Herbal Medicines Compendium

Published on *Herbal Medicines Compendium* (https://hmc.usp.org)

Sphaeranthus indicus Aerial Parts

Proposed For Comment Version 0.2

***Sphaeranthus indicus* Aerial Parts**

DEFINITION

The article consists of aerial parts of *Sphaeranthus indicus* L. (Family: Asteraceae), gathered shortly before or during flowering. It contains NLT 0.14% of sphaeranthanolide, calculated on the dried basis.

SYNONYMS

Sphaeranthus hirtus Willd.
Sphaeranthus mollis Roxb.
Sphaeranthus mollis Roxb. ex DC.

POTENTIAL CONFOUNDING MATERIALS

None known

SELECTED COMMON NAMES

English: East Indian globe thistle
Hindi: Gorakhmundi, mundi
Malayalam: Adakkamanian, mirangani

CONSTITUENTS OF INTEREST

Sesquiterpene glycoside: Sphaeranthanolide
Sterol glycoside: 7-hydroxyfrullanolide

IDENTIFICATION

- **A. Botanical Characteristics**

Macroscopic:

Inflorescence (capitula): compound head, solitary, terminal on branches, 1–1.6 cm in diameter, globose or globose-ovoid, pink to purple, ebracteate, peduncled; peduncle 1–4.5 cm long, stout, glandular-pubescent, with toothed wings. Partial heads small, sessile, numerous, heterogamous of few florets, aggregated on a large, common receptacle-forming head inflorescence (capitulum). Ivolucres of partial heads, 4 mm long, campanulate, margin toothed; bracts persistent, linear, acuminate, subpaleaceous, ciliate on the upper half. Florets all tubular; marginal and central florets with dark purple corolla; female 6–9, 3-toothed, filiform; 2–3 disc florets hermaphrodite, with 5-toothed corolla, the latter much swollen in the lower half. Anther bases sagittate. Style arms shortly bifid or entire.

Fruit: achene, surmounted by the corolla which consists of hair, bristles and teeth margin;

monocarpellate, indehiscent, obconical in shape, very minute, 0.7-1.3 mm in leangh and 0.4-0.5 mm in width, occasionally attached with long slender style, dark brown in color.

Leaf: dried leaves are brittle, shrivelled, sessile, alternate, spathulate to ovate-oblong, 1-5 cm in length and 4-22 mm in width, obtuse to subacute, serrate to dentate, decurrent; surface highly pubescent, upper dark green with obscure veins, lower paler.

Microscopic:

Inflorescence: The bract in surface view is composed of thin-walled, elongated, rectangular- to polygonal-shaped parenchyma cells; marginal cells are elongated to hair-like projections. Vascular strand in the center of spiral elements. A few monoclinic calcium oxalate crystals and starch grains are observed in cells. The corolla is semitransparent, of rectangular to polygonal parenchyma cells, showing the presence of few crystals. The anthers are dark yellow in color; the cells 2–3 times more elongated than width, thin-walled, a few showing oval-spherical-shaped starch grains. Filament cells are comparatively smaller, rectangular, and parenchymatous. The pollen grains are spherical, spinous. The gynoecium syncarpous have two carpels; ovary superior, oval-shaped, unilocular, with basal placentation; style short; stigma sessile. Ovary wall is multi-layered, 4–6 cells in thickness; cells polygonal, thin-walled. Starch grains are few; locule pentagonal.

Transverse section of leaf: Passing through midrib is dorsiventrally convex, 3 to 4 centrally located meristeles arranged in row and a narrow band of collenchyma underneath both the epidermii bearing trichomes are seen, the lateral laminar extensions being dorsiventral. Upper and lower epidermis embedded with stomata, covered with thin cuticle and bearing plenty of simple and glandular trichomes, simple trichomes being occassionally branched with 3 to 4 arms but majority of them are uniseriate, 3 to 4 celled, thick walled, straight or knee shaped, some with swollen base of embedded at places with collapsed cells, glandular trichomes are short or long with unicellular or multicellular stalk and multicellular head. Midrib shows centrally located 3 to 4 conjoint collateral vascular bundles sheathed dorsiventrally with sclerenchymatous band, embedded in rows in the parenchymatous ground tissue; 3 to 6 layers of collenchymatous cells underneath both the epidermii are seen, lamina is narrow, dorsiventral, shows a layer of narrow band of palisade underneath the upper epidermis followed by 4 to 5 rows of spongy parenchyma traversed with obliquely cut vascular tissues.

Transverse section of fruit: Oval in outline shows an outler hairy epicarp and an inner centrally located cotyledon encircled by endosperm and the outer seed coat layer.

Longitudinal section of fruit: Along with the corolla shows obconical seed, attached at the base with short remains of pedicel; centrally located seed and hairy parenchymatous, parallely running, elongated narrow cells bears trichomes at places of corolla.

- **B. Thin-Layer Chromatography**

Standard solution A: 1 mg/mL of USP Sphaeranthanolide RS in methanol

Standard solution B: 0.1 g of USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS. Mix with 10 mL of methanol, sonicate for 10 min, and centrifuge. Use the supernatant.

Sample solution: Mix 0.5 g of *Sphaeranthus indicus* Aerial Parts, finely powdered and accurately weighed, with 5 mL of methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system

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

Adsorbent: Use a suitable chromatographic material with an average particle size of 5 µm (HPTLC plates).

Application volume: 5 µL each of *Standard solution A*, *Standard solution B*, and *Sample solution*, as 8-mm bands

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

Developing solvent system: Dichloromethane and methanol (70: 9.2)

Developing distance: 7 cm

Derivatization reagent: Sulfuric acid reagent created by carefully adding 20 mL of sulfuric acid to 180 mL of ice-cooled methanol.

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 the plate with *Derivatization reagent* by dipping, heat for 5 min at 105°, and examine under visible light.

System suitability: Under visible light, the chromatogram of *Standard solution B* exhibits a dark brown band right above the origin in position and color similar to the sphaeranthanolide band in the chromatogram of *Standard solution A*. Six additional bands appear with increasing order of R_F: a broad dark brown band near the origin, a light purple band near one half of the lower half, a purple band right above the half, a strong blue band above the purple band, a blue/purple band, and a strong reddish-brown band near the front.

Acceptance criteria: Under visible light, the chromatogram of *Sample solution* exhibits a band due to sphaeranthanolide corresponding in color and R_F to the band in the chromatogram of *Standard solution A*. The following bands, with increasing R_F, correspond to the similar bands in the chromatogram of *Standard solution B:* a broad dark brown band near the origin, a light purple band near one half of the lower half, a purple band right above the half, a strong blue band above the purple band, and a strong reddish-brown band near the front.

- **C. HPLC**

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

Acceptance criteria: The chromatogram of the *Sample solution* exhibits a peak at the retention time corresponding to the peak due to sphaeranthanolide in *Standard solution B*.

ASSAY

- **Content of Sphaeranthanolide**

Solution A: Acetonitrile

Solution B: Water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	5	95
20	100	0
25	100	0
27	5	95
30	5	95

Standard solution A: 0.01 mg/mL of USP Sphaeranthanolide RS in methanol

Standard solution B: Dissolve 0.25 g of USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS in 20 mL of methanol by shaking for 2 h at 60°. Cool to room temperature, pass through filter paper into a 50-mL volumetric flask, and fill with methanol. Dilute with methanol to obtain a solution with a concentration of about 0.5 mg/mL. Pass the solution through a membrane filter of 0.45-µm pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer about 2.50 g of *Sphaeranthus indicus* Aerial Parts, finely powdered and accurately weighed, to a flask, add 20 mL of methanol, and shake for 2 h at 60°. Cool to room temperature, pass through filter paper into a 50-mL volumetric flask, and fill with methanol. Dilute with methanol to obtain a solution with a concentration of about 5 mg/mL. Pass the solution through a membrane filter of 0.45-µm pore size, discarding the first few mL of the filtrate.

Chromatographic system

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

Detector: UV 206 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Zorbax XDB C_{18})

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

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

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS being used.

Tailing factor: NMT 2.0 for the sphaeranthanolide peak, *Standard solution A*

Relative standard deviation: NMT 2.0%, *Standard solution A*

Analysis

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

Calculate the percentage of sphaeranthanolide in the portion of *Sphaeranthus indicus* Aerial Parts taken:

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

r_U = peak area for sphaeranthanolide from the *Sample solution*

r_S = peak area for sphaeranthanolide from *Standard solution A*

C_S = concentration of USP Sphaeranthanolide RS in *Standard solution A* (mg/mL)

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

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

Acceptance criteria: NLT 0.14% on the dried basis

CONTAMINANTS

• Elemental Impurities—Procedures <233>

Acceptance criteria

Arsenic: NMT 2.0 µg/g

Cadmium: NMT 0.5 µg/g

Lead: NMT 5 µg/g

Mercury: NMT 0.2 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>**

Sample: 1 g of *Sphaeranthus indicus* Aerial Parts, finely powdered

Analysis: Dry the *Sample* at 105° for 2 h.

Acceptance criteria: NMT 15%

• **Articles of Botanical Origin,** *Total Ash* **<561>:** NMT 20%

• **Articles of Botanical Origin,** *Acid-Insoluble Ash* **<561>:** NMT 10%

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 part(s) of the plant contained in the article.

• **USP Reference Standards <11>**

USP Sphaeranthanolide RS

USP *Sphaeranthus indicus* Aerial Parts Dry Extract RS
