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Research Article

PRELIMINARY PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING OF *BHUMYAMALAKI* (*PHYLLANTHUS AMARUS* SCHUMACH. & THONN.)

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ABSTRACT

Phyllanthus amarus Schumach. & Thonn. is an annual plant distributed throughout India. In Ayurvedic classics, it is identified under the names *Bhumyamalaki* or *Tamalaki* and stands out as a significant therapeutic agent in Ayurvedic medicines. Pharmacognostic studies are important for identification and for determining the quality and purity of crude drugs. The aim of the study was to evaluate the preliminary pharmacognostic characters of *Phyllanthus amarus* Schumach. & Thonn. The pharmacognostic evaluation included a thorough examination of the macroscopic, microscopic, and physicochemical characteristics. The assessed parameters comprised foreign matter, moisture content, total ash, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, fibre content and sugar contents, qualitative chemical analysis and High-Performance Thin Layer Chromatography. Macroscopic study showed a taproot system with a straight, cylindrical, light brown root and abundant secondary and tertiary rootlets. The stem was slender, glabrous, exhibiting a smooth and green surface. The leaves were simple, elliptic-oblong in shape with entire margin, arranged alternately. The microscopic examination of the root revealed starch grains and crystals present notably in both the cortex and phloem regions. The stem exhibited Calcium oxalate crystals in the pith region. Numerous anisocytic stomata were observed in the lower epidermis of leaf with stomatal index 25.18%. The water-soluble extractive was found to be more than alcohol soluble extractive. Total sugar was found to be 5.6%. HPTLC at 575nm showed 9 peaks with Rf value ranging from 0.04 to 0.9. With these research findings, it becomes possible to establish pharmacognostic standards for the plant. This, in turn, simplifies the process of identifying and ensuring the purity and quality of *Phyllanthus amarus* Schumach. & Thonn.

INTRODUCTION

Bhumyamalaki, initially highlighted in *Brihatrayees* for addressing respiratory system disorders, later found its application in hepatobiliary diseases according to Nighantus and Keraleeya textbooks. Recognized for its *Rasayana* properties, this plant is a crucial ingredient in formulations such as *Chyavanaprasa, Amritaprasa Ghrita,* and *Jivantyadi Churna*. The versatility in conditions indicated underscores its significance in traditional medicine.

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The plant is distributed throughout India mainly in tropical and sub-tropical parts of the country. It grows as weed in the cultivated fields and are also found near water bodies and by the roadside. Researches have proven the anti-inflammatory, antioxidant, antiviral, antimicrobial, anti-diabetic, nephroprotective, diuretic, hepatoprotective, anti-carcinogenic and anti-fibromyalgic activities of different parts of the plant[1]. According to WHO, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any test are undertaken[2]. The present study aims to assess the pharmacognostic and phytochemical parameters of the plant, contributing to the authentication of the plant.

Plant Description

Phyllanthus amarus Schumach. & Thonn. belonging to the family Phyllanthaceae (APG Ⅳ, 2016) is an annual herb 60 to 75 cm tall, quite glabrous, distributed throughout India

Taxonomical positioning of the plant is as follows: [3]

Kingdom: Plantae

Phylum: Tracheophyta

Class: Equisetopsida

Order: Malpighiales

Family: Phyllanthaceae

Genus: Phyllanthus

Species: amarus

MATERIALS AND METHODS

Plant Material

The whole plant materials of *Phyllanthus amarus* Schumach. & Thonn. were collected from Thiruvananthapuram and Alappuzha districts. The samples were authenticated for botanical identity at the Pharmacognosy Unit, Government Ayurveda College, Thiruvananthapuram. The fresh samples were cleaned, shade dried and stored in standard condition.

Macroscopic evaluation of *Phyllanthus amarus* Schumach. & Thonn.

Fresh plant was taken for macroscopic examination. Organoleptic characteristics of *Phyllanthus amarus* Schumach. & Thonn., as colour, features, surface characters, shape, and odour was analysed

Microscopic evaluation of *Phyllanthus amarus* Schumach. & Thonn.

Fine transverse sections of the root, stem and leaf were taken and stained with safranin, was mounted in glycerine and observed under microscope. Various identifying characters and cell composition were recorded and micro-photographed.

Powder microscopy

Sufficient amount of powder of dried whole plant was mounted in glycerin. The slide was then examined under digital microscope. Objectives 40x and 100x were used for all observations and diagnostic features were photographed.

Preliminary Physicochemical Evaluation

The preliminary physicochemical analysis of whole plant of *Phyllanthus amarus* Schumach. & Thonn was conducted following the established procedures outlined in Ayurveda Pharmacopoeia of India and WHO guidelines. Parameters such as moisture content, different ash values, volatile oil content, various extractive values like water-soluble and alcohol-soluble extractives, fibre content, and sugar content were assessed.

Preliminary Phytochemical Evaluation

Preliminary phytochemical analysis was done with the methanolic extract of *Phyllanthus amarus* Schumach. & Thonn to detect the presence of various secondary metabolites, including alkaloids, flavonoids, phenols, saponins, steroids and tannins.

Qualitative Analysis for presence of Phytochemicals

Test for Alkaloids

Methanolic extract of whole plant was evaporated and to the residue, dil. HCl was added and filtered. To the filtrate Dragendroff's reagent was added, an orange brown precipitate indicates the presence of alkaloids.[4]

Test for Flavonoids

The residue of methanolic extract of whole plant was dissolved in alcohol. Concentrated HCl and Magnesium ribbon were added to it. A reddish brown colour indicates the presence of flavonoids.[4]

Test for Phenols

Methanolic extract of *Phyllanthus amarus* Schumach. & Thonn was evaporated and the residue of extract was dissolved in alcohol and neutral $FeCl_3$. A violet colour indicates the presence of phenols.[4]

Test for Saponins

A few drops of sodium bicarbonate solution was added to the methanolic extract and shaken well. Formation of a honey comb like frothy appearance confirms the presence of saponins.[4]

Test for Steroids

Steroids were detected by evaporating the methanolic extract of whole plant in a watch glass and to the residue, acetic anhydride and conc. H_2SO_4 were added through the sides. The change of colours from yellow to brown indicates the presence of steroids.[4]

Test for Tannins

10g of the powder of whole plant of *Phyllanthus amarus* Schumach. & Thonn was weighed and transferred into a 250ml round bottomed flask. 100ml of distilled water was added and refluxed for one hour. The solution was filtered hot using an ordinary filter paper into a conical flask and made up to 100ml. This was then transferred into a standard flask and 2ml of lead acetate solution was added, appearance of a precipitate indicates the presence of tannins.[4]

Chromatography - High Performance Thin Layer Chromatography

Developing solvent system

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system Toluene:Ethyl acetate: Formic acid (4:6:0.1) for the extract. The optimum

separations of constituents were achieved using the solvent system.

Sample application

The extracts were applied as different tracks of different concentrations of width 8mm each on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of Chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10cm × 10cm) pre-saturated with the mobile phase selected.

Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Visualizer and the images were captured under UV light at 254nm and 366nm.

Densitometry

The plate was scanned at 254nm and 366nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation

The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105°C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575nm and the R_f values and finger print data were documented.

RESULTS AND DISCUSIION

Macroscopic evaluation of *Phyllanthus amarus* Schumach. & Thonn. (Fig 1)

The macroscopic characters of samples of root, stem and leaf of *Phyllanthus amarus* Schumach. & Thonn. was evaluated. The observations are summarized in Table 1, 2 and 3

Root

Table 1: Macroscopic evaluation of root of Phyllanthus amarus Schumach. & Thonn

S.no	Characters	Observations
1	Type	Tap root
2	Shape	Cylindrical straight
3	Diameter	Length: 4-7 cm Diameter: 1-3 mm
4	Colour	Light brown
5	Surface	Rough with numerous secondary and tertiary roots
6	Fracture	Fibrous
7	Odour	Pleasant

Stem

Table 2: Macroscopic evaluation of stem of Phyllanthus amarus Schumach. & Thonn

S.no	Characters	Observations
1	Type	Slender, glabrous
2	Shape	Cylindrical
3	Size	Length: 10-18 cm Diameter: 1-2 mm
4	Branching	Profuse towards the upper region
5	Colour	Greenish
6	Surface	Smooth
7	Fracture	Short except for the bark
8	Odour	Characteristic

Leaf

Table 3: Macroscopic evaluation of leaf of Phyllanthus amarus Schumach. & Thonn

S.no	Characters	Observations
1	Type	Simple
2	Arrangement	Alternate
3	Petiole	Subsessile
6	Shape	Oblong or elliptic oblong

7	Colour	Adaxial surface – Green Abaxial surface – Pale green
9	Margin	Entire
10	Apex	Obtuse and shortly mucronate
11	Base	Obtuse or rounded
12	Venation	Unicostate reticulate
13	Odour	Characteristic

FIGURES

Figure 1: *Phyllanthus amarus* Schumach. & Thonn

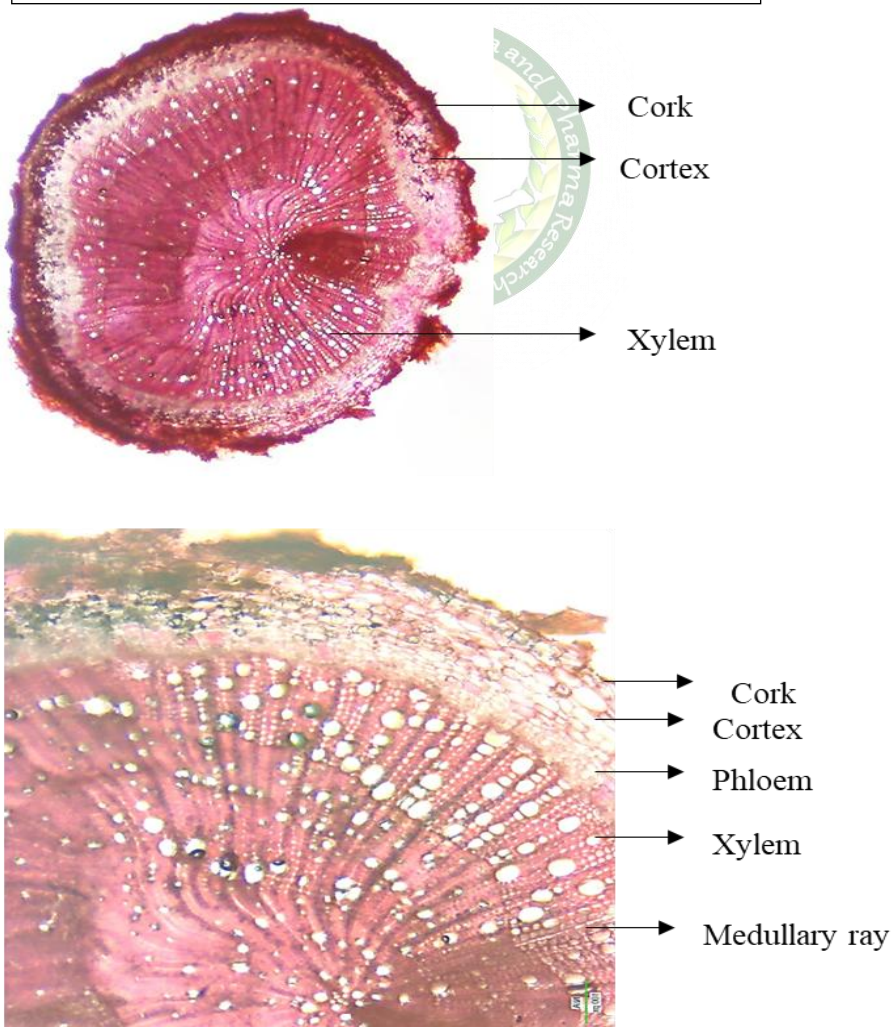


Figure 2: Transverse section of root of *Phyllanthus amarus* Schumach. & Thonn

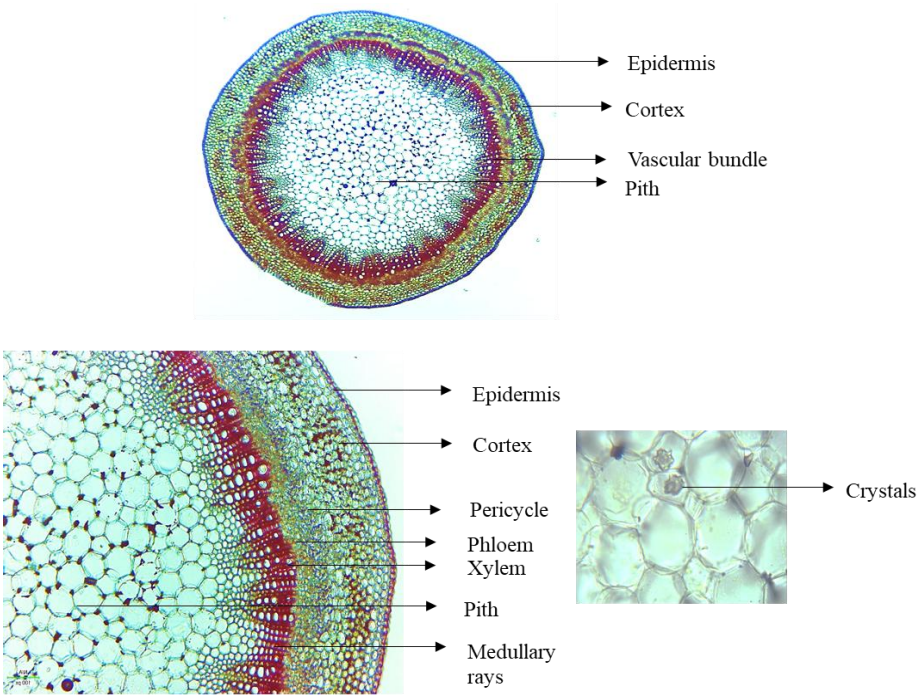


Figure 3: Transverse section of stem of *Phyllanthus amarus* Schumach. & Thonn

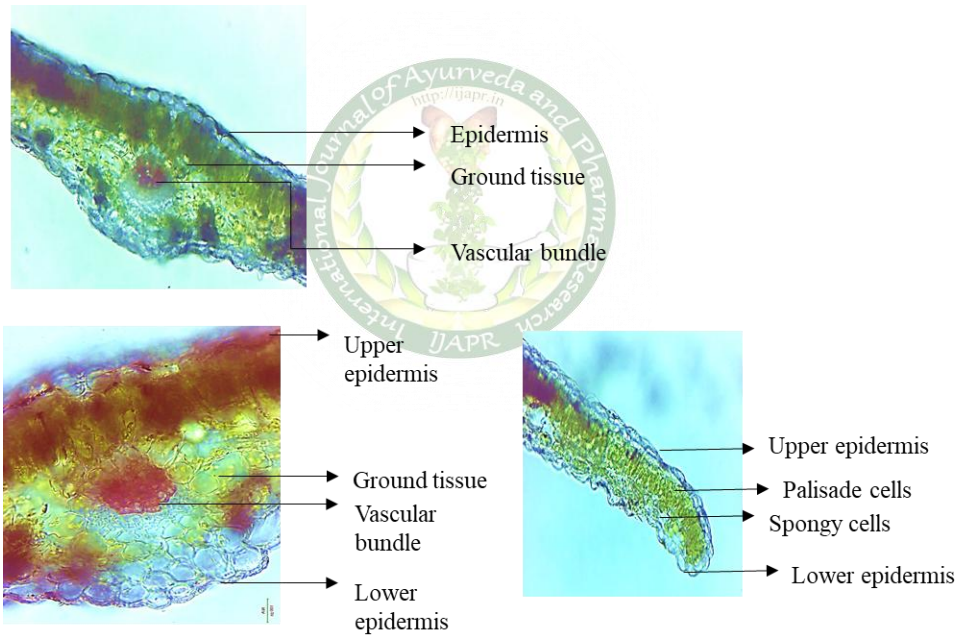


Figure 4: Transverse section of leaf of *Phyllanthus amarus* Schumach. & Thonn

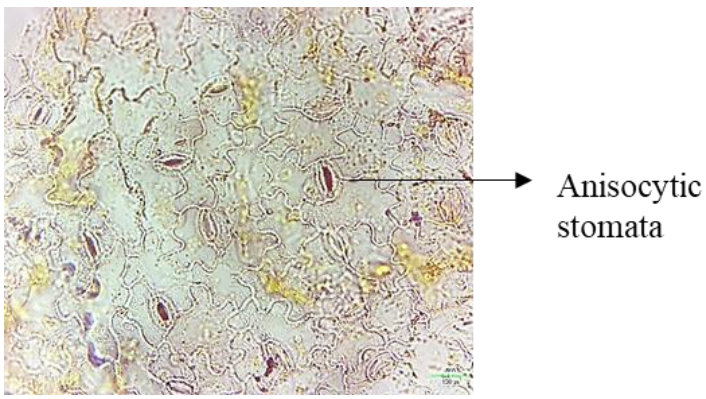
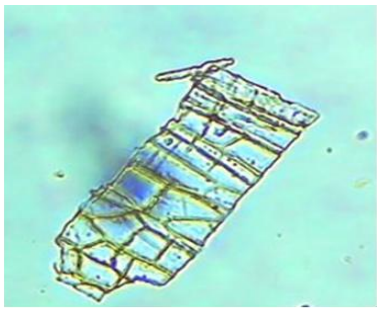


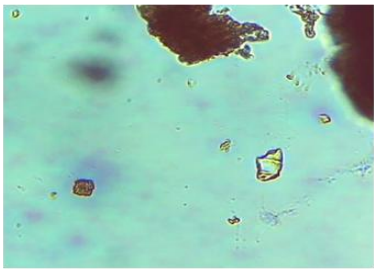
Figure 5: Anisocytic stomata on the lower epidermis



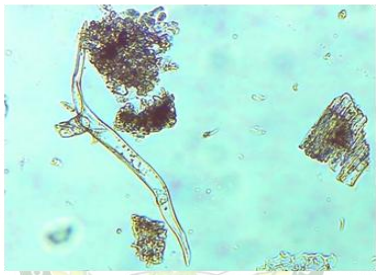
a. Epidermal cells

b. Sclereids with crystals

c. Phloem elements



d. Crystals



e. Group of stone cells , Vascular elements & Fibres

Figure 6: Powder Microscopy of whole plant of *Phyllanthus amarus* Schumach. & Thonn

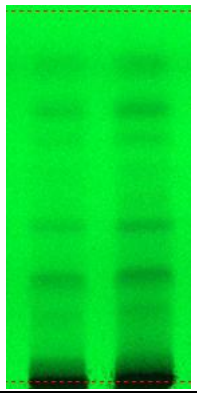
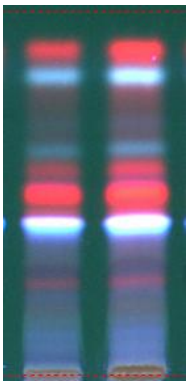
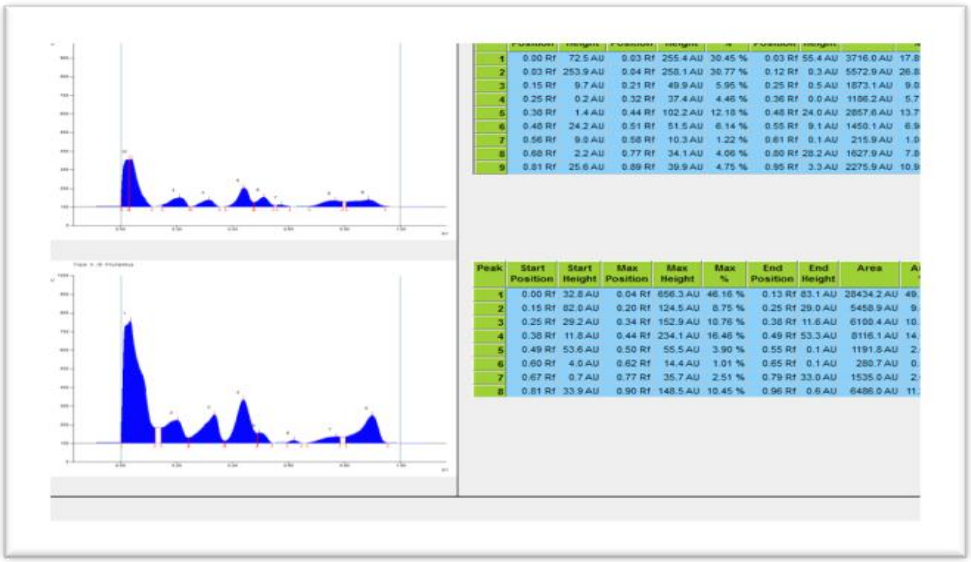
		
Under UV short	Under UV long	Under white light after derivatisation

Figure 7: HPTLC plate visualized under UV short, UV long and white light

254 nm

366 nm



575 nm

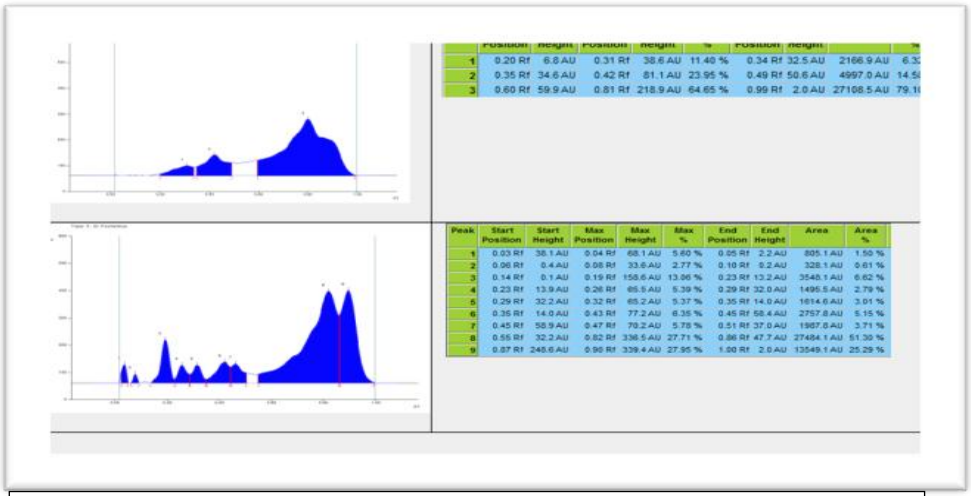


Figure 8: Graphical representation of Track 1 and 2 in HPTLC

Microscopic evaluation of *Phyllanthus amarus* Schumach. & Thonn.

Root (Fig 2)

- TS of the root showed an outer cork 4-6 layered, made up of rectangular tangentially elongated cells filled with reddish brown content.
- The secondary cortex was made up of 6-8 layers of thin walled tangentially elongated parenchymatous cells and patches of sclereids.
- The Vascular region contained a narrow zone of secondary phloem and very wide zone of secondary xylem traversed by medullary rays.
- Phloem was composed of sieve elements and phloem parenchyma.
- Xylem was composed of vessels, tracheids, fibres and xylem parenchyma.
- Starch grains and crystals were observed in the cortex and phloem region.

Stem (Fig 3)

- TS of the stem showed a single layered epidermis made up of tangentially elongated cells.
- Cortex was composed of 4-6 layers of oval, tangentially elongated, thin-walled parenchymatous cells.
- Pericycle showed several tangentially elongated strands of lignified fibres.
- Secondary phloem was narrow composed of sieve elements and phloem parenchyma.
- Secondary xylem was composed of vessels, fibres and parenchyma traversed by medullary rays.
- Centrally located pith consisted of parenchymatous cells, which are thin-walled, circular to oval in shape
- Calcium oxalate crystals were observed in the pith region

Leaf (Fig 4)

Midrib

- The TS of the midrib region of leaf showed upper and lower epidermis enclosing the ground tissue with the vascular bundle in the centre.
- The epidermis was covered with thin cuticle.
- The ground tissue was composed of thin-walled parenchymatous cells and a vascular bundle at the centre.

Lamina

- Lamina showed epidermis on both upper and lower surfaces.
- A single layered Palisade tissue is present below upper epidermis. The remaining portion is occupied by loosely arranged spongy parenchyma cells with a few containing calcium oxalate crystals.

Stomata (Fig 5)

- Numerous Anisocytic types of stomata were present on both upper and lower epidermis (amphistomatic) but more on the lower surface.
- Stomatal index of the lower epidermis was calculated as 25.18%.

Powder microscopy of whole plant of *Phyllanthus amarus* Schumach. & Thonn. (Fig 6)

The powder microscopy of *Phyllanthus amarus* Schumach. & Thonn. revealed the presence of epidermal cells, sclereids, crystals, phloem elements, stone cells, vascular elements and fibres.

Preliminary Physicochemical evaluation of whole plant of *Phyllanthus amarus* Schumach. & Thonn.

Preliminary physicochemical analysis was done and the results are summarized in Table 4

Table 4: Results of Preliminary Physicochemical evaluation of whole plant of *Phyllanthus amarus* Schumach. & Thonn

Parameters	Observations
Foreign matter	Nil
Loss on drying	2.7423%
Volatile oil	Nil
Water soluble extractive	15.808%
Alcohol soluble extractive	11.52%
Total ash	10.6%
Acid insoluble ash	3.51%
Total sugar	5.6%
Reducing sugar	5.76%
Fibre content	14%

Preliminary Phytocochemical evaluation of whole plant of *Phyllanthus amarus* Schumach. & Thonn.

Preliminary phytochemical analysis was done and the results are summarized in Table 5

Table 5: Results of Preliminary Phytocochemical evaluation of whole plant of Phyllanthus amarus Schumach. & Thonn

Constituents	Observation
Alkaloids	+++
Flavonoid	-
Phenol	+++
Saponin	-
Steroid	++
Tannin	+++

+++ - High presence, ++ - Moderate presence

High Performance Thin Layer Chromatography Analysis (Fig 7, Fig 8)

HPTLC analysis of methanolic extract of whole plant of Phyllanthus amarus Schumach. & Thonn. was done with the solvent system Toluene: Ethyl acetate: Formic acid in the ratio 4: 6: 0.1

Track 1 & 2 *Phyllanthus amarus* (5µl & 10µl respectively)

At 254nm

- The chromatogram scanned for Track 1 showed 8 peaks with Rf values ranging from 0.04 to 0.88 with areas 29.33% and 19.77% respectively.
- The chromatogram scanned for Track 2 showed 9 peaks with Rf values ranging from 0.04 to 0.88 with areas 37.96% and 12.49% respectively.

At 366nm

- The chromatogram scanned for Track 1 showed 9 peaks with Rf values ranging from 0.03 to 0.89 with areas 17.89% and 10.95% respectively.
- The chromatogram scanned for Track 2 showed 8 peaks with Rf values ranging from 0.04 to 0.90 with areas 49.36% and 11.26% respectively.

At 575nm

- The chromatogram scanned for Track 1 showed 3 peaks with Rf values ranging from 0.31 to 0.81 with areas 6.32% and 79.1% respectively.
- The chromatogram scanned for Track 2 showed 9 peaks with Rf values ranging from 0.04 to 0.90 with areas 1.50% and 25.29% respectively.

CONCLUSION

The research findings enable the establishment of pharmacognostic standards for Phyllanthus amarus Schumach. & Thonn., streamlining the identification process and ensuring its purity and quality. These findings may contribute to the standardization and identification of Phyllanthus amarus-based drugs utilized in Ayurveda, laying a solid foundation for further research in this field.

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