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Senna Leaf

DEFINITION

Senna Leaf consists of the dried leaflet of *Senna alexandrina* Mill., also known as *Cassia acutifolia* Delile (Alexandrian senna) or *C. angustifolia* Vahl (Tinnevelly senna), (Fam. Fabaceae). Senna Leaf contains NLT 2.5% of anthraquinone glucosides, calculated as sennosides, on the dried basis.

IDENTIFICATION

• **A.**

Potassium hydroxide solution: 100 mg/mL of potassium hydroxide in alcohol

Sample: 500 mg of finely powdered Senna Leaf

Analysis: Add 10 mL of *Potassium hydroxide solution* to the *Sample*. Boil for 2 min, dilute with 10 mL of water, and filter. Acidify the filtrate with hydrochloric acid. Shake with ether, remove the ether layer, and shake with 5 mL of 6 N ammonium hydroxide.

Acceptance criteria: The aqueous layer is orange or bluish red.

ASSAY

• **Procedure**

[Note—Conduct all sample preparations with minimal exposure to subdued light, and use low-actinic glassware to protect solutions from light.]

Ferric chloride solution: 105 mg/mL of ferric chloride

Methanolic magnesium acetate solution: 5 mg/mL of magnesium acetate in methanol

Sodium bicarbonate solution: 5 mg/mL of sodium bicarbonate

Standard solution: 0.13 mg/mL of USP Sennosides RS in *Sodium bicarbonate solution*

Sample solution: Weigh and pulverize 10 g of Senna Leaf. Transfer 0.15 g to a 100-mL round-bottom flask. Add 30 mL of water. Mix, weigh, attach a condenser, and reflux in a water bath for 15 min. Cool to room temperature, weigh, and adjust to the original weight with water. Centrifuge, and transfer 20.0 mL of the supernatant to a 150-mL separatory funnel. Add 0.1 mL of diluted hydrochloric acid, and shake with three 15-mL quantities of chloroform. Allow to separate, and discard the chloroform layer after each addition. Add about 0.1 g of sodium bicarbonate, shake for 3 min, and centrifuge. Use the supernatant as the *Sample solution*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Wavelength: 515 nm

Cell: Quartz

Blank: Methanol

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer 10.0 mL each of the *Standard solution* and the *Sample solution* to separate 100-mL round-bottom flasks equipped with condensers. Add 20 mL of *Ferric chloride solution,* and mix. Reflux in a water bath for 20 min. Add 1 mL of hydrochloric acid, and reflux for an additional 20 min, with frequent shaking, to dissolve the precipitates. Cool to room temperature, transfer the mixtures to separate 100-mL separatory funnels, and shake with three 25-mL quantities of ether previously used to rinse the flasks. Combine the ether extracts, mix, and wash with two 15-mL quantities of water. Transfer the ether layers to separate 100-mL volumetric flasks. Dilute with ether to volume, and mix. Evaporate 10.0 mL of the ether extracts to dryness, and dissolve the residue in 10.0 mL of *Methanolic magnesium acetate solution*. Determine the absorbance of the resulting solution from the *Standard solution* and *Sample solution*, with a suitable spectrophotometer fitted with matched quartz cells, using the *Blank*.

Calculate the percentage of sennosides in the portion of Senna Leaf taken:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/W) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Sennosides RS in the *Standard solution* (mg/mL)

V = volume of water to which powdered Senna Leaf was added, 30 mL

W = weight of powdered Senna Leaf (mg)

Acceptance criteria: NLT 2.5% of anthraquinone glucosides, calculated as sennosides, on the dried basis

CONTAMINANTS

• **Microbial Enumeration Tests ⟨2021⟩:** The total 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 count does not exceed 10^3 cfu/g.

• **Absence of Specified Microorganisms ⟨2022⟩,** *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

• **Articles of Botanical Origin ⟨561⟩,** *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

• **Botanical Characteristics**

Macroscopic

Unground Alexandrian senna leaf: Inequilaterally lanceolate or lance-ovate leaflets, frequently broken; 1.5–3.5 cm in length and 5–10 mm in width, unequal at the base, with very short, stout petiolules. The leaflets are acutely cuspidate, entire, brittle, and subcoriaceous, with short and somewhat appressed hairs, few on the upper surface, more numerous on the lower surface, where they occur spreading on the midrib, especially on its lower part. The color is weak yellow to light grayish green to pale olive. The odor is characteristic.

Unground Tinnevelly senna leaf: Usually unbroken leaflets, 2–5 cm in length and 6–15 mm in width; acute at the apex; and slightly hairy. The color of the leaves is weak yellow to pale olive.

Powdered Senna Leaf: Dusky greenish yellow to light olive brown

Microscopic

Transverse section: Senna Leaf shows polygonal epidermal cells with straight walls and frequently containing mucilage; numerous, broadly elliptical stomata mostly 20–35 µm in length, usually bordered by two neighbor-cells with their long axes parallel to that of the stoma, and rarely, though more frequently in Alexandrian senna leaf, a third epidermal cell at the end of the stoma. The hairs are nonglandular, one-celled, conical, often curved, with thick papillose walls, 100–350 µm in length. Palisade cells in a single layer underlie both surfaces except in the midrib region where they occur only beneath the upper epidermis. A meristele occurs in the midrib composed of several radially arranged fibrovascular bundles, the latter separated by narrow vascular rays and supported above and below by arcs of lignified pericyclic fibers. Calcium oxalate occurs in rosette aggregates in the spongy parenchyma and in six- to eight-sided prisms in the crystal fibers, which lie on the outer surface of each group of pericyclic fibers.

Powdered Senna Leaf: Fragments of veins bearing lignified vessels, tracheids, and crystal fibers, isolated hairs, masses of palisade and spongy parenchyma, fragments of epidermis with stomata, free calcium oxalate rosette aggregates, and prisms 10–20 µm in length. In powdered Alexandrian senna leaf, the hairs are more numerous than in powdered Tinnevelly senna leaf.

• **Articles of Botanical Origin ⟨561⟩,** *Methods of Analysis, Foreign Organic Matter*: The amount of senna stems is NMT 8.0%, and the amount of senna pods or other foreign organic matter is NMT 2.0%.

• **Articles of Botanical Origin ⟨561⟩,** *Methods of Analysis, Total Ash*: NMT 12.0%

• **Articles of Botanical Origin ⟨561⟩,** *Methods of Analysis, Acid-Insoluble Ash*: NMT 3.0%

• **Loss on Drying ⟨731⟩**

Sample: 1.0 g of finely powdered Senna Leaf

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

Acceptance criteria: It loses NMT 12.0% of its weight.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve against attack by insects and rodents. Store protected from light and moisture at room temperature.

• **Labeling:** The label states the Latin binomial and, following the official name, the part(s) of the plant contained in the article.

• **USP Reference Standards ⟨11⟩**

USP Sennosides RS

Topic/Question	Contact	Expert Committee
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REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

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