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Ziziphus jujuba Fruit Powder

Proposed For Comment Version 0.2

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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 triterpenic acids consisting of betulinic acid, oleanolic acid, ursolic acid, and their 3-ketone analogs, betulonic acid, oleanonic acid, and ursonic acid.

POTENTIAL CONFOUNDING MATERIALS

Ziziphus jujuba var. *Spinosa* (Bunge) Hu ex H. F. Chou Powder

CONSTITUENTS OF INTEREST

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

Cyclic nucleotides: cAMP and cGMP

Flavonoid glycosides: Rutin and quercetin 3-*O*-robinobioside

Polysaccharides: Water-soluble and alcohol-insoluble polysaccharides

IDENTIFICATION

- **A. Botanical Characteristics**

  Macroscopic: Yellowish-brown color

  Microscopic: Sclereids are subpolygonal, 14–70 µm in diameter, some of them are fibrously elongated up to 260 µm long; contain yellowish-brown contents; pit canals observed. Lignified fibers are 12–27 µm in diameter; pit dot bordered, pit striation oblique slit-like or crossed. Reticulate vessel and bordered pitted vessel are 11–60 µm in diameter.

- **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 Dry Extract RS in ethyl acetate. Macerate for 1 h and sonicate for 15 min. Filter and use the filtrate.

  Sample solution: Sonicate about 1 g of *Ziziphus jujuba* Fruit Powder in 10 mL of hexanes for 15 min and filter. Discard the filtrate and 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 filtrate to dryness under reduced pressure at a temperature not to exceed 50°. Dissolve the residue in 1 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, 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 366 nm.

  System suitability: Under visible light, 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*, a violet band in the lower-third section, and an intense violet band and a faint brown band right below the solvent front in the upper-third section.

  Under UV light at 366 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 *Standard solution B*, and the following bands corresponding to similar bands in *Standard solution C*: a violet band in the lower-third section; and an intense violet band and a faint brown band right below the solvent front in the upper-third section.

Under UV light at 366 nm, the chromatogram of the *Sample solution* exhibits a yellow band corresponding to the band due to 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 *Standard solution C*.

- **C. HPLC for Triterpenic Acids**

Solution A: 5.0 mg/mL of ammonium acetate in water

Solution B: Methanol

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

Standard solution A: 0.1 mg/mL of USP Betulinic Acid RS and 0.5 mg/mL of USP Oleanolic Acid RS in methanol

Standard solution B: Dissolve 500 mg of USP *Ziziphus jujuba* Fruit Dry Extract RS in 10 mL of ethyl acetate in a glass-stoppered conical flask, and sonicate for 60 min. Cool to room temperature, centrifuge, take 5 mL of the supernatant, 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 glass-stoppered conical flask. Accurately add 100 mL of ethyl acetate, sonicate for 60 min, and cool to room temperature. 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.

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)

Column temperature: 25 ± 1°

Flow rate: 0.6 mL/min

Injection volume: 20 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 *Ziziphus jujuba* Fruit Dry Extract RS being used.

Resolution: NLT 1.5 between betulinic acid and oleanolic acid peaks, *Standard solution A*

Tailing factor: NMT 2.0 for betulinic acid and oleanolic acid peaks, *Standard solution A*

Relative standard deviation: NMT 2.0% for the betulinic acid and oleanolic acid peaks, *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 *Ziziphus jujuba* Fruit Dry Extract RS being used, identify the retention time of the peaks corresponding to betulinic acid, oleanolic acid, ursolic acid, and their 3-ketone analogs, betulonic acid, oleanonic acid, and ursonic acid in the *Sample solution*. The approximate relative retention times for the peaks due to betulinic acid, oleanolic acid, ursolic acid, betulonic acid, oleanonic acid, and ursonic acid are 1.0, 1.10, 1.18, 1.22, 1.28, and 1.35, respectively.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits the principal peaks related to triterpenic acids for betulinic acid, oleanolic acid, ursolic acid, betulonic acid, oleanonic acid, and ursonic acid at retention times corresponding to the same triterpenic acids in *Standard solution B* and the reference chromatogram provided with the lot of USP *Ziziphus jujuba* Fruit Dry Extract RS, with the peak for betulinic acid being higher than the peak for oleanolic acid. It may show a smaller, minor peak due to pomonic acid with an approximate relative retention time of 0.42 with respect to betulinic acid (difference with *Ziziphus jujuba* var. *Spinosa* fruit for which the pomonic acid peak is more abundant than that for betulinic acid).

ASSAY

- **TO COME**

[A suitable assay for this plant material has not yet been established. Comments and suggestions are encouraged.]

CONTAMINANTS

- **Elemental Impurities—Procedure <233>**

Acceptance criteria

Arsenic: NMT 2.0 µg/g

Cadmium: NMT 0.3 µg/g

Lead: NMT 5.0 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—Nutritional and Dietary Supplements <2021>:** 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.
- **Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements <2022>:** 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%
- **Content of Adenosine 3',5'-Cyclic Monophosphate**

Solution A: 0.05 mol Potassium dihydrogen phosphate in 1000 mL 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 adjust to the initial weight by adding *Solvent* if needed. Mix, pass through a nylon filter of 0.45-µm pore size, and discard 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)

Column temperature: 30 ± 1°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

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 at room temperature for 12 h.]

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*.

Calculate the percentage of adenosine 3',5'-cyclic monophosphate ($C_{10}H_{12}N_2O_6P$) in the portion of *Ziziphus jujuba* Fruit Powder taken:

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

r_U = peak area of adenosine 3',5'-cyclic monophosphate from the *Sample solution*

r_S = peak area of adenosine 3',5'-cyclic monophosphate from the *Standard solution*

C_S = concentration of adenosine 3',5'-cyclic monophosphate in the *Standard solution* (µg/mL)

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

W = weight of *Ziziphus jujuba* Fruit Powder taken to prepare the *Sample solution (*g)

Acceptance criteria: NLT 100 µg/g of adenosine 3',5'-cyclic monophosphate on the dried basis

- **Content of Water-soluble Polysaccharide**

Reagent solution A: 5% solution of phenol in water

Reagent solution B: concentrated sulfuric acid

Standard stock solution: 0.1 mg/mL of USP Dextrose RS in water

Standard solutions: Separately transfer 0.2, 0.4, 0.6, 0.8, 1.0 mL of the *Standard stock solution* to 10-mL test tubes. Add water up to

2.0 ml for each.

Sample stock solution: Accurately transfer 500 mg g of *Ziziphus jujuba* Fruit powder to a round-bottom flask, and add 100 mL of 80% ethanol. Heat under reflux for 1 hour, filter immediately, and wash the residue left in the round-bottom flask and then using the washing to wash the filter paper on the filter using 80% hot ethanol for 3 times, 30 mL for each. Transfer the filter paper with the residue to the round-bottom flask, and add 150 ml of water, heat under reflux for 2 hours, filter immediately into a flask. Wash the residue left in the round-bottom flask and then using the washing to wash the filter paper on the filter using hot water for 3 times, 30 mL for each, combine the filtrate and washings. After cooling to room temperature, quantitatively transfer the solution to a 250-mL volumetric flask, rinse the flask with small amount of water and transfer the rinsing to the volumetric flask, add water to volume, and mix well.

Sample solution: Transfer 1.0 mL of *Sample stock solution* to a 10-mL test tube, add 1.0 ml of water.

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* 851 [1].)

Mode: Vis

Wavelength: 490 nm

System suitability

Samples: *Standard solutions*

Suitability requirements

Relative standard deviation: NMT 2.0% determined from the absorbance of *Standard solutions* in repeated determinations.

Correlation coefficient: NLT 0.995 for the regression line as determined in *Analysis*

Analysis

Samples: *Standard solutions* and *Sample solution*

Add 1.0 mL of *Reagent solution A* to each of *Sample solution* and *Standard solutions*, mix well. Then along the tube wall rapidly add 7.0 mL of *Reagent solution B* to each of *Sample solution* and *Standard solutions*, mix well. After standing for 10 minutes, warm the test tubes on a water bath at 40° for 15 min (tightly close the tube during the processing), immediately cool the solutions to room temperature in an ice bath. Determine the absorbance of the solutions obtained from the *Standard solutions* and the *Sample solution,* using 2 mL water plus 1.0 mL *Reagent solution A* and 7.0 mL *Reagent solution B* as a blank. Plot the absorbance versus the concentrations, in mg/mL, of dextrose from the *Standard solutions*, and determine the regression line using a least-squares analysis.

Determine the concentration, *C*, in mg/mL, of the dextrose in the *Sample solution* by regression line.

Calculate the percentages of polysaccharide in the portion of *Ziziphus jujuba* Fruit Powder taken:

$$\text{Result} = 2CV/W \times 100$$

C = concentration of dextrose in the *Sample solution* obtained from the graphs (mg/mL)

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

W = weight of *Ziziphus jujuba* Fruit Powder taken to prepare the *Sample stock solution* (mg)

Acceptance criteria: NLT 2.0% and NMT 3.7% of total polysaccharide, calculated as dextrose ($C_6H_{12}O_6$), on the dried basis.

• **Loss on Drying <731>**

Sample: 1.0 g of *Ziziphus jujuba* Fruit Powder

Analysis: Dry the *Sample* at 105° for 4 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%

• **Articles of Botanical Origin,** *Water-Soluble Extractives* **<561>:** NLT 17.0%

• **Articles of Botanical Origin,** *Alcohol-Insoluble Extractives* **<561>:** NLT 19.0%

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(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 Dextrose RS

USP *Ziziphus jujuba* Fruit Dry Extract RS
