Lagerstroemia speciosa Leaf

Final Authorized Version 1.0

Lagerstroemia speciosa Leaf

DEFINITION
The article consists of the dried leaves of Lagerstroemia speciosa (L.) Pers. (Family Lythraceae). It contains NLT 0.2% of corosolic acid, on the dried basis.

SYNONYMS
Adambea glabra Lam.  
Lagerstroemia augusta Wall.  
Lagerstroemia flos-reginae Retz.  
Lagerstroemia macrocarpa Wall.  
Lagerstroemia major Retz.  
Lagerstroemia munchausia Willd.  
Lagerstroemia plicifolia Stokes  
Lagerstroemia reginae Roxb.  
Munchausia speciosa L.

POTENTIAL CONFOUNDING MATERIALS
Terminalia cuneata Roth

SELECTED COMMON NAMES
Bangali: jarul  
Burmese: Gawkng-uchyamang  
English: Banaba, pride-of-India, queen's crape-myrtle, giant crape-myrtle  
Filipino: Banaba  
Javanese: Ketangi, bungur, bungur tekuyung (Sumatra)  
Malay: Bungor raya, bongor biru, tibabah  
Portuguese: Resedá-flor-da-rainha, resedá-gigante (Brazil)  
Spanish: Reina de las flores, banaba, orgullo de la India  
Thai: Chuang-muu, tabaek dam, inthanin nam

CONSTITUENTS OF INTEREST
Pentacyclic triterpene acids: Corosolic acid, virgatic acid, asiatic acid, ursolic acid, and oleanolic acid

IDENTIFICATION
• A. BOTANICAL CHARACTERISTICS
  Macroscopic: The leaf varies in shape from lanceolate, oblong-lanceolate, oblong, to elliptic ovate; olive green to yellowish-brown; margin entire or slightly wavy; base acute; apex acute to acuminate; leathery texture; up to 34 cm long and 11 cm wide; petiolate, petiole up to 1 cm long.
  Microscopic  
  Transverse section of the midrib: A layer of upper epidermis, rectangular to round cells covered with thin cuticle; a few layers of collenchyma cells; numerous layers of parenchyma cells, with large intercellular spaces, some cells contain cluster crystals of calcium oxalate, groups of lignified fiber bundles and secretory canals appear scattered in the parenchyma zone; bicollateral vascular bundle encircled by continuous sheath of fibers accompanied by sclerenchyma and cells containing cluster crystals of calcium oxalate; secretory canals occur between the vascular bundles; numerous layers of parenchyma cells, with large intercellular spaces, some containing cluster crystals of calcium oxalate; a few layers of collenchyma; a layer of lower epidermal cells.

  Transverse section of the lamina: A layer of upper epidermis, rectangular cells about twice as large as those of the lower epidermis, some cells are secretory cells, which tend to protrude into the mesophyll and sometimes appear to be below the upper epidermis; two layers of rectangular palisade cells; 4–6 layers of parenchyma cells with larger intercellular spaces, some containing
prisms of calcium oxalate, other cells containing cluster crystals of calcium oxalate, groups of vascular bundles appear scattered in the parenchyma zone; lower epidermis showing stomata

**B. Thin-Layer Chromatography**

**Standard solution A**: 0.2 mg/mL of USP Corosolic Acid RS in methanol

**Standard solution B**: 10 mg/mL of USP Lagerstroemia speciosa Leaf Dry Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

**Sample solution**: Sonicate about 0.2 g of Lagerstroemia speciosa Leaf, finely powdered, in 10 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**: 6 µL each of Standard solution A and Standard solution B and 8 µL Sample solution, as 8-mm bands
- **Relative humidity**: Condition the plate to a relative humidity of about 33% using a suitable device.
- **Temperature**: 25°C
- **Developing solvent system**: Toluene, ethyl acetate, and glacial acetic acid (55: 45: 0.5)
- **Developing distance**: 6 cm
- **Derivatization reagent**: Anisaldehyde reagent—85 mL of ice-cooled methanol mixed with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of p-anisaldehyde

**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 an un-saturated chamber, remove the plate from the chamber, and dry. Treat with Derivatization reagent, and heat for 3 min at 100°C. Examine under visible light.

**System suitability**: Under visible light, the chromatogram of Standard solution B exhibits the most intense band, a violet or blue band, with similar Rf and color as the corosolic acid band in the chromatogram of Standard solution A; a blue band close to the start (about Rf 0.1), consistent with asiatic acid; two minor blue bands in between corosolic and asiatic; above the band due to corosolic acid a minor blue band due to virgatic acid and just below the latter, a minor brown band. Standard solution B also exhibits at about three-fourths of the chromatogram two minor violet bands, separated, the band with the lower Rf corresponds to oleanolic acid.

**Acceptance criteria**: Under visible light, the chromatogram of the Sample solution exhibits the most intense band as a violet band corresponding in color and Rf to the band due to corosolic acid in the chromatogram of Standard Solution A, and the following bands corresponding to similar bands in Standard solution B: a minor blue band close to the start (about Rf 0.1); a minor brownish band above the corosolic acid; and a minor violet band at about three-fourths of the chromatogram.

**C. HPLC**

**Analysis**: Proceed as directed in the test for Content of Corosolic Acid.

**Acceptance criteria**: The chromatogram of the Sample solution exhibits a group of three peaks, the one in the center is the most intense of the group and occurs at a retention time corresponding to that of corosolic acid in the chromatogram of Standard solution A, the peak that elutes before corosolic acid has about one-half to one-third of the intensity of that of corosolic acid, and the peak eluting after corosolic acid has the lesser intensity of the three and is consistent with virgatic acid. A minor peak due to oleanolic acid elutes later in the chromatogram.

**ASSAY**

**• CONTENT OF COROSOLIC ACID**

**Solution A**: 0.1% Phosphoric acid in water

**Solution B**: Acetonitrile

**Mobile phase**: A mixture of Solution A and Solution B (4:6)

**Standard solution A**: 0.1 mg/mL of USP Corosolic Acid RS in methanol

**Standard solution B**: 5.0 mg/mL of USP Lagerstroemia speciosa Leaf Dry Extract RS in methanol, sonicate if necessary. Before injection, pass through a membrane filter of 0.45-µm or finer pore size. Discard the first few mL of the filtrate.

**Sample solution**: Transfer about 5.0 g of Lagerstroemia speciosa Leaf, finely powdered and accurately weighed, to a round-bottom flask. Add 75 mL of methanol and reflux for 15 min, set aside to settle, and decant the supernatant. Repeat the extraction three more times and combine the extract, filter, concentrate under reduced pressure, transfer to a 100-mL volumetric flask, adjust with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.45-µm or finer pore size. Discard the first few mL of the filtrate.

**Chromatographic system**

(See Chromatography [621], System Suitability.)

- **Detector**: UV 205 nm

- **Column**: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Hibar® 250-4.6; Lichrospher® 100, RP-18e; or Luna C18 100A)
Flow rate: 1.6 mL/min
Injection volume: 20 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 Lagerstroemia speciosa Leaf Dry Extract RS [2] being used.
Resolution: NLT 1.5 between the corosolic acid peak and the peak before, Standard solution B
Tailing factor: NMT 2.0 for the corosolic acid peak, Standard solution A
Relative standard deviation: NMT 2.0%, determined from the corosolic acid peak in repeated injections, Standard solution A

Analysis
Samples: Standard solution A, Standard solution B, and Sample solution
Using the chromatograms of Standard solution A, Standard solution B, and the reference chromatogram provided with the lot of USP Lagerstroemia speciosa Leaf Dry Extract RS [2] being used, identify the retention time of the peaks corresponding to corosolic acid, virgatic acid, and oleanolic acid in the Sample solution chromatogram. The approximate relative retention times of the different peaks for corosolic acid, virgatic acid, and oleanolic acid are 1.0, 1.1, and 3.2, respectively.

Calculate the percentage of corosolic acid in the portion of Lagerstroemia speciosa Leaf taken:

\[ \text{Result} = \left( \frac{r_U}{r_S} \right) \times C_S \times \left( \frac{V}{W} \right) \times 100 \]

- \( r_U \) = peak area of corosolic acid from the Sample solution
- \( r_S \) = peak area of corosolic acid from Standard solution A
- \( C_S \) = concentration of corosolic acid in Standard solution A (mg/mL)
- \( V \) = volume of the Sample solution (mL)
- \( W \) = weight of Lagerstroemia speciosa Leaf taken to prepare the Sample solution (mg)

Acceptance criteria: NLT 0.2% of corosolic acid, calculated on dried basis

CONTAMINANTS
- **Elemental Impurities—Procedures <233> [4]**
  - 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 [5]**, General Method for Pesticide Residues Analysis <561>: Meets the requirements
- **Microbial Enumeration Tests <61> [6]**: 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> [7]**: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS
- **Articles of Botanical Origin [5]**, Foreign Organic Matter <561>: NMT 2.0%
- **Articles of Botanical Origin [5]**, Alcohol-Soluble Extractives, Method 1 <561>: NLT 10.0%
- **Articles of Botanical Origin [5]**, Water-Soluble Extractives, Method 1 <561>: NLT 18.0%
- **Loss on Drying <731> [8]**
  - Analysis: Dry 2.0 g of Lagerstroemia speciosa Leaf, finely powdered, at 105° for 2 h.
  - Acceptance criteria: NMT 10.0%
- **Articles of Botanical Origin [5]**, Total Ash <561>
  - Analysis: 2.0 g of Lagerstroemia speciosa Leaf, finely powdered
  - Acceptance criteria: NMT 7.0%
- **Articles of Botanical Origin [5]**, Acid-Insoluble Ash <561>
  - Analysis: 4.0 g of Lagerstroemia speciosa Leaf, finely powdered
  - Acceptance criteria: NMT 2.0%

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 part of the plant contained in the article.

• **USP Reference Standards <11>** [9]
  - USP Corosolic Acid RS [1]
  - USP Lagerstroemia speciosa Leaf Dry Extract RS [2]