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Phyllanthus amarus Aerial Parts

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***Phyllanthus amarus* Aerial Parts**

DEFINITION

The article consists of the dried aerial parts of *Phyllanthus amarus* Schumach (Family Euphorbiaceae) collected during fruiting stage. It contains NLT 0.25% of lignans calculated as the sum of phyllanthin and hypophyllanthin on the dried basis.

SYNONYMS

Diasperus nanus (Hook. f.) Kuntze
Phyllanthus amarus Schumach. & Thonn.
Phyllanthus nanus Hook. f.
Phyllanthus niruri var. *amarus* (Schumach. & Thonn.) Leandri
Phyllanthus niruri var. *scabrellus* (Webb) Müll. Arg.
Phyllanthus scabrellus Webb
Phyllanthus swartzii Kostel.

POTENTIAL CONFOUNDING MATERIALS

Related *Phyllanthus* species including *P. debilis* Klein ex Willd., *P. fraternus* G. L. Webster, *P. maderaspatensis* L., *P. niruri* L., *P. urinaria* L., and *P. virgatus* G. Forst.

SELECTED COMMON NAMES

African: Ahlivi (Togo); bomagua kéné, bounou, bounou honlin (Ivory Coast); hinlinwe (West Africa); mokichinento (East Africa); tsekulemegbe (Togo)
English: Carry-me-seed, black catnip, child pick-a-back, gale of wind, gulf leaf flower, hurricane weed, shatterstone, stone breaker
French: Poudre de plomb (Ivory Coast)
German: Weisse Blatt-blume, bittere blattblüte
Indian: Bhuiamla (Bengali), bhui amala (Hindi), nilanelli (Kannada), bhumyaamalaki (Sanskrit), kizhukai nelli (Tamil)
Japanese: キダチコミカンソウ (kidachi komi kansou)
Javanese: Memeniran, meniran
Malay: Dukung anak, dukung-dukung anak, amin buah, rami buah, turi hutan, meniran
Portuguese: Quebra-pedra, arrebenta pedra
Spanish: Chancapiedra, rompepiedra, sarandí blanco.
Vietnamese: Diệp hạ châu đắng

CONSTITUENTS OF INTEREST

Lignans: Hypophyllanthin, phyllanthin, niranthin, and phyltetralin

Alkaloids: Securinine, norsecurinine, and isobubbialine

IDENTIFICATION

- **A. Botanic Characteristics**

Macroscopic: Erect annual herb, 10–60 cm high; stem rounded and hairy (distinction from *P. debilis* and *P. maderaspatensis*, angular and glabrous; and *P. virgatus,* rounded with two protrusions and glabrous); leaves on main stem are reduced to scales (cataphyll leaves) (distinction from *P. maderaspatensis* and *P. virgatus*, absent) and turn black at maturity (distinction from *P. debilis*, stay green); secondary branchlets present (distinction from *P. maderaspatensis* and *P. virgatus*, absent), short, extend at right angles, each carrying 15–30 leaves; leaves, simple, alternate, 4–12 mm long, 2–5 mm wide, short petiolate, oblong (distinction from *P. debilis*, narrowly elliptic in upper part and cuneate at base; *P. maderaspatensis*, spathulate; and *P. virgatus*, oblong-elliptic), apex mucronate, margin entire (distinction from *P. fraternus* with serrate margin; and *P. maderaspatensis* with crenate margin and bulbous at the base), base often slightly asymmetric, have a green upper surface raised at the midrib and a pale green lower surface with prominent midrib and secondary veins; flowers minute, yellowish, greenish, or whitish, unisexual, axillary on secondary branchlets, 1–2 and sometimes 3 per axil, first 1–2 internodes of each branchlet bear 1–2 male flowers, the rest have male and female flowers, each flower has five sepals (distinction from *P. debilis*, *P. fraternus*, *P. maderaspatensis* and *P. virgatus*, all with flowers having six sepals); fruits are flattened, globose spherical capsules, straw color, 3-loculed, about 2 mm in diameter; seeds usually two per locule, light brown, about 0.9 mm long, triangular with 6–7 longitudinal ribs and many transverse striations on the back. Compendial article often is green to yellowish-green mass composed mostly of leaves, branchlets, fruits, and stem fragments.

Microscopic:

Transverse section of stem: The transverse section is circular, shows epidermis covered with thick cuticle, embedded with stomata, at places bearing papilla; a narrow band of chlorenchymatous hypodermis; about 15 layers of cortex cells, thick wall, contain chloroplast, some contain calcium oxalate crystals, inner 7–10 layers are made of thick-wall cells interrupted at regular interval by parenchyma cells; a layer of parenchyma cells containing starch grains; phloem, 7–10 layers of thin-wall cells; groups of xylem vessels; pith, multilayer of thin-wall cells, few contain calcium oxalate crystals.

Transverse section of branchlet: The transverse section is circular; 6–8 layers of cortex, thick-wall cells, most contain chloroplast and a few calcium oxalate crystals, after 3–4 layers there is a layer of cells containing starch grains, followed by 2–3 layers of fiber cells interrupted by cortex parenchyma; phloem 5–7 layers of thin-wall cells; groups of xylem vessels; pith, multilayer of thin-wall cells, containing chloroplasts.

Transverse section of leaf: Upper epidermis, one row of subrectangular cells, mostly tangentially elongated, sometimes with convex outer walls, covered with thin cuticle, at places it is pappilose and embedded with stomata; 1–4 rows of collenchymas cells, below the upper and lower epidermis in the midrib region; a single layer of palisade cells, which occupy nearly half of the space between the upper and lower epidermis and appearing across the upper part of the midrib, some cells contain prismatic and rosette clusters of calcium oxalate; 3–5 layers of parenchyma cells, a few contain prisms of calcium oxalate; vascular bundles, collateral; meristele of the midrib consists of radiate xylem and an arch of phloem; fiber bundles, outside the phloem, with thickened walls; lower epidermis, subrounded or subrectangular cells.

Tansverse section of Fruit: Diagrammatic TS is circular in outline with inconspicuous 3-ridged margin, shows narrow pericarp surrounding three locules occupying the major portion of the fruit each has two triangular endospermic seeds. Detailed TS shows the outermost layer of tangentially arranged, narrow,

rectangular cells of epicarp covered with thin cuticle; a layer of hypodermis consisting of large oval, tangentially arranged parenchymatous cells; a layer of small rectangular to square, radially arranged chlorenchymatous cells; 3–4 rows of collapsed parenchymatous cells; innermost layer of the pericarp composed of radially arranged, compactly placed, large rectangular thick-wall parenchymatous cells; a layer of endocarp of radially arranged parenchymatous cells.

Transverse section of Seed: Testa consists of an outer layer of lens shaped giant cells, a sclereide layer, and an innermost layer of cells containing pigment; endosperm, wide, parenchymatous, full of oil globules, microrosette crystals of calcium oxalate and aleurone grains; cotyledons are narrow, consisting of upper and lower epidermis, enclosing mesophyll cells.

- **B. Thin-Layer Chromatography**

Standard solution A: 1.0 mg/mL of USP Phyllanthin RS in methanol

Standard solution B: 10 mg/mL of USP Powdered *Phyllanthus amarus* Extract RS in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Sample solution: Sonicate about 0.5 g of *Phyllanthus amarus* Aerial Parts, finely powdered, in 5 mL of methanol for 10 min, centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* <621>*, Thin-Layer Chromatography.*)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)

Application volume: 8 µL each of *Standard solution A* and *Standard solution B* and 4 µL of *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device

Temperature: 25°

Developing solvent system: Hexane and ethyl acetate (2:1)

Developing distance: 6 cm

Derivatization reagent: A solution of 10% sulfuric acid in methanol. [Note—Prepare fresh. Keep alcohol cold over ice, carefully and gradually add sulfuric acid.]

Analysis

Samples: *Standard solution A, Standard solution B,* and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry. Treat with *Derivatization reagent*, heat for 3 min at 120°, and examine under visible light and UV light at 366 nm.

System suitability: Under UV light at 366 nm, the chromatogram of *Standard solution B* exhibits, in the lower-third section, three bands that are clearly separated: a dark band corresponding in color and R_F to the phyllanthin band in the chromatogram of *Standard solution A*; a dark band due to hypophyllanthin at an R_F higher than that of phyllanthin; and a dark band at an R_F higher than that of hypophyllanthin. The last band is the most intense band in the chromatogram. The chromatogram also exhibits a light gray band at about the middle of the chromatogram. Similar profile is observed under visible light with three bands clearly separated in the lower-third section: two brown bands, due to phyllanthin and hypophyllanthin, and a brownish-violet band (most intense) at an R_F higher than that of hypophyllanthin. The chromatogram also exhibits a brownish-violet band at about the middle of the chromatogram.

Acceptance criteria: Under visible light, the chromatogram of the *Sample solution* exhibits a band due to phyllanthin corresponding in color and in R_F to the band in the chromatogram of *Standard solution A*, and the following bands corresponding to similar bands in the chromatogram of *Standard solution B*: a brown band due to hypophyllanthin, at an R_F higher than that of phyllanthin; a brownish-violet band at an R_F higher than that of hypophyllanthin; and a brownish-violet band at about the middle of the chromatogram. Additional bands detected in the *Sample solution* chromatogram include two olive-green bands: one at an R_F below that of phyllanthin and the other at about the middle of the chromatogram.

Under UV light at 366 nm, the *Sample solution* exhibits four red bands: two at R_F similar to those of the two olive-green bands detected under visible light, one at an R_F similar to that of hypophyllanthin, and a red band at about the middle of the chromatogram. It also exhibits a dark band at an R_F higher than that of hypophyllanthin, corresponding to a similar band in the chromatogram of *Standard solution B*.

- **C. HPLC**

Analysis: Proceed as directed in the *Assay* for *Content of Lignans.*

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to phyllanthin, hypophyllanthin, and niranthin in the chromatogram of *Standard solution B*. The most prominant peak is that of phyllanthin.

ASSAY

- **Content of Lignans**

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, mix, and filter.

Mobile phase: Acetonitrile and *Solution A* (4:6)

Standard solution A: 0.1 mg/mL of USP Phyllanthin RS in methanol

Standard solution B: Sonicate a portion of USP Powdered *Phyllanthus amarus* Extract RS in methanol to obtain a solution having a concentration of about 5.0 mg/mL. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer about 3.0 g of *Phyllanthus amarus* Aerial Parts, finely powdered and accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 50 mL of methanol, reflux in a water bath for about 20 min, allow to settle, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, and filter. Concentrate the filtrate under vacuum, and dilute with methanol to 100 mL. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Luna C18 and Inertsil ODS-3)

Column temperature: 25±1°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Powdered *Phyllanthus amarus* Extract RS being used.

Resolution: NLT 1.0 between the phyllanthin and hypophyllanthin peaks, *Standard solution B*

Tailing factor: NMT 1.5 for the phyllanthin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% determined from the phyllanthin peak in repeated injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

[Note—*Standard solution A*, *Standard solution B*, and *Sample solution* are stable for 48 h at room temperature.]

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP Powdered *Phyllanthus amarus* Extract RS being used, identify the peaks corresponding to phyllanthin, hypophyllanthin, and niranthin. The approximate relative retention times, relative to phyllanthin, are provided in *Table 1*.

Table 1

Analyte	Relative Retention Times
Phyllanthin	1.00
Hypophyllanthin	1.08
Niranthin	1.48

Separately calculate the percentages of phyllanthin and hypophyllanthin in the portion of *Phyllanthus amarus* Aerial Parts taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak response of the analyte from the *Sample solution*

r_S = peak response of phyllanthin from *Standard solution A*

C_S = concentration of phyllanthin in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of *Phyllanthus amarus* Aerial Parts taken to prepare the *Sample solution* (mg)

F = conversion factors for the analytes; 1.00 for phyllanthin and 0.75 for hypophyllanthin

Calculate the content of the lignans as the sum of the percentages of phyllanthin and hypophyllanthin.

Acceptance criteria: NLT 0.25% on the dried basis

CONTAMINANTS

• **Elemental Impurities—Procedures <233>**

Acceptance criteria

Arsenic: NMT 2.0 µg/g

Cadmium: NMT 1.0 µg/g

Lead: NMT 5.0 µg/g

Mercury: NMT 1.0 µg/g

• **Articles of Botanical Origin,** *General Method for Pesticide Residues Analysis* **<561>:** Meets the requirements

• **Microbial Enumeration Tests <61>:** The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g.

• **Tests for Specified Microorganisms <62>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **Articles of Botanical Origin,** *Foreign Organic Matter* **<561>:** NMT 2.0%
- **Loss on Drying <731>**
  Analysis: Dry 1.0 g of *Phyllanthus amarus* Aerial Parts, finely powdered, at 105° for 2 h.
  Acceptance criteria: NMT 12.0%
- **Articles of Botanical Origin,** *Total Ash* **<561>**
  Analysis: 2.0 g of *Phyllanthus amarus* Aerial Parts, finely powdered
  Acceptance criteria: NMT 8.0%
- **Articles of Botanical Origin,** *Acid-Insoluble Ash* **<561>**
  Analysis: 2.0 g of *Phyllanthus amarus* Aerial Parts, finely powdered
  Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and the parts of the plant contained in the article.
- **USP Reference Standards <11>**
  USP Phyllanthin RS [1]
  USP Powdered *Phyllanthus amarus* Extract RS [2]
