Figure 1  A photograph of Plumbagin Zeylanicae Radix

A. Plumbagin Zeylanicae Radix     B. Magnified image of root
C. Magnified image of transverse section of root
1. NAMES

Official Name: Plumbaginis Zeylanicae Radix

Chinese Name: 白花丹

Chinese Phonetic Name: Baihuadan

2. SOURCE

Plumbaginis Zeylanicae Radix is the dried root of Plumbago zeylanica L. (Plumbaginaceae). The root is collected all the year round, foreign matter removed, then dried under the sun to obtain Plumbaginis Zeylanicae Radix.

3. DESCRIPTION

Main root cylindrical, texture hard, usually branched, slightly curved, with numerous rootlets. Externally greyish-brown to brownish-yellow, smooth or rough, with longitudinal wrinkles and rootlet scars. Wood yellowish. Fracture showed several concentric rings and closely arranged radial striations. Ray white and distinct. Odour slight; taste pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Cork consists of several layers of cells, containing brownish-red contents. Cortex consists of several layers of polygonal parenchymatous cells, filled with starch granules and brownish-red contents. Cortical fibres visible, scattered singly or in bundles. Phloem distinct. Cambium in a ring. Xylem broad. Vessels scattered singly or in groups, arranged radially. Xylem ray consists of 1-2 rows of cells (Fig. 2).
Plumbaginis Zeylanicae Radix

**Powder**

Colour yellow to brown. Xylem fibres scattered or in bundles, walls slightly thickened, with distinct pits and pit canals; white under the polarized microscope. Cortical fibres long, walls thickened; yellowish-white under the polarized microscope. Cork cells subpolygonal or subrectangular, walls thickened. Stone cells usually scattered singly, 30-175 μm long, 24-85 μm in diameter, walls 5-18 μm thick, with striations, distinct pits and pit canals; yellowish-white under the polarized microscope. Bordered-pitted vessels visible, 13-77 μm in diameter. Starch granules numerous, mainly simple starch granules, subrounded, 4-24 μm in diameter; black and cruciate-shaped under the polarized microscope (Fig. 3).
Figure 2  Microscopic features of transverse section of Plumbaginis Zeylanicae Radix

A. Sketch     B. Section illustration     C. Cortical fibres
D. Brownish-red contents (→) and starch granules (→)


Figure 3  Microscopic features of powder of Plumbaginis Zeylanicae Radix


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


a. Features under the light microscope   b. Features under the polarized microscope
4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

Standard solution

*Plumbagin standard solution*
Weigh 1.0 mg of plumbagin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system
Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and glacial acetic acid (10:1:0.5, v/v).

Test solution
Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of methanol. Sonicate (350 W) the mixture for 15 min. Centrifuge at about 6000 × g for 5 min. Filter through a 0.45-µm nylon filter.

Procedure
Carry out the method by using a HPTLC silica gel F<sub>254</sub> plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately plumbagin standard solution (2 μL) and the test solution (10 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 6 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<sub>f</sub> value by using the equation as indicated in Appendix IV (A).

![Chemical structure of plumbagin](Figure 4)

**Figure 4** Chemical structure of plumbagin
4.2 Thin-Layer Chromatographic Identification

**Standard solution**

Plumbagin standard solution (Fig. 4) weighing 1.0 mg of plumbagin CRS and dissolve in 1 mL of methanol.

**Developing solvent system**

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate, and glacial acetic acid (10:1:0.5, v/v).

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of methanol. Sonicate (350 W) the mixture for 15 min. Centrifuge at about 6000 × g for 5 min. Filter through a 0.45-µm nylon filter.

**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 plumbagin standard solution (2 μL) and the test solution (10 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 6 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).

**Figure 4**

Chemical structure of plumbagin

**Figure 5**

A reference HPTLC chromatogram of Plumbaginis Zeylanicae Radix extract observed under UV light (366 nm)

1. Plumbagin standard solution     2. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the \( R_f \) value, corresponding to that of plumbagin (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

**Standard solution**

Plumbagin standard solution for fingerprinting, Std-FP (150 mg/L)

Weigh 1.5 mg of plumbagin CRS and dissolve in 10 mL of methanol.

**Test solution**

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (120 W) the mixture for 45 min. Centrifuge at about 4000 × g for 10 min. Filter through a 0.45-µm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (270 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 µm particle size). The column temperature is maintained at 30ºC during the separation. 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>Water (% v/v)</th>
<th>Acetonitrile (% v/v)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 22</td>
<td>65</td>
<td>35</td>
<td>isocratic</td>
</tr>
<tr>
<td>22 – 27</td>
<td>65 → 50</td>
<td>35 → 50</td>
<td>linear gradient</td>
</tr>
<tr>
<td>27 – 45</td>
<td>50 → 45</td>
<td>50 → 55</td>
<td>linear gradient</td>
</tr>
<tr>
<td>45 – 60</td>
<td>45 → 40</td>
<td>55 → 60</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

System suitability requirements

Perform at least five replicate injections, each using 20 µL of plumbagin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of plumbagin should not be more than 5.0%; the RSD of the retention time of plumbagin peak should not be more than 2.0%; the column efficiency determined from plumbagin peak should not be less than 8500 theoretical plates.

The R value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject plumbagin Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of plumbagin peak in the chromatogram of plumbagin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify plumbagin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of plumbagin Std-FP. The retention times of plumbagin 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 four characteristic peaks of Plumbaginis Zeylanicae Radix extract are listed in Table 2.
Table 2  The RRTs and acceptable ranges of the four characteristic peaks of Plumbaginis Zeylanicae Radix extract

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (marker, plumbagin)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.91 ± 0.03</td>
<td>± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>2.76 ± 0.03</td>
<td>± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>2.92 ± 0.03</td>
<td>± 0.03</td>
</tr>
</tbody>
</table>

Figure 6  A reference fingerprint chromatogram of Plumbaginis Zeylanicae 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 Sulphur Dioxide Residues (Appendix XVI): meet the requirements.

5.5 Foreign Matter (Appendix VIII): not more than 3.0%.
5.6 Ash *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 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 6.0%.
Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

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

**Standard solution**

*Plumbagin standard stock solution, Std-Stock (500 mg/L)*

Weigh accurately 5.0 mg of plumbagin CRS and dissolve in 10 mL of methanol.

*Plumbagin standard solution for assay, Std-AS*

Measure accurately the volume of the plumbagin Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 1.5, 12.5, 50, 100, 200 mg/L for plumbagin.

**Test solution**

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (50%). Sonicate (120 W) the mixture for 15 min. Centrifuge at about 4000 × g for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). Filter through a 0.45-µm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (270 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 µm particle size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of water and acetonitrile (65:35, v/v). The elution time is about 30 min.
System suitability requirements
Perform at least five replicate injections, each using 20 µL of plumbagin Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of plumbagin should not be more than 5.0%; the RSD of the retention time of plumbagin peak should not be more than 2.0%; the column efficiency determined from plumbagin peak should not be less than 8500 theoretical plates.

The $R$ value between plumbagin 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 plumbagin Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of plumbagin against the corresponding concentrations of plumbagin Std-AS. Obtain the slope, y-intercept and the $r^2$ value from the 5-point calibration curve.

Procedure
Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify plumbagin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of plumbagin Std-AS. The retention times of plumbagin 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 plumbagin in the test solution, and calculate the percentage content of plumbagin in the sample by using the equations as indicated in Appendix IV (B).

Limits
The sample contains not less than 0.10% of plumbagin ($C_{11}H_{8}O_3$), calculated with reference to the dried substance.