Rhizoma Belamcandae

Figure 1   A photograph of Rhizoma Belamcandae
1. **NAMES**

   Official Name: Rhizoma Belamcandae

   Chinese Name: 射干

   Chinese Phonetic Name: Shegan

2. **SOURCE**

   Rhizoma Belamcandae is the dried rhizome of *Belamcanda chinensis* (L.) DC. (Iridaceae). The rhizome is collected in early spring when the plant is budding, or in late autumn when the aerial part is withering. Fibrous roots and soil removed, washed clean, then dried under the sun to obtain Rhizoma Belamcandae.

3. **DESCRIPTION**

   Irregularly nodulated, 2-7 cm long, 10-20 mm in diameter. Externally yellowish-brown, dark brown to blackish-brown, shrunken, with profuse annular striations. Several dish-shaped and sunken stem scars are found on the upper part, occasionally with remains of stem bases, while remains of thin roots and root scars are found on the lower part. Texture hard; fracture yellow, granular. Odour slight; taste bitter and slightly pungent (Fig. 1).

4. **IDENTIFICATION**

   4.1 **Microscopic Identification (Appendix III)**

   **Transverse section**

   Cork consists of several layers of irregularly shaped cells. Cortex marked by sparsely scattered leaf-trace vascular bundles; endodermis indistinct. Vascular bundles in the stele are of amphivasal and collateral types, densely arranged near the outer edge. Parenchyma cells contain columnar crystals of calcium oxalate, as well as starch granules and oil drops (Fig. 2).

   **Powder**

   Colour yellowish-brown. Columnar crystals of calcium oxalate abundant, mostly broken; intact crystals 49-388 μm long. Starch granules simple, globose to ellipsoid, 2-14 μm in diameter, the hilum dotted; compound granules rare. Reticulate, spiral and bordered-pitted vessels frequent, 9-39 μm in diameter. Fibres mostly in bundles, slender, the end truncated.
Parenchyma cells subglobose to ellipsoid, their wall slightly thickened or beaded, with simple pits. Cork cells brown, polygonal in surface view, the wall slightly sinuous, some cells contain brown masses (Fig. 3).

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

**Standard solution**

*Irisflorentin standard solution*

Weigh 1.0 mg of irisflorentin CRS (Fig. 4) and dissolve in 2 mL of methanol.

**Developing solvent system**

Prepare a mixture of petroleum ether (60-80°C) and ethyl acetate (1:1, v/v).

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 2000 × g for 10 min. Collect the supernatant.

**Procedure**

Carry out the method by using a HPTLC silica gel F$_{254}$ plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately irisflorentin standard solution (4 μL) and the test solution (8 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 7 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the $R_f$ values by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the $R_f$ value, corresponding to those of irisflorentin.
Figure 2  Microscopic features of transverse section of Rhizoma Belamcandae

A. Sketch  B. Section illustration  C. Vascular bundle

9. Amphivasal vascular bundles  10. Leaf-trace vascular bundles
Figure 3  Microscopic features of powder of Rhizoma Belamcandae

5. Parenchyma cells  6. Cork cells  
a. Features under the light microscope  b. Features under the polarized microscope
Figure 4 Chemical structures of (i) irisflorentin (ii) tectoridin

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

**Standard solutions**

*Tectoridin standard solution for fingerprinting Std-FP (20 mg/L)*

Weigh 2.0 mg of tectoridin CRS (Fig.4) and dissolve in 100 mL of ethanol (70%).

*Irisflorentin standard solution for fingerprinting, Std-FP (10 mg/L)*

Weigh 1.0 mg of irisflorentin CRS and dissolve in 100 mL of ethanol (70%).

**Test solution**

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × g for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatant. Make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (266 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 1  Chromatographic system conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.05% Phosphoric acid (%), v/v</th>
<th>Acetonitrile (%), v/v</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–15</td>
<td>82→80</td>
<td>18→20</td>
<td>linear gradient</td>
</tr>
<tr>
<td>15–25</td>
<td>80→67</td>
<td>20→33</td>
<td>linear gradient</td>
</tr>
<tr>
<td>25–45</td>
<td>67→60</td>
<td>33→40</td>
<td>linear gradient</td>
</tr>
<tr>
<td>45–60</td>
<td>60→47</td>
<td>40→53</td>
<td>linear gradient</td>
</tr>
<tr>
<td>60–65</td>
<td>47</td>
<td>53</td>
<td>isocratic</td>
</tr>
</tbody>
</table>

System suitability requirements
Perform at least five replicate injections, each using 10 μL of tectoridin Std-FP and irisflorentin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of tectoridin and irisflorentin should not be more than 5.0%; the RSD of the retention times of tectoridin and irisflorentin peaks should not be more than 2.0%; the column efficiencies determined from tectoridin and irisflorentin peaks should not be less than 3500 and 20000 theoretical plates respectively.

The R value between peak 1 and peak 2 in the chromatogram of the test solution should not be less than 1.0 and the R value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig.5).

Procedure
Separately inject tectoridin Std-FP, irisflorentin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of tectoridin and irisflorentin peaks in the chromatograms of the corresponding Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify tectoridin and irisflorentin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatograms of the corresponding Std-FP. The retention times of tectoridin and irisflorentin peaks in the chromatograms of the test solution and the corresponding Std-FP 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 four characteristic peaks of Rhizoma Belamcandae extract are listed in Table 2.
Table 2  The RRTs and acceptable ranges of the four characteristic peaks of Rhizoma Belamcandae extract

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (marker 1, tectoridin)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.44 (vs peak 4)</td>
<td>±0.03</td>
</tr>
<tr>
<td>3</td>
<td>0.49 (vs peak 4)</td>
<td>±0.03</td>
</tr>
<tr>
<td>4 (marker 2, irisflorentin)</td>
<td>1.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 5  A reference fingerprint chromatogram of Rhizoma Belamcandae extract

For positive identification, the sample must give the above four 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 Foreign Matter (Appendix VIII): not more than 2.0%.

5.5 Ash (Appendix IX)

Total ash: not more than 7.0%.
Acid-insoluble ash: not more than 1.0%.

5.6 Water Content (Appendix X): not more than 10.0%.
6. **EXTRACTIVES** *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 21.0%.
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**

*Mixed tectoridin and irissflorentin standard stock solution, Std-Stock (20 mg/L for tectoridin and 10 mg/L for irissflorentin)*

Weigh accurately 5.0 mg of tectoridin CRS and 2.5 mg of irissflorentin CRS and dissolve in 250 mL of ethanol (70%).

*Mixed tectoridin and irissflorentin standard solution for assay, Std-AS*

Measure accurately the volume of the mixed tectoridin and irissflorentin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 2, 4, 8, 16, 20 mg/L for tectoridin and 1, 2, 4, 8, 10 mg/L for irissflorentin.

**Test solution**

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × g for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatant. Make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (266 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 3) –

<table>
<thead>
<tr>
<th>Time (min)</th>
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<th>Acetonitrile (% v/v)</th>
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<td>47</td>
<td>53</td>
<td>isocratic</td>
</tr>
</tbody>
</table>
System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed tectoridin and irisflorentin Std-AS (16 mg/L for tectoridin and 8 mg/L for irisflorentin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of tectoridin and irisflorentin should not be more than 5.0%; the RSD of the retention times of tectoridin peak and irisflorentin peak should not be more than 2.0%; the column efficiencies determined from tectoridin and irisflorentin peaks should not be less than 3500 and 20000 theoretical plates respectively.

The R value between tectoridin peak and the closest peak in the chromatogram of the test solution should not be less than 1.4 and the R value between irisflorentin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed tectoridin and irisflorentin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of tectoridin and irisflorentin against the corresponding concentrations of the mixed tectoridin and irisflorentin Std-AS. Obtain the slopes, y-intercepts and the r² values from the corresponding 5-point calibration curves.

Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify tectoridin and irisflorentin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed tectoridin and irisflorentin Std-AS. The retention times of tectoridin and irisflorentin peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of tectoridin and irisflorentin in the test solution, and calculate the percentage contents of tectoridin and irisflorentin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.14% of tectoridin (C₂₂H₂₂O₁₁) and not less than 0.10% of irisflorentin (C₂₀H₁₈O₈), calculated with reference to the dried substance.