Picrorhiza kurrooa Root and Rhizome

Proposed For Comment Version 0.2

Picrorhiza kurrooa Root and Rhizome

DEFINITION
The article consists of the dried root and rhizome of *Picrorhiza kurrooa* Royle (Family Plantaginaceae). It contains NLT 3.5% of iridoid glycosides calculated as the sum of picroside I and picroside II on the anhydrous basis.

SYNONYMS
The synonym status of the species names *Picrorhiza kurrooa* Royle and *Picrorhiza kurroa* Royle ex Benth. is unresolved at this time. Previously this species was placed in the Family Scrophulariaceae.

POTENTIAL CONFOUNDING MATERIALS
*Picrorhiza scrophulariiflora* Pennell

SELECTED COMMON NAMES
Chinese: 胡簧莲
English: Picrorhiza
Hindi: Kutki
Korean: 호황련
Sanskrit: Tiktā, Kavī, Sutiktaka
Tamil: Katuka rohini

CONSTITUENTS OF INTEREST
Iridoid glycosides: Picroside I and picroside II

IDENTIFICATION
• A. BOTANICAL CHARACTERISTICS
  Macroscopic
  Rhizome: 2.5–12.0 cm Long and 0.3–1.0 cm thick, subcylindrical, straight or slightly curved, externally grayish-brown, surface rough due to longitudinal wrinkles, circular scars of roots and bud scales, sometimes roots attached, tip ends in a growing bud surrounded by a tufted crown of leaves, in-place cork exfoliates exposing dark cortex; fracture, short.
  Root: Thin, cylindrical, 5–10 cm long and 0.5–1.0 mm in diameter, straight or slightly curved with a few longitudinal wrinkles and dotted scars, mostly attached with rhizomes, dusty-grey; fracture short, inner surface black with whitish xylem.

  Microscopic
  Rhizome portion: 20–25 Layers of cork consisting of tangentially elongated, suberized cells; cortex cambium 1–2 layers; cortex single-layered or absent, primary cortex persists in some cases, 1 or 2 small vascular bundles present in the cortex. Vascular bundles surrounded by fibrous bundle sheath. Secondary phloem composed of parenchyma cells and a few scattered fibers. Cambium 2–4 layered. Secondary xylem consists of vessels, tracheids, fibers, and parenchyma cells. Vessels vary in size and shape, have transverse oblique articulation; tracheids long, thick-walled, lignified, more or less cylindrical with blunt tapering ends. Starch grains abundant, 25–105 μm in diameter.
  Root portion: When young, shows 1-layered epidermis, some epidermal cells elongate forming unicellular hairs. Hypodermis single-layered. Cortex 8–14 layered, consisting of oval to polygonal, thick-walled parenchymatous cells. Primary stele, tetrarch to heptarch, enclosed by a single-layered pericycle and single-layered thick-walled cells of endodermis. Mature roots show 4–15 layers of cork, 1–2 layers of cortex cambium. Vessels vary in size and shape, some cylindrical with tail-like, tapering ends; some drum shaped with perforation on end walls or lateral walls. Tracheids cylindrical with tapering pointed ends.

• B. THIN-LAYER CHROMATOGRAPHY
  Standard solution A: 0.5 mg/mL of USP Picroside I RS in methanol
  Standard solution B: 80 mg/mL of USP Picrorhiza kurrooa Root and Rhizome Dry Extract RS in methanol
  Sample solution: Sonicate 2 g of *Picrorhiza kurrooa* Root and Rhizome, finely powdered and accurately weighed, in 25 mL of methanol for 10 min, centrifuge, and use 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: 10 µL each of Standard solution A, Standard solution B, and Sample solution, as 10-mm bands
Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.
Developing solvent system: Ethyl acetate, methanol, and water (82: 10: 0.8)
Developing distance: 6 cm
Derivatization reagent: Anisaldehyde-sulfuric acid reagent

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 with Derivatization reagent, heat for 3 min at 100°, and examine under visible light.

System suitability: Under visible light, Standard solution B exhibits, in the lower half, about seven dark brown bands with the most intense two bands close to the upper section of the lower half; the band with higher R<sub>f</sub> corresponds to the band due to picroside I in Standard solution A.

Acceptance criteria: Under visible light, the Sample solution exhibits a strong dark brown band in the middle corresponding to the band due to picroside I in Standard solution A. The Sample solution exhibits, in the lower half, about seven dark brown bands, with the second strongest band having lower R<sub>f</sub> than picroside I, corresponding to the band due to picroside II in Standard solution A.

• C. HPLC
Analysis: Proceed as directed in the Assay for Content of Iridoid Glycosides.
Acceptance criteria: The Sample solution exhibits peaks at the retention times corresponding to the peaks due to picroside I and picroside II of Standard solution B.

ASSAY
• CONTENT OF IRIDOID GLYCOSIDES
Solution A: Dissolve 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH<sub>2</sub>PO<sub>4</sub>) in 900 mL of water and add 0.5 mL of orthophosphoric acid. Dilute with water to 1000 mL.
Solution B: Acetonitrile
Mobile phase: See Table 1.

Table 1

<table>
<thead>
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<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
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<tbody>
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<td>15</td>
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<td>30</td>
<td>85</td>
<td>15</td>
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</tbody>
</table>

Standard solution A: 0.015 mg/mL of USP Picroside I RS in methanol
Standard solution B: 0.6 mg/mL USP Picrorhiza kurrooa Root and Rhizome 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 0.5 g of Picrorhiza kurrooa Root and Rhizome, finely powdered and accurately weighed, to a flask, add 50 mL methanol, and reflux for 20 min. Repeat 4–5 times for exhaustive extraction. Combine each extract and dilute with methanol to 100 mL.

Chromatographic system
(See Chromatography <621>, System Suitability.)
Detector: UV 263 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Luna 5-µ C18 (2)-100A)
Flow rate: 1.5 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 Picrorhiza kurrooa Root and Rhizome Dry Extract RS being used.

Resolution: NLT 2.0 between picroside I and picroside II

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.5%

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 Picrorhiza kurrooa Root and Rhizome Dry Extract RS being used, identify the retention times of the peaks corresponding to picroside I and picroside II. The approximate relative retention times of the peaks for picroside I and picroside II are 1.00 and 0.77, respectively.

Calculate the percentage of iridoid glycosides in the portion of Picrorhiza kurrooa Root and Rhizome taken:

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

- \( r_U \): response from the Sample solution
- \( r_S \): response from Standard solution A
- \( C_s \): concentration of Standard solution A (mg/mL)
- \( V \): volume of the Sample solution (mL)
- \( W \): weight of Picrorhiza kurrooa Root and Rhizome taken to prepare the Sample solution (mg)
- \( F \): conversion factor for the analyte; 1.0 for picroside I, 1.68 for picroside II

Acceptance criteria: NLT 3.5% on the anhydrous basis

CONTAMINANTS

- Elemental Impurities—Procedures <233>
  - Arsenic: NMT 2.0 µg/g
  - Cadmium: NMT 0.5 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
- Articles of Botanical Origin, Test for Aflatoxins <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%
- Articles of Botanical Origin, Alcohol-Soluble Extractives, Method 1 <561>: NLT 10.0%
- Articles of Botanical Origin, Water-Soluble Extractives, Method 2 <561>: NLT 20.0%
- Articles of Botanical Origin, Total Ash <561>
  - Analysis: 2.0 g of Picrorhiza kurrooa Root and Rhizome, finely powdered
  - Acceptance criteria: NMT 7.0%
- Articles of Botanical Origin, Acid-Insoluble Ash <561>
  - Analysis: 2.0 g of Picrorhiza kurrooa Root and Rhizome, finely powdered
  - Acceptance criteria: NMT 1.0%
- Water Determination, Method III <921>: NMT 10.0%

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> [1]
  - USP Aflatoxins RS [2]
  - USP *Picrorhiza kurrooa* Root and Rhizome Dry Extract RS
  - USP Picroside I RS