Figure 1  A photograph of Polygoni Cuspidati Rhizoma et Radix
1. **NAMES**

Official Name: Polygoni Cuspidati Rhizoma et Radix

Chinese Name: 虎杖

Chinese Phonetic Name: Huzhang

2. **SOURCE**

Polygoni Cuspidati Rhizoma et Radix is the dried rhizome and root of *Polygonum cuspidatum* Sieb. et Zucc. (Polygonaceae). The rhizome and root are collected in spring and autumn, the rootlets removed, washed clean, cut into short sections or thick slices when fresh, then dried under the sun to obtain Polygoni Cuspidati Rhizoma et Radix.

3. **DESCRIPTION**

Mostly irregular thick slices, 0.5-9 cm long, 5-27 mm in diameter or pieces in short, cylindrical sections. Externally yellowish-brown, showing longitudinal wrinkles and rootlet scars. In transversely cut surface, bark relatively thin, easily separated from wood, wood broad, yellowish-brown with radial rays. Pith in the rhizome septated or hollowed. Texture hard. Odour slight; taste slightly bitter and astringent (Fig. 1).

4. **IDENTIFICATION**

4.1 **Microscopic Identification** *(Appendix III)*

**Transverse section**

**Rhizome:** Cork consists of several layers of cells, brownish-red. Cortex narrow, scattered with clusters of calcium oxalate. Pericycle fibre bundles arranged in the outer part of phloem. Phloem scattered with clusters of calcium oxalate and fibre bundles. Cambium in a ring. Xylem relatively broad. Phloem rays and xylem rays, 2-9 rows of cells, arranged radially, originated from the pith parenchyma. Pith parenchymatous cells contain starch granules (Fig. 2).

**Root** (8 mm in diameter): without pith compared with rhizome (Fig. 3).
**Powder**

Colour yellowish-brown. Clusters of calcium oxalate in rosette shape, abundant, 25-100 µm in diameter; polychromatic under the polarized microscope. Stone cells somewhat cylindrical in shape, 25-45 µm in diameter, pale yellow, with pits, branched at one end. Xylem fibres mostly in bundles, 25-50 µm in diameter, with pits. Cork cells brownish-yellow, subpolygonal to subsquare. Xylem ray parenchymatous cells subsquare to rectangular, the cell walls thickened, bead-like, with pits. Vessel elements mostly bordered-pitted, 30-150 µm in diameter. Starch granules abundant, simple granules rounded to ellipsoid, 3-22 µm in diameter, hilum dotted, cruciate or V-shaped; compound granules composed of 2-4 units; black and cruciate in shape under the polarized microscope (Fig. 4).

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

**Standard solution**

*Polydatin standard solution*

Weigh 1.3 mg of polydatin CRS (Fig. 5) and dissolve in 25 mL of ethanol.

**Developing solvent system**

Prepare a mixture of water, ethanol, ethyl acetate and acetic acid (4:2:1:0.5, v/v).

**Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol. Sonicate (240 W) the mixture for 30 min. Filter the mixture.

**Procedure**

Carry out the method by using a TLC polyamide plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately polydatin standard solution and the test solution (2 µL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC 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 (366 nm). Calculate the $R_f$ value 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 polydatin.
Figure 2  Microscopic features of transverse section of rhizome of Polygoni Cuspidati Rhizoma et Radix

A. Sketch   B. Section illustration   C. Pericycle fibre bundle
D. Clusters of calcium oxalate

7. Pith   8. Clusters of calcium oxalate
Figure 3  Microscopic features of transverse section of root of Polygoni Cuspidati Rhizoma et Radix

A. Sketch  B. Section illustration  C. Pericycle fibre bundle
D. Clusters of calcium oxalate

7. Clusters of calcium oxalate
Figure 4  Microscopic features of powder of Polygoni Cuspidati Rhizoma et Radix

5. Xylem ray parenchymatous cells  6. Bordered-pitted vessels  7. Starch granules

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

**Standard solutions**

Polydatin standard solution for fingerprinting, Std-FP (120 mg/L)

Weigh 1.2 mg of polydatin CRS and dissolve in 10 mL of ethanol.

Resveratrol standard solution for fingerprinting, Std-FP (130 mg/L)

Weigh 1.3 mg of resveratrol CRS (Fig. 5) and dissolve in 10 mL of ethanol.

**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. Sonicate (240 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 5 min. Filter through a 0.45-µm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (240 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) –

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**Figure 5** Chemical structures of (i) polydatin and (ii) resveratrol
Table 1  Chromatographic system conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Water (% v/v)</th>
<th>Acetonitrile (% v/v)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 20</td>
<td>84</td>
<td>16</td>
<td>isocratic</td>
</tr>
<tr>
<td>20 – 40</td>
<td>84 → 74</td>
<td>16 → 26</td>
<td>linear gradient</td>
</tr>
<tr>
<td>40 – 60</td>
<td>74</td>
<td>26</td>
<td>isocratic</td>
</tr>
</tbody>
</table>

System suitability requirements
Perform at least five replicate injections, each using 10 µL of polydatin Std-FP and resveratrol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of polydatin and resveratrol should not be more than 5.0%; the RSD of the retention times of polydatin and resveratrol peaks should not be more than 2.0%; the column efficiencies determined from polydatin and resveratrol peaks should not be less than 8000 and 70000 theoretical plates respectively.

The R value between peak 1 and the closest peak; and the R value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure
Separately inject polydatin Std-FP, resveratrol Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of polydatin and resveratrol peaks in the chromatograms of polydatin Std-FP, resveratrol Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify polydatin and resveratrol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of polydatin Std-FP and resveratrol Std-FP. The retention times of polydatin and resveratrol 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 Polygoni Cuspidati Rhizoma et Radix extract are listed in Table 2.
Table 2  The RRTs and acceptable ranges of the four characteristic peaks of Polygoni Cuspidati Rhizoma et Radix extract

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (polydatin)</td>
<td>0.43 ± 0.03</td>
<td>± 0.03</td>
</tr>
<tr>
<td>2 (marker, resveratrol)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.15 ± 0.03</td>
<td>± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>1.21 ± 0.03</td>
<td>± 0.03</td>
</tr>
</tbody>
</table>

Figure 6  A reference fingerprint chromatogram of Polygoni Cuspidati Rhizoma et Radix 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. 6).

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 1.0%.

5.5 Ash (Appendix IX)

Total ash: not more than 4.5%.

Acid-insoluble ash: not more than 1.0%.
5.6 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

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

Water-soluble extractives (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. **ASSAY**

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

**Standard solution**

*Mixed polydatin and resveratrol standard stock solution, Std-Stock (600 mg/L for polydatin and 660 mg/L for resveratrol)*

Weigh accurately 3.0 mg of polydatin CRS and 3.3 mg of resveratrol CRS, and dissolve in 5 mL of ethanol.

*Mixed polydatin and resveratrol standard solution for assay, Std-AS*

Measure accurately the volume of the mixed polydatin and resveratrol Std-Stock, dilute with ethanol to produce a series of solutions of 4.8, 9.6, 36, 60, 108 mg/L for polydatin and 5.28, 10.56, 39.6, 66, 118.8 mg/L for resveratrol.

**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. 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. Add 5 mL of ethanol to the residue. Sonicate (240 W) the mixture for 30 min. Filter and transfer the filtrate to the 25-mL volumetric flask and make up to the mark with ethanol. Filter through a 0.45-µm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (306 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 3  Chromatographic system conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Water (%, v/v)</th>
<th>Acetonitrile (%, v/v)</th>
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<tr>
<td>13 – 30</td>
<td>70</td>
<td>30</td>
<td>isocratic</td>
</tr>
</tbody>
</table>

**System suitability requirements**
Perform at least five replicate injections, each using 10 µL of the mixed polydatin and resveratrol Std-AS (36 mg/L for polydatin and 39.6 mg/L for resveratrol). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of polydatin and resveratrol should not be more than 5.0%; the RSD of the retention times of polydatin and resveratrol peaks should not be more than 2.0%; the column efficiencies determined from polydatin and resveratrol peaks should not be less than 5000 and 50000 theoretical plates respectively.

The $R$ value between polydatin peak and the closest peak; and the $R$ value between resveratrol 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 polydatin and resveratrol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of polydatin and resveratrol against the corresponding concentrations of the mixed polydatin and resveratrol Std-AS. Obtain the slopes, y-intercepts and the $r^2$ 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 polydatin and resveratrol peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed polydatin and resveratrol Std-AS. The retention times of polydatin and resveratrol peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of polydatin and resveratrol in the test solution, and calculate the percentage contents of polydatin and resveratrol in the sample by using the equations indicated in Appendix IV(B).

**Limits**
The sample contains not less than 1.1% of the total content of polydatin ($\text{C}_{20}\text{H}_{22}\text{O}_8$) and resveratrol ($\text{C}_{14}\text{H}_{12}\text{O}_3$), calculated with reference to the dried substance.