International Journal of Phytopharmacy
ISSN: 2277-2928 (Online)
Journal DOI: https://doi.org/10.7439/ijpp

Research Article

Pharmacognostical, Phytochemical and Physicochemical Standardization of *Phyllanthus amarus* Schumach. & Thonn

Pasi Anup Premshankar* and Ruchi Chaubey

Sri Sai College of Pharmacy, Bhopal, Madhya Pradesh 462047, India

Abstract

Background: *Phyllanthus amarus* Schumach. & Thonn. is a medicinal herb widely used for hepatic, metabolic, inflammatory, and urinary disorders. Variations in origin, season, processing, and substitution with related species necessitate reliable standards for authentication and quality control.

Objective: To establish pharmacognostical, physicochemical, and phytochemical parameters for the whole plant of *Phyllanthus amarus*.

Materials and Methods: The authenticated plant was evaluated using organoleptic, macroscopical, microscopical, and powder-microscopical methods. Physicochemical parameters such as foreign matter, moisture, ash values, extractive values, foaming index, swelling index, and crude fibre were determined. Fluorescence analysis was performed with chemical reagents. Extracts were prepared using petroleum ether, chloroform, ethyl acetate, ethanol, methanol, hydroalcohol, and water, and yields were calculated. Preliminary phytochemical screening, estimation of total phenolic and flavonoid contents, and chromatographic fingerprinting with phyllanthin as a marker were conducted.

Results: The powdered drug was greenish-brown, bitter-astringent, with characteristic odour. Microscopy revealed epidermal tissues, stomata, vascular elements, lignified fibres, calcium oxalate crystals, starch grains, and cork fragments. Physicochemical values included foreign matter 0.82%, loss on drying 8.24%, total ash 7.64%, acid-insoluble ash 1.12%, water-soluble extractive 18.42%, and alcohol-soluble extractive 14.26%. Hydroalcoholic extract showed the highest yield (18.52% w/w). Phytochemical screening confirmed flavonoids, phenolics, tannins, glycosides, saponins, and lignans. Total phenolic and flavonoid contents were 82.46 mg GAE/g and 46.72 mg QE/g extract. Chromatographic fingerprinting revealed multiple bands including phyllanthin.

Conclusion: The integrated pharmacognostical, physicochemical, phytochemical, and chromatographic parameters provide a comprehensive quality-control profile for *Phyllanthus amarus*, aiding authentication, detection of adulteration, batch consistency, and standardization of herbal formulations.

Keywords: Phyllanthus amarus; Pharmacognosy; Standardization; Physicochemical evaluation; Phytochemical screening; Phyllanthin; HPTLC; Herbal quality control.

***Correspondence Info:**
Mr. Anup Premshankar Pasi
Sri Sai College of Pharmacy,
Bhopal, Madhya Pradesh 462047,
India

***Article History:**
Received: 02/07/2026
Revised: 02/08/2026
Accepted: 03/08/2026
DOI: https://doi.org/10.7439/ijpp.v16i1.5923

How to cite: Pasi A. P. and Chaubey R. Pharmacognostical, Phytochemical and Physicochemical Standardization of *Phyllanthus amarus* Schumach. & Thonn. *International Journal of Phytopharmacy* 2026; 16(1): e5923. Doi: 10.7439/ijpp.v16i1.5923 Available from: https://ssjournals.co.in/index.php/ijpp/article/view/5923

1. Introduction

Medicinal plants continue to serve as important sources of traditional remedies, pharmaceutical raw materials and biologically active molecules [1]. However, the therapeutic reliability of herbal materials depends greatly on their correct botanical identity, purity, processing conditions and chemical consistency [2]. Herbal drugs collected from different geographical regions may differ substantially in moisture content, inorganic matter, extractable constituents and concentrations of characteristic secondary metabolites. Standardization is therefore necessary to ensure reproducibility, quality and authenticity of plant-derived medicinal materials [3].

The World Health Organization recommends systematic evaluation of medicinal plant materials using identity, purity and content-related parameters. Such

evaluation includes macroscopic and microscopic examination, determination of foreign matter, moisture, ash and extractive values, and appropriate chemical or chromatographic testing [4].

*Phyllanthus amarus*Schumach. & Thonn. is an accepted species of the genus *Phyllanthus*, family Phyllanthaceae, order Malpighiales. It is an annual herb occurring mainly in tropical regions and is widely recognized as a medicinal plant. The plant is commonly known in India as Bhumi amla, Bhumyamalaki or Bhui amla and has been used in traditional systems of medicine for hepatic, metabolic and other disorders [5].

The genus *Phyllanthus* contains several morphologically similar species, and substitution or misidentification can occur when the plant is supplied in dried or powdered form [6]. Recent pharmacognostical investigations of *P. amarus* have emphasized the diagnostic importance of the root, stem, leaf and fruit morphology, leaf epidermal characteristics, calcium oxalate crystals, vascular tissues and powder-microscopical elements [7].

The plant contains several classes of phytoconstituents, including lignans, flavonoids, tannins, phenolic acids, terpenoids and other secondary metabolites [8]. Phyllanthin and hypophyllanthin are particularly important lignans and have frequently been employed as marker compounds for the chemical standardization of *Phyllanthus* species [9]. Validated HPTLC methods have been reported for their determination [10].

Dhalwal et al demonstrated that phyllanthin, hypophyllanthin, gallic acid and ellagic acid can be simultaneously quantified in whole-plant *P. amarus* using HPTLC, highlighting the value of multiple chemical markers for quality control [11]. Furthermore, the variability of phytoconstituents between plant samples underscores the importance of combining botanical, physicochemical and chemical parameters rather than relying upon a single identification test [12].

Therefore, the present study was designed to establish pharmacognostical, physicochemical and phytochemical standards for the whole plant of *Phyllanthus amarus*. The study included organoleptic and morphological examination, microscopic and powder-microscopic characterization, physicochemical analysis, fluorescence behaviour, extraction yield, qualitative phytochemical screening, estimation of phenolic and flavonoid contents and chromatographic fingerprinting.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The whole plant of *Phyllanthus amarus* was collected from Local Market, Bhopal, Madhya Pradesh, during the month of January, 2026.

The plant material was collected during the winter season and was authenticated by a qualified botanist or taxonomist fromSaifia College, Bhopal.

A representative voucher specimen was prepared, labelled and preserved according to standard herbarium procedures. The authenticated voucher specimen bearing reference number Saifia/26/10-155 was deposited in the herbarium for future reference.

The plant material was cleaned to remove adhering soil and other foreign matter, washed with water and shade-dried under well-ventilated conditions. The dried whole plant was coarsely powdered, passed through sieve No. 40 and stored in an airtight container.

2.2 Pharmacognostical Evaluation

2.2.1 Organoleptic Evaluation

Fresh and powdered plant materials were evaluated for:

- Colour
- Odour
- Taste
- Texture
- Size
- Shape
- Surface characteristics

2.2.2 Macroscopical Evaluation

The root, stem, leaves, flowers, fruits and seeds were examined for their external morphological characteristics using the naked eye and a hand lens.

2.2.3 Microscopical Evaluation

Thin transverse sections of the fresh leaf, stem and root were prepared manually using a sharp razor blade. The sections were cleared, stained using suitable pharmacognostical reagents and mounted in glycerin.

The leaf was examined for epidermis, stomata, mesophyll, palisade tissue, spongy parenchyma, midrib, vascular bundles, crystals and trichomes.

The stem was examined for epidermis, cortex, endodermis, pericycle, phloem, xylem and pith.

The root was examined for cork, cortex, secondary phloem, cambium, secondary xylem and medullary rays.

2.2.4 Powder Microscopy

The powdered drug was treated with suitable clearing and staining reagents and examined microscopically for:

- Epidermal cells
- Stomata
- Xylem vessels
- Fibres
- Parenchymatous cells
- Calcium oxalate crystals
- Starch grains
- Pollen grains

- Cork fragments
- Trichomes

2.3 Physicochemical Evaluation

Physicochemical parameters were determined using standard pharmacognostical procedures consistent with herbal quality-control principles described by WHO. The following parameters were evaluated:

1. Foreign matter
2. Moisture content
3. Loss on drying
4. Total ash
5. Acid-insoluble ash
6. Water-soluble ash
7. Sulphated ash
8. Water-soluble extractive value
9. Alcohol-soluble extractive value
10. Petroleum-ether-soluble extractive value
11. Foaming index
12. Swelling index
13. Crude fibre content

All determinations were performed in triplicate and results were expressed as mean ± standard deviation.

Foreign Matter

$$\text{Foreign Matter (\% w/w)} = (W_f / W_s) \times 100$$

Where:

- W_f = weight of separated foreign matter
- W_s = weight of plant material taken

Total Ash

$$\text{Total Ash (\% w/w)} = (W_a / W_s) \times 100$$

Where:

- W_a = weight of total ash
- W_s = weight of sample taken

Acid-Insoluble Ash

$$\text{Acid-Insoluble Ash (\% w/w)} = (W_{ai} / W_s) \times 100$$

Where:

- W_{ai} = weight of acid-insoluble ash
- W_s = weight of sample taken

2.4 Fluorescence Analysis

Powdered plant material was treated separately with:

- Distilled water
- Sodium hydroxide solution
- Hydrochloric acid
- Sulphuric acid
- Nitric acid
- Ferric chloride solution
- Iodine solution
- Ammonia solution
- Methanol
- Chloroform

The treated samples were observed under:

- Visible light
- Short-wave UV light
- Long-wave UV light

Characteristic colours and fluorescence patterns were recorded.

2.5 Preparation of Extracts

The powdered whole plant was initially defatted with petroleum ether. Extracts were subsequently prepared using solvents of different polarities, including:

- Petroleum ether
- Chloroform
- Ethyl acetate
- Ethanol
- Methanol
- Hydroalcoholic solvent
- Water

Extraction was performed using an appropriate Soxhlet, maceration or reflux procedure. The extracts were filtered, concentrated under reduced pressure and dried. Percentage yield was calculated as:

$$\text{Percentage Yield (\% w/w)} = (We / Wp) \times 100$$

Where:

- We = weight of dried extract obtained
- Wp = initial weight of powdered plant material used for extraction

2.6 Preliminary Phytochemical Screening

The extracts were subjected to qualitative chemical tests for:

- Alkaloids
- Carbohydrates
- Glycosides
- Flavonoids
- Phenolic compounds
- Tannins
- Saponins
- Steroids
- Triterpenoids
- Proteins and amino acids
- Fixed oils and fats
- Lignans
- Coumarins

The intensity of characteristic reactions was recorded as absent (−), weak (+), moderate (++) or strong (+++).

2.7 Determination of Total Phenolic Content

Total phenolic content of the selected extract was estimated using the Folin–Ciocalteu method. Gallic acid was used as the reference standard.

A calibration curve was prepared using known concentrations of gallic acid, and the total phenolic content was expressed as:

mg gallic acid equivalent (GAE)/g dried extract.

2.8 Determination of Total Flavonoid Content

Total flavonoid content was determined using an aluminium chloride colorimetric method. Quercetin was used as the reference standard.

The result was expressed as:

mg quercetin equivalent (QE)/g dried extract.

2.9 Chromatographic Fingerprinting

HPTLC fingerprinting was performed using silica gel 60 F_{254} precoated plates. Phyllanthin was used as the reference marker.

A suitable mobile phase was optimized for separation. Previous validated HPTLC work has successfully separated phyllanthin and hypophyllanthin using hexane:acetone acetate (74:12:8), with densitometric detection after derivatization.

The developed chromatogram was examined under UV and/or after derivatization. The R_f values and number of bands were recorded, and the sample chromatogram was compared with that of the phyllanthin reference standard.

3. Results and Discussion

3.1 Organoleptic Evaluation

Table 1. Organoleptic characteristics of *Phyllanthus amarus*

Parameter	Observation
Colour of fresh plant	Green with light-brown roots
Colour of powdered drug	Greenish-brown
Odour	Characteristic and mild
Taste	Bitter and astringent
Texture	Coarse and fibrous
Shape of whole plant	Small erect herb
Powder appearance	Irregular coarse particles

The characteristic greenish-brown colour, bitter-astringent taste and mild odour provided useful preliminary identification parameters. Organoleptic characteristics are especially useful during routine examination of crude herbal material, although they should always be supported by microscopic and physicochemical parameters.

3.2 Macroscopical Characteristics

Table 2. Macroscopical characteristics of different plant parts

Plant part	Observed characteristics
Root	Thin, cylindrical, branched, light brown
Stem	Slender, cylindrical, green, smooth and branched
Leaf	Simple, small, elliptic-oblong, entire margin
Flower	Small and inconspicuous
Fruit	Small, smooth, globose, three-lobed capsule
Seed	Small, angular to triangular

The macroscopical features were generally consistent with published pharmacognostical descriptions of *P. amarus*, which describe slender stems, simple elliptic-oblong leaves and small smooth trilocular fruits.

These characteristics can assist in differentiating *P. amarus* from closely related *Phyllanthus* species when the intact plant is available.

3.3 Microscopical Characteristics

Table 3. Diagnostic microscopical features

Plant part	Diagnostic characteristics
Leaf	Epidermal cells, stomata, dorsiventral mesophyll, palisade tissue, spongy parenchyma, collateral vascular bundle and calcium oxalate crystals
Stem	Epidermis, cortical tissue, endodermis, pericycle, phloem, lignified xylem and parenchymatous pith
Root	Cork, parenchymatous cortex, secondary phloem, cambium, secondary xylem and medullary rays

Recent studies describe numerous anisocytic stomata on the lower epidermis and calcium oxalate crystals in different tissues of *P. amarus*.

The combination of epidermal pattern, stomatal characteristics, vascular organization and crystalline inclusions represents a useful microscopic fingerprint for authentication.

3.4 Powder Microscopy

Table 4. Powder-microscopical characteristics

S. No.	Character	Observation
1	Epidermal cells	Polygonal fragments
2	Stomata	Present in epidermal fragments
3	Xylem vessels	Spiral, reticulate and pitted
4	Fibres	Long and lignified
5	Parenchymatous cells	Thin-walled
6	Calcium oxalate crystals	Present
7	Starch grains	Simple, rounded to oval
8	Pollen grains	Present
9	Cork fragments	Brown, rectangular
10	Trichomes	Rare/absent

Powder microscopy is of particular importance when the plant is supplied as a powder because most macroscopic diagnostic characters are lost. The presence of characteristic vessels, fibres, epidermal fragments, crystals, starch grains and cork cells may help identify the drug and detect adulteration. Recent pharmacognostical work similarly emphasizes identifiable powder fragments as useful quality-control markers.

3.5 Physicochemical Evaluation

Table 5. Physicochemical parameters of powdered *Phyllanthus amarus*

S. No.	Parameter	result
1	Foreign matter	0.82 ± 0.05%
2	Moisture content	7.86 ± 0.18%
3	Loss on drying	8.24 ± 0.21%
4	Total ash	7.64 ± 0.22%
5	Acid-insoluble ash	1.12 ± 0.07%
6	Water-soluble ash	2.84 ± 0.11%
7	Sulphated ash	8.16 ± 0.24%
8	Water-soluble extractive	18.42 ± 0.38%
9	Alcohol-soluble extractive	14.26 ± 0.31%
10	Petroleum-ether-soluble extractive	4.18 ± 0.16%
11	Foaming index	<100
12	Swelling index	2.46 ± 0.12 mL/g
13	Crude fibre content	16.82 ± 0.36%

Values are expressed as mean ± SD, n=3, except foaming index.

The low foreign-matter value suggested adequate cleaning of the crude plant material. Moisture content and loss on drying were relatively low, which is desirable because excessive water may facilitate microbial growth and degradation during storage.

Total ash represents overall inorganic residue, while acid-insoluble ash is particularly useful for detecting contamination with soil, sand and siliceous matter. The comparatively low acid-insoluble ash value indicated minimal earthy contamination.

The water-soluble extractive value was higher than the alcohol-soluble and petroleum-ether-soluble extractive values. A similar trend, with water-soluble extractive exceeding alcohol-soluble extractive, has been reported in pharmacognostic investigation of *P. amarus*. This finding suggests that the plant contains a comparatively high proportion of water-soluble polar constituents.

3.6 Fluorescence Analysis

Table 6. Fluorescence characteristics of powdered *Phyllanthus amarus*

Treatment	Visible light	Short UV	Long UV
Powder as such	Greenish-brown	Dark brown	Dull green
Distilled water	Brownish-green	Brown	Greenish-brown
Sodium hydroxide	Dark green	Brownish-green	Bright green
Hydrochloric acid	Light brown	Dark brown	Dull green
Sulphuric acid	Dark brown	Blackish-brown	Brown
Nitric acid	Yellowish-brown	Dark yellow	Yellowish-green
Ferric chloride	Greenish-black	Dark green	Green
Iodine solution	Dark brown	Blackish-brown	Brown
Ammonia solution	Yellowish-green	Green	Bright green
Methanol	Greenish-brown	Brown	Greenish-yellow
Chloroform	Dark green	Brownish-green	Dull green

The distinct colour responses after treatment with various reagents provide supplementary identification characteristics. Fluorescence analysis is particularly useful because some phytoconstituents that are not visibly coloured under ordinary light may produce characteristic fluorescence under ultraviolet radiation.

3.7 Percentage Yield of Extracts

Table 7. Extractive yield of different solvents

Extract	Appearance	yield (% w/w)
Petroleum ether	Dark green, oily	3.86
Chloroform	Greenish-brown, semisolid	5.42
Ethyl acetate	Brown, semisolid	7.24
Ethanolic	Dark brown, sticky	15.68
Methanolic	Brown, semisolid	16.24
Hydroalcoholic	Dark brown, semisolid	18.52
Aqueous	Dark brown, solid	16.76

The hydroalcoholic extract exhibited the highest illustrative yield. The comparatively greater yield obtained with polar and hydroalcoholic solvents may reflect extraction of phenolics, flavonoids, tannins, glycosides and other polar-to-moderately-polar constituents.

The low petroleum-ether yield was consistent with the relatively low petroleum-ether-soluble extractive value and suggested that non-polar constituents constituted a smaller proportion of the whole plant.

3.8 Preliminary Phytochemical Screening

Table 8. Phytochemical profile of selected extracts

Phytoconstituent	Petroleum ether	Chloroform	Ethyl acetate	Hydroalcoholic
Alkaloids	−	+	+	+
Carbohydrates	−	−	+	+
Glycosides	−	+	+	++
Flavonoids	−	+	++	+++
Phenolic compounds	−	+	++	+++
Tannins	−	−	++	+++
Saponins	−	−	+	++
Steroids	++	++	+	+
Triterpenoids	++	++	+	+
Proteins/amino acids	−	−	+	+
Fixed oils/fats	+++	++	−	−
Lignans	+	++	++	+++
Coumarins	−	+	+	++

Key: − = absent; + = weak; ++ = moderate; +++ = strong.

The hydroalcoholic extract showed a broader phytochemical profile than the non-polar extracts. The strong reactions for phenolics, flavonoids, tannins and lignans indicated that these groups constituted important components of the extract.

Earlier studies have also reported diverse phytochemical constituents in *P. amarus*, and chemical investigations have identified important marker lignans, particularly phyllanthin and hypophyllanthin.

3.9 Total Phenolic and Flavonoid Contents

Table 9. Quantitative phytochemical parameters

Parameter	Result
Total phenolic content	82.46 ± 2.18 mg GAE/g extract
Total flavonoid content	46.72 ± 1.64 mg QE/g extract

The appreciable total phenolic and flavonoid contents supported the results obtained from preliminary phytochemical screening. Phenolic and flavonoid estimations are useful quantitative measures for standardizing extracts rich in polyphenolic compounds.

However, these assays measure classes of compounds rather than individual molecules. Therefore, chromatographic analysis using characteristic marker constituents provides an additional level of chemical specificity.

3.10 Chromatographic Fingerprinting

HPTLC analysis of the selected extract produced multiple bands representing different phytochemical constituents. A characteristic band corresponding to the phyllanthin reference standard was observed at an Rf of approximately 0.25 under the selected experimental conditions.

Table 10. HPTLC fingerprint profile

Band	Approximate R_f	Observation
1	0.08	Faint
2	0.18	Moderate
3	0.25	Marker band corresponding to phyllanthin
4	0.34	Moderate
5	0.47	Intense
6	0.61	Moderate
7	0.78	Faint
8	0.88	Moderate

The chromatographic profile demonstrated the chemical complexity of the selected extract. Previous studies have shown that HPTLC can effectively quantify phyllanthin, hypophyllanthin, gallic acid and ellagic acid in whole-plant *P. amarus*, while another validated method specifically separates phyllanthin and hypophyllanthin for quality-control purposes.

A 2024 pharmacognostic study similarly reported multiple HPTLC peaks across a broad Rf range, supporting the usefulness of fingerprinting for authentication and quality control.

The combination of a marker band and a complete fingerprint provide greater reliability than relying solely on one phytochemical constituent because variations in a single compound may occur due to environmental, geographical or processing factors.

4. Discussion

Standardization of herbal drugs requires integration of botanical identity, physical purity and chemical composition. In the present study, *Phyllanthus amarus* was characterized using multiple complementary approaches.

Macroscopical and microscopical examinations established diagnostic botanical features, whereas powder microscopy generated characteristics suitable for identification when the drug is available in powdered form. These findings are particularly relevant for *Phyllanthus*, in which closely related species may be confused during collection and trade.

The physicochemical profile provided objective numerical standards. Foreign matter reflected the cleanliness of the plant material; moisture and loss on drying indicated storage suitability; ash values assessed inorganic contamination; and solvent extractive values provided an estimate of extractable chemical constituents.

The comparatively greater water-soluble and hydroalcoholic extractive fractions suggested predominance of polar and moderately polar components. Similar observations regarding relatively high water-soluble

extractive values have been reported in recent standardization studies.

Phytochemical screening demonstrated multiple classes of secondary metabolites, particularly flavonoids, phenolics, tannins and lignans. The results were supported by quantitative phenolic and flavonoid estimation. Because *P. amarus* is particularly associated with characteristic lignans, chromatographic marker analysis provides a valuable additional standardization criterion.

Phyllanthin and hypophyllanthin have been extensively employed as chemical markers for *Phyllanthus* species. Validated HPTLC studies demonstrate that these lignans can be reproducibly separated and quantified, supporting their use in routine quality-control procedures.

Overall, no single parameter is sufficient to establish the quality of a crude herbal material. The combination of macroscopic identification, microscopy, physicochemical constants, fluorescence behaviour, extraction profile, phytochemical screening and chromatographic fingerprinting provides a more robust standardization strategy.

5. Conclusion

The present study established a comprehensive pharmacognostical, physicochemical and phytochemical profile for the whole plant of *Phyllanthus amarus*Schumach. & Thonn.

The characteristic macroscopical and microscopical features provided reliable botanical identification parameters, while powder microscopy offered useful diagnostic markers for powdered crude drug. Physicochemical parameters, including moisture, ash and extractive values, provided measurable indices of purity and quality.

The hydroalcoholic extract exhibited the highest illustrative extraction yield and showed a broad range of phytoconstituents, particularly phenolic compounds, flavonoids, tannins, glycosides and lignans. Quantitative estimation indicated appreciable phenolic and flavonoid contents.

Chromatographic fingerprinting further supported the chemical standardization of the plant and demonstrated the usefulness of phyllanthin as a marker constituent.

The combined parameters generated in this study may therefore be useful for:

- Authentication of *Phyllanthus amarus*.
- Detection of adulteration and substitution.
- Evaluation of crude-drug purity.
- Batch-to-batch comparison.
- Standardization of extracts.
- Quality control of herbal formulations containing *P. amarus*.

Further work using validated quantitative HPTLC, HPLC or LC–MS methods with multiple markers such as phyllanthin, hypophyllanthin, gallic acid and ellagic acid would strengthen the chemical standardization of the plant.

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