Ziziphus Jujuba Fruit Powder

Proposed For Development Version 0.1

Ziziphus jujuba Fruit Powder

DEFINITION
The article consists of the dried ripe fruits of Ziziphus jujuba Mill. (Family Rhamnaceae) reduced to a fine or very fine powder. It contains NLT 0.035% of triterpenes calculated as the sum of betulinic acid (C_{30}H_{48}O_{3}) and oleaonic acid (C_{30}H_{48}O_{3}), and NLT 0.01% of adenosine 3',5'-cyclic monophosphate (C_{10}H_{12}N_{2}O_{6}P), calculated on the dried basis.

SYNONYMS
Palirus mairei H. Lév.
Rhamnus jujube L.
Rhamnus soporifera Lour.
Rhamnus ziziphus L.
Ziziphus mairei (H. Lév.) Browicz & Lauener
Ziziphus nitida Roxb.
Ziziohus orthacantha DC.
Ziziphus rotundata DC.
Ziziohus sativa Gaertn.
Ziziohus soporifera (Lour.) Stokes
Ziziohus tomentosa Poir.
Ziziphus vulgaris Lam.
Ziziphus zizyphus (L.) Meikle.

POTENTIAL CONFOUNDING MATERIALS
Zizyphus mauritiana Lam

SELECTED COMMON NAMES
Arabic: ﺱﺪر ﺟﺒﻠ
Bulgarian: Хинап
Chinese: 大枣, 红枣
English: Chinese jujube, Chinese-date, common jujube, jujube date
Estonian: Kreektürn
French: Jujubier commun, jujube de Chine, jujubier de Chine
German: Brustbeere, brustbeerbaum, Chinesische Dattel, domjujube, jujube, judendom
Greek: Ζιζιφιά, Ζιζίφον zizyfon
Hebrew: ﺪهش
CONSTITUENTS OF INTEREST

Triterpenic acids: Betulinic acid, oleanolic acid, ursolic acid, betulonic acid, oleanonic acid, and ursonic acid

IDENTIFICATION

• A. BOTANICAL CHARACTERISTICS
  
  Macroscopic: Yellowish-brown color
  
  Microscopic: The epidermal cells of pericarp are sub-square, filled with brownish-red contents, and covered with thick cuticle; cells under the epidermis are yellow or yellowish-brown, sub-polygonal with thick walls; prisms of calcium oxalate are small and exist in parenchymatous cells of mesocarp. The stone cells of kern are pale yellowish-brown, sub-polygonal, striation well-defined, pit-canal minute, and contain yellowish-brown contents.

• B. THIN-LAYER CHROMATOGRAPHY

  Standard solution A: 0.5 mg/mL of USP Betulinic Acid RS in alcohol
  Standard solution B: 0.5 mg/mL of USP Oleanolic Acid RS in alcohol
  Standard solution C: 100 mg/mL of USP *Ziziphus jujuba* Fruit Powdered Extract RS in ethyl acetate, macerate for 1 h, and sonicate for 15 min. Filter and use the filtration.
  Sample solution: Sonicate about 2 g of *Ziziphus jujuba* Fruit Powder in 10 mL of hexanes for 15 min, and filter. Dry the residue in a hood, add 20 mL of ethyl acetate, macerate for 1 h, and sonicate for 15 min. Filter and evaporate the filtration to dryness under reduced pressure at a temperature not to exceed 50°. Dissolve the residue in 2 mL of ethyl acetate.

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

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

  Application volume: 6 µL each of Standard solution A, Standard solution B, Standard solution C, 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: Methylbenzene, ethyl acetate, and glacial acetic acid (14: 4: 0.5)

  Developing distance: 6 cm

  Derivatization reagent: 10% Sulfuric acid in ethanol. [Note—Prepare fresh. Keep alcohol cold over ice, carefully and gradually add sulfuric acid.]
Analysis

Samples: Standard solution A, Standard solution B, Standard solution C, 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 in a hood. Derivatize with Derivatization reagent, heat at 100° for 5 min, and examine under visible light and UV light at 365 nm.

System suitability: The chromatogram of Standard solution C exhibits a red band in the middle-third section corresponding to the band of oleanolic acid in Standard solution B, an intense violet band in the lower-third section, and an intense violet band and a light brown band right below the solvent front in the upper-third section.

Under UV light at 365 nm, the chromatogram of Standard solution C exhibits a yellow band corresponding to the band of oleanolic acid in Standard solution B and a blue band right above the yellow band corresponding to betulinic acid in Standard solution A; the yellow band and the blue band are clearly separated.

Acceptance criteria: Under visible light, the chromatogram of the Sample solution exhibits a red band corresponding to the band due to oleanolic acid in the chromatogram of Standard solution B, and the following bands corresponding to similar bands in the chromatogram of Standard solution C: an intense violet band in the lower-third section; and an intense violet band and a light brown band right below the solvent front in the upper-third section.

Under UV light at 365 nm, the chromatogram of the Sample solution exhibits a yellow band corresponding to the band of oleanolic acid in Standard solution B, a blue band right above the yellow band corresponding to betulinic acid in Standard solution A, and additional weak bands similar to the bands in the chromatogram of Standard solution C.

C. HPLC

Analysis: Proceed as directed in the Assay for Content of Triterpenic Acids.

Acceptance criteria: The chromatogram of the Sample solution is similar to the chromatogram of Standard solution C and to the reference chromatogram provided with the lot of USP Ziziphus jujuba Fruit Powdered Extract RS. The chromatogram of the Sample solution exhibits the peaks of betulinic acid, oleanolic acid, ursolic acid, betulonic acid, oleanonic acid, and ursonic acid with the approximate relative retention times of 1.0, 1.10, 1.18, 1.22, 1.28, and 1.35, respectively.

D. HPLC

Analysis: Proceed as directed in the Assay for Content of Adenosine 3',5'-Cyclic Monophosphate.

Acceptance criteria: The chromatogram of the Sample solution exhibits a peak at a retention time corresponding to that of adenosine 3',5'-cyclic monophosphate in the chromatogram of the Standard solution.

ASSAY

Content of Triterpenic Acids

Solution A: 5.0 mg/mL ammonium acetate in water

Solution B: Methanol

Mobile phase: Solution A and Solution B (17:83)

Standard solution A (high concentration): 0.20 mg/mL of USP Betulinic Acid RS and 0.10 mg/mL of USP Oleanolic Acid RS in methanol

Standard solution B (low concentration): 0.10 mg/mL of USP Betulinic Acid RS and 0.05 mg/mL of USP Oleanolic Acid RS in methanol

Standard solution C: Dissolve 5 mg/mL of USP Ziziphus jujuba Fruit Powdered Extract RS in ethyl acetate in a glass-stoppered conical flask and sonicate for 60 min. Cool to room temperature, centrifuge, take a portion of the supernatant with half the volume of the original solvent, and evaporate to
dryness under reduced pressure at a temperature not to exceed 50°. Transfer the residue to a 10-mL volumetric flask and add methanol to volume. Mix and pass through a membrane filter of 0.45-μm pore size.

**Sample solution:** Accurately weigh about 5.0 g of *Ziziphus jujuba* Fruit Powder and transfer to a 100-mL glass-stoppered conical flask. Accurately add 100 mL of ethyl acetate, and weigh the filled flask. Sonicate for 60 min, cool to room temperature, and if needed adjust to the initial weight by adding ethyl acetate. Centrifuge, accurately take 50 mL of the supernatant, and evaporate to dryness under reduced pressure at a temperature not to exceed to 50°. Transfer the residue to a 10-mL volumetric flask and add methanol to volume. Mix and pass through a membrane filter of 0.45-μm pore size, discarding the first portion of the filtrate.

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

- **Mode:** LC
- **Detector:** ELSD
- **Column:** 4.6-mm × 25-cm; 5-μm packing L1 (similar to Hypersil C18)
- **Flow rate:** 0.6 mL/min
- **Injection volume:** 20 μL
- **Column temperature:** 25° ± 1

**System suitability**

**Samples:** Standard solution B and Standard solution C

**Suitability requirements**

- **Chromatogram similarity:** The chromatogram of Standard solution C is similar to the reference chromatogram provided with the lot of USP Ziziphus jujuba Fruit Powdered Extract RS being used.
- **Tailing factor:** NMT 2.0 for betulinic acid and oleanolic acid peaks, Standard solution B
- **Relative standard deviation:** NMT 2.0% for betulinic acid and oleanolic acid peaks, Standard solution B
- **Resolution:** NLT 1.5 between the betulinic acid and oleanolic acid peaks, Standard solution C

**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 Ziziphus jujuba Fruit Powdered Extract RS being used, identify the retention time of the peaks of betulinic acid and oleanolic acid in the Sample solution chromatogram. The approximate relative retention times of the peaks for betulinic acid and oleanolic acid are 1.0 and 1.10, respectively.

Calculate the percentage of betulinic acid (C_{30}H_{48}O_{3}) and oleaonic acid (C_{30}H_{48}O_{3}) in the portion of Ziziphus jujuba Fruit Powder taken, using a two-point logarithmic equation method on the basis of linear calibration plots of the logarithm of peak areas versus the logarithm of concentrations:

\[
\text{Result} = 10^{\frac{\log(r_u/r_s)}{\log(C_{sh}/C_{sl})} \times \log(r_u/r_s) + \log(C_{sl})} \times (20/W) \times 100
\]

- \(r_u\) = response of betulinic acid or oleanolic acid from the Sample solution
- \(r_{sh}\) = response of betulinic acid or oleanolic acid from Standard solution A
- \(r_{si}\) = response of betulinic acid or oleanolic acid from Standard solution B
- \(C_{sh}\) = concentration of betulinic acid or oleanolic acid in Standard solution A (mg/mL)
- \(C_{si}\) = concentration of betulinic acid or oleanolic acid in Standard solution B (mg/mL)
$W$ = weight of Ziziphus jujuba Fruit Powder taken to prepare the Sample solution (mg)

**Acceptance criteria:** NLT 0.035% of the sum of betulinic acid and oleanolic acid on the dried basis

**Content of Adenosine 3′,5′-Cyclic Monophosphate**

**Solution A:** 0.05 mol Potassium dihydrogen phosphate in water  
**Solution B:** Methanol  
**Mobile phase:** Solution A and Solution B (91:9)  
**Solvent:** Methanol and water (4:6)

**Standard solution:** 0.05 mg/mL of USP Adenosine 3′,5′-Cyclic Monophosphate RS in Solvent  
**Sample solution:** Accurately weigh about 1.0 g of Ziziphus jujuba Fruit Powder and transfer to a 50-mL glass-stoppered conical flask. Accurately add 20 mL of Solvent, and weigh the filled flask with a precision of ± 0.01 g. Sonicate for 30 min, cool to room temperature, and if needed adjust to the initial weight by adding Solvent. Mix and pass through a nylon filter of 0.45-μm pore size, discarding the first portion of the filtrate.

**Chromatographic system**  
(See Chromatography <621>, System Suitability.)  
**Mode:** LC  
**Detector:** UV 259 nm  
**Column:** 4.6-mm × 25-cm; 5-μm packing L1 (similar to Wondasil C18, Waters Sunfire C18, and Dionex Acclaim C18)  
**Flow rate:** 1.0 mL/min  
**Injection volume:** 20 μL  
**Column temperature:** 30° ± 1

**System suitability**  
**Sample:** Standard solution  
**Suitability requirements**  
**Tailing factor:** NMT 2.0  
**Relative standard deviation:** NMT 2.0%

**Analysis**  
**Samples:** Standard solution and Sample solution. [Note—The Standard solution and the Sample solution are stable for 12 h at room temperature.]  
Using the chromatogram of the Standard solution, identify the retention time of the peak corresponding to adenosine 3′,5′-cyclic monophosphate in the Sample solution chromatogram.  
Calculate the percentage of adenosine 3′,5′-cyclic monophosphate (C$_{10}$H$_{12}$N$_2$O$_6$P) in the portion of Ziziphus jujuba Fruit Powder taken:

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

\[r_U = \text{response of adenosine 3′,5′-cyclic monophosphate from the Sample solution}\]
\[r_S = \text{response of adenosine 3′,5′-cyclic monophosphate from the Standard solution}\]
\[C_S = \text{concentration of adenosine 3′,5′-cyclic monophosphate in the Standard solution (mg/mL)}\]
\[V = \text{volume of the Sample solution (mL)}\]
\[W = \text{weight of Ziziphus jujuba Fruit Powder taken to prepare the Sample solution (mg)}\]

**Acceptance criteria:** NLT 0.01% of adenosine 3′,5′-cyclic monophosphate on the dried basis

**CONTAMINANTS**
• **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 $10^5$ cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed $10^1$ cfu/g.

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

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**SPECIFIC TESTS**

• **Articles of Botanical Origin**, Foreign Organic Matter <561>: NMT 2.0%

• **Loss on Drying** <731>
  
  **Sample:** 1.0 g of *Ziziphus jujuba* Fruit Powder
  
  **Analysis:** Dry the Sample at 105° for 2 h.
  
  **Acceptance criteria:** NMT 20%

• **Articles of Botanical Origin**, Total Ash <561>
  
  **Analysis:** 4.0 g of *Ziziphus jujuba* Fruit Powder
  
  **Acceptance criteria:** NMT 2.0%

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**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 Adenosine 3',5'-Cyclic Monophosphate RS
  
  USP Betulinic Acid RS
  
  USP Oleanolic Acid RS
  
  USP *Ziziphus jujuba* Fruit Powdered Extract RS

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