Figure 1  A photograph of Radix Puerariae Lobatae
1. NAMES

Official Name: Radix Puerariae Lobatae

Chinese Name: 葛根

Chinese Phonetic Name: Gegen

2. SOURCE

Radix Puerariae Lobatae is the dried root of *Pueraria lobata* (Willd.) Ohwi (Fabaceae). The root is collected in autumn or winter, cut into thick slices or into small cubes when fresh, then dried under the sun to obtain Radix Puerariae Lobatae.

3. DESCRIPTION

Usually in thick, longitudinally cut slice, or in small irregular cube, variable in size. Longitudinally cut slice 0.4-36.2 cm in length, 0.4-10 cm in width, 0.2-5.4 cm in thickness. Externally brown, with longitudinal wrinkles. Transverse section rough, pale yellowish-brown, several concentric annular rings indistinctly visible. Texture tough, pliable and strongly fibrous. Odourless; taste, slightly sweetish (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork broad, consisting of several rows of densely arranged cork cells. Cortex narrow. Fibre bundles are the major tissue elements, associated with the vessels, surrounded by parenchyma cells containing prisms of calcium oxalate, appearing as crystal fibres. Phloem and xylem are arranged alternately as hetero-vascular bundles, forming 1-3 concentric rings; vessels relatively large, numerous, densely and alternately arranged with fibre bundles, rays narrow, the parenchyma cells containing a few starch granules (Fig. 2).

Powder

Colour pale brown to yellowish-white. Starch granules numerous, mainly of the simple type, but compound granules are sometimes found. Simple granules spherical, hemispherical or ellipsoidal, 2-53 µm in diameter, with dotted, cleft, or asteroidal hilum; compound granules composed of 2-12 units, 8-87 µm in diameter. Fibre mostly in bundles, with thickened and lignified walls,
surrounded by parenchyma cells containing prisms of calcium oxalate, parenchyma cells with thickened and lignified walls appear as crystal fibres. Bordered-pitted vessels large and numerous, densely packed. Pigment cells yellowish-brown, more-or-less oblong, usually scattered near fibres and vessels (Fig. 3).

4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

**Standard solution**

*Puerarin standard solution*

Weigh 1.0 mg of puerarin CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

**Developing solvent system**

Prepare a mixture of ethyl acetate, ethanol and water (12:3:1, v/v).

**Test solution**

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 10 min. Filter through a filter paper.

**Procedure**

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately puerarin standard solution (1 µL) and the test solution (0.5 µL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 nm). Calculate the \( R_f \) value by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the \( R_f \) value, corresponding to that of puerarin.
Figure 2  Microscopic features of transverse section of *Radix Puerariae Lobatae*

A. Sketch  B. Section illustration  C. Fibre bundle

7. Prism of calcium oxalate

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Figure 3  Microscopic features of powder of *Radix Puerariae Lobatae*

5. Cork cells  6. Pigment cells

a. Features under the light microscope  b. Features under the polarized microscope
Figure 3  Microscopic features of powder of Radix Puerariae Lobatae

5. Cork cells  6. Pigment cells

a. Features under the light microscope  b. Features under the polarized microscope
4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

**Standard solution**

_Puerarin standard solution for fingerprinting, Std-FP (100 mg/L)_{2}

Weigh 1.0 mg of puerarin CRS and dissolve in 10 mL of ethanol (70%).

**Test solution**

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Filter through a 0.45-µm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (250 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.1% Formic acid (%)</th>
<th>Acetonitrile (%)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 40</td>
<td>90 → 65</td>
<td>10 → 35</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

**System suitability requirements**

Perform at least five replicate injections, each using 1 µL of puerarin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of puerarin should not be more than 5.0%; the RSD of the retention time of puerarin peak should not be more than 2.0%; the column efficiency determined from puerarin peak should not be less than 30000 theoretical plates.
The $R$ value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

**Procedure**

Separately inject puerarin Std–FP and the test solution (1 µL each) into the HPLC system and record the chromatograms. Measure the retention time of puerarin peak in the chromatogram of puerarin Std–FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify puerarin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of puerarin Std–FP. The retention times of puerarin peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the five characteristic peaks of Radix Puerariae Lobatae extract are listed in Table 2.

**Table 2  The RRTs and acceptable ranges of the five characteristic peaks of Radix Puerariae Lobatae extract**

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.56</td>
<td>± 0.03</td>
</tr>
<tr>
<td>2 (marker, puerarin)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.05</td>
<td>± 0.03</td>
</tr>
<tr>
<td>4 (Daidzin)</td>
<td>1.33</td>
<td>± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>1.86</td>
<td>± 0.08</td>
</tr>
</tbody>
</table>

Figure 5  A reference fingerprint chromatogram of Radix Puerariae Lobatae extract
For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

5. **TESTS**

5.1 **Heavy Metals** *(Appendix V)*: meet the requirements.

5.2 **Pesticide Residues** *(Appendix VI)*: meet the requirements.

5.3 **Mycotoxins** *(Appendix VII)*: meet the requirements.

5.4 **Sulphur Dioxide Residues** *(Appendix XV)*: meet the requirements.

5.5 **Foreign Matter** *(Appendix VIII)*: not more than 1.0%.

5.6 **Ash** *(Appendix IX)*

   Total ash: not more than 6.5%.
   Acid-insoluble ash: not more than 1.0%.

5.7 **Water Content** *(Appendix X)*: not more than 12.0%.

6. **EXTRACTIVES** *(Appendix XI)*

   Water-soluble extractives (hot extraction method): not less than 46.0%.
   Ethanol-soluble extractives (hot extraction method): not less than 22.0%.

7. **ASSAY**

   Carry out the method as directed in Appendix IV(B).

   **Standard solution**

   *Puerarin standard stock solution, Std-Stock (800 mg/L)*

   Weigh accurately 8.0 mg of puerarin CRS and dissolve in 10 mL of ethanol (30%).

   *Puerarin standard solution for assay, Std-AS*

   Measure accurately the volume of the puerarin Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 16, 40, 80, 160, 240 mg/L for puerarin.

   **Test solution**

   Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add
Radix Puerariae Lobatae

15 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Centrifuge at about 3000 × g for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the extracts and make up to the mark with ethanol (70%). Mix and filter through a 0.45-µm RC filter.

Chromatographic system
The liquid chromatograph is equipped with a DAD (250 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of acetonitrile and water (10:90, v/v). The elution time is about 25 min.

System suitability requirements
Perform at least five replicate injections, each using 5 µL of puerarin Std-AS (80 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of puerarin should not be more than 5.0%; the RSD of the retention time of puerarin peak should not be more than 2.0%; the column efficiency determined from puerarin peak should not be less than 7000 theoretical plates.

The R value between puerarin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve
Inject a series of puerarin Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of puerarin against the corresponding concentrations of puerarin Std-AS. Obtain the slope, y-intercept and the $r^2$ value from the 5-point calibration curve.

Procedure
Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify puerarin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of puerarin Std-AS. The retention times of puerarin peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of puerarin in the test solution, and calculate the percentage content of puerarin in the sample by using the equations indicated in Appendix IV(B).

Limits
The sample contains not less than 2.6 % of puerarin ($C_{21}H_{20}O_9$), calculated with reference to the dried substance.