruri* Linn. *Panchanga*.

Sl no	Media	Solubility
1	Methanol	Sparingly Soluble
2	Ethanol	Sparingly Soluble
3	Xylene	Insoluble
4	Chloroform	Insoluble
5	Distilled water	Soluble
6	Hydrochloric acid	Soluble

Preparation of Aqueous Extract

The Aqueous extract of *Phyllanthus niruri* Linn. *Panchanga* was successfully obtained by Soxhlet extraction.^[13] The extract was **dark brown in colour, semisolid** in consistency, and possessed a **characteristic odour**. The concentrated extract was preserved in an airtight container and used for preliminary phytochemical screening and HPLC fingerprint analysis.

Preliminary Phytochemical Screening

The Aqueous extract was subjected to preliminary qualitative phytochemical screening to detect alkaloids, carbohydrates, proteins, amino acids, glycosides, flavonoids, tannins, phenolic compounds, saponins, steroids, triterpenoids, and fixed oils and fats using standard methods.^[5,14] The results are presented in **Table 4**.

Table 4: Preliminary phytochemical constituents detected in the aqueous extract of *Phyllanthus niruri* Linn. *Panchanga*.

Sl no	Tests	Results
1.	Test for Alkaloids Wagner's Test	Present
2.	Test for Carbohydrates Benedicts test	Absent
3.	Test for Tannins	Absent
4.	Test for Steroids Salkowski Test	Present
5.	Test for Triterpenoids Salkowski Test	Present
6.	Test for Flavonoids Lead Acetate Test	Present
7.	Test for Proteins Millions Biuret Test	Present
8.	Test for Saponins Foam test	Present
9.	Test for Caretenoids	Absent

HPLC Fingerprint Analysis

HPLC fingerprint analysis of the aqueous extract of *Phyllanthus niruri* Linn. *Panchanga* demonstrated a characteristic chromatographic profile comprising seven well-resolved peaks at different retention times. The highest peak area percentage (27.8%) was observed at a retention time of 10.20 min (**major bioactive constituents, such as lignans (e.g., phyllanthin-like compounds or flavonoids.)** followed by peaks at 7.85

min (20.5%) (**less polar, more hydrophobic compounds.** Possibly corresponds to **lignans, ellagitannins, or related polyphenolic compounds.**) and 5.40 min (17.0%), indicating the presence of multiple phytochemical constituents. The detailed chromatographic profile is presented in **Table 5**, and the representative HPLC chromatogram is shown in **Figure 17**.

Table 5: HPLC fingerprint profile of the aqueous extract of *Phyllanthus niruri* Linn. *Panchanga*.

Peak no	Retention time(min)	Peak area	Area (%)
1	2.15	12,500	8.2
2	3.78	18,200	12.1
3	5.40	25,600	17.0
4	7.85	31,000	20.5
5	10.20	42,000	27.8
6	12.65	15,000	9.9
7	15.30	8,000	4.5

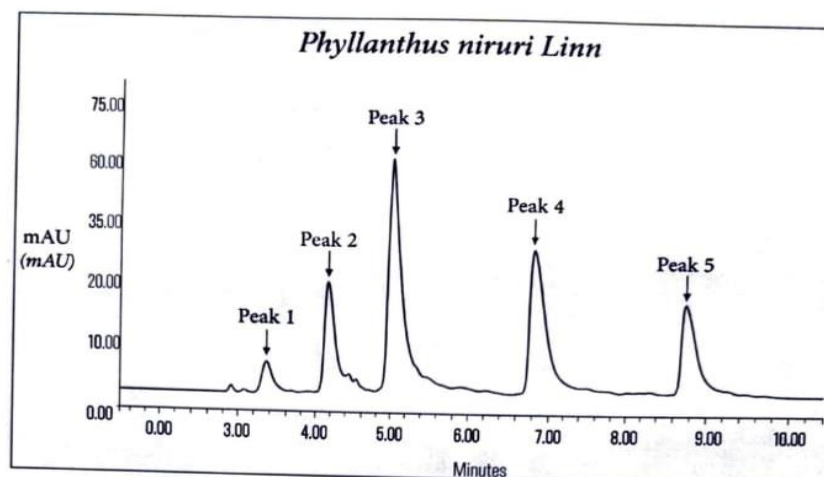


Figure 17: HPLC chromatogram of the aqueous extract of *Phyllanthus niruri* Linn. *Panchanga*.

DISCUSSION

The present study established pharmacognostical, physicochemical, phytochemical, and HPLC fingerprint standards for *Phyllanthus niruri* Linn. Panchanga, providing scientific evidence for its authentication and quality assessment. Organoleptic evaluation revealed characteristic features such as a pale green to dark green colour, characteristic herbal odour, and bitter, slightly astringent taste, which are useful for preliminary identification of the crude drug.

Macroscopic examination showed that *P. niruri* is a small erect annual herb with a slender branched stem, simple oblong leaves, greenish axillary flowers, and globose capsules containing triangular brown seeds. These morphological features are characteristic of the species and facilitate its identification in the fresh state.

Microscopic evaluation revealed characteristic diagnostic features such as a typical dicot root with radial vascular bundles and exarch xylem, and a dicot stem with collateral open vascular bundles, sclerenchymatous pericycle, medullary rays, and parenchymatous pith. The presence of starch grains and calcium oxalate crystals further supports the authentication of the crude drug.

The physicochemical parameters indicated good quality of the raw material. A loss on drying value of 10% suggests low moisture content and better storage stability, while the total ash value (16%), pH (6.85), and specific gravity (1.012) may serve as useful reference standards for quality control. The solubility profile demonstrated better solubility in water than in organic solvents, supporting the traditional use of aqueous preparations in Ayurveda.

Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, steroids, triterpenoids, saponins, and proteins, indicating the presence of diverse bioactive constituents responsible for the therapeutic potential of *P. niruri*. HPLC fingerprint analysis generated seven well-resolved peaks, with the major peak observed at a retention time of 10.20 min, providing a characteristic chromatographic profile that can serve as a reference for authentication and batch-to-batch quality evaluation.

Overall, the integration of pharmacognostical, physicochemical, phytochemical, and chromatographic findings provides a comprehensive quality control profile for *Phyllanthus niruri* Linn. Panchanga and establishes baseline standards for its authentication and standardization in herbal formulations.

CONCLUSION

The present study successfully established comprehensive pharmacognostical, physicochemical, phytochemical, and HPLC fingerprint standards for *Phyllanthus niruri* Linn. Panchanga. Characteristic organoleptic, macroscopic, and microscopic features

confirmed the botanical identity of the plant, while physicochemical parameters demonstrated acceptable quality attributes suitable for herbal drug standardization. Preliminary phytochemical screening revealed the presence of important bioactive constituents, and HPLC fingerprint analysis generated a characteristic chromatographic profile that may serve as a reliable reference for authentication and quality control. Collectively, these findings provide scientifically validated baseline standards for the identification, purity assessment, and standardization of *Phyllanthus niruri* Linn. Panchanga and support its use as a quality-assured raw material in Ayurvedic and herbal pharmaceutical formulations. Future studies involving quantitative estimation of marker compounds and advanced spectroscopic characterization may further strengthen its pharmacopoeial standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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