Figure 1  A photograph of Sinopodophylli Radix et Rhizoma

A. Sinopodophylli Radix et Rhizoma
B. Magnified image of transverse section of rhizome
C. Magnified image of transverse section of root
1. NAMES

Official Name: Sinopodophylli Radix et Rhizoma

Chinese Name: 桃兒七

Chinese Phonetic Name: Taoerqi

2. SOURCE

Sinopodophylli Radix et Rhizoma is the dried root and rhizome of *Sinopodophyllum hexandrum* (Royle) Ying (Berberidaceae). The root and rhizome are collected in spring and autumn, soil removed, washed clean, then dried under the sun to obtain Sinopodophylli Radix et Rhizoma.

3. DESCRIPTION

Root slender, cylindrical, 10-25 cm long, 2-4 mm in diameter; externally brownish-yellow to brown, with longitudinal wrinkles and rootlet scars; texture fragile, easily broken; fracture even, whitish or yellowish-white, starchy, wood in the central small, pale yellow or yellow. Rhizome transversely nodulated, 2-8 cm long, 8-18 mm in diameter; externally reddish-brown, brown or greyish-brown; apex with scars of the stem or remnants of the stem base; texture hard. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

**Transverse section**

*Root*: Epidermis consists of 1 layer of subsquare to subrectangular cells, walls thickened and lignified. Hypodermis consists of 1 layer of subsquare or subrectangular cells, cells relatively large, walls thin. Cortex broad, consisting of more than 10 layers of cells, cells filled with starch granules. Endodermis distinct, consisting of 1 layer of cells. Stele small. Phloem bundles arranged alternatively with primary xylem, in tetrarch or pentarch configuration, vessels large, polygonal [Fig. 2 (i)].
Rhizome: Cork cells rectangular or subsquare, wall slightly thick, obviously lignified. Cortex broad, composed of large parenchymatous cells, cells contain starch granules. Vascular bundles collateral. Phloem cells mostly shrunken. Cambium distinct. Xylem composed of vessels and parenchymatous cells, xylem vessels scattered singly or in groups, arranged radially. Pith relatively large, parenchymatous cells subrounded [Fig. 2 (ii)].

Powder
Colour whitish to yellowish-white. Starch granules numerous, single starch granules subspherical or semicircular, 7-15 µm in diameter, hilum pointed, V-shaped or slit-shaped; black and cruciate-shaped under the polarized microscope; compound starch granules mostly composed of 2-4 units. Epidermal cells pale brownish-yellow, subrectangular to elongated-polygonal in surface view, walls unevenly thickened. Vessels scattered singly or in groups, mostly of reticulate or pitted vessels, few scalariform, spiral or annular vessels, 14-45 µm in diameter (Fig. 3).
**Figure 2 (i)** Microscopic features of transverse section of root of Sinopodophylli Radix et Rhizoma

A. Sketch  B. Section illustration  C. Stele

Figure 2 (ii)  Microscopic features of transverse section of rhizome of Sinopodophylli Radix et Rhizoma

A. Sketch  B. Section illustration

Figure 3  Microscopic features of powder of Sinopodophylli Radix et Rhizoma


a. Features under the light microscope   b. Features under the polarized microscope

4.2 Thin-Layer Chromatographic Identification

[Appendix IV(A)]

Standard solutions

Deoxypodophyllotoxin standard solution
Weigh 6.0 mg of deoxypodophyllotoxin CRS (Fig. 4) and dissolve in 5 mL of methanol.

Podophyllotoxin standard solution
Weigh 6.0 mg of podophyllotoxin CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system
Prepare a mixture of cyclohexane and ethyl acetate (1:1, v/v).

Spray reagent
Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution
Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (250 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol.

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 deoxypodophyllotoxin standard solution, podophyllotoxin standard solution and the test solution (5 μL each) 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 8.5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 10 min). Examine the plate under visible light. Calculate the \( R_f \) values by using the equation as indicated in Appendix IV (A).
4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

**Standard solutions**

*Deoxypodophyllotoxin standard solution*
Weigh 6.0 mg of deoxypodophyllotoxin CRS (Fig. 4) and dissolve in 5 mL of methanol.

*Podophyllotoxin standard solution*
Weigh 6.0 mg of podophyllotoxin CRS (Fig. 4) and dissolve in 5 mL of methanol.

**Developing solvent system**
Prepare a mixture of cyclohexane and ethyl acetate (1:1, v/v).

**Spray reagent**
Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

**Test solution**
Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (250 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol.

**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 deoxypodophyllotoxin standard solution, podophyllotoxin standard solution and the test solution (5 μL each) 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 8.5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 105°C until the spots or bands become visible (about 10 min). Examine the plate under visible light. 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 $R_f$ values, corresponding to those of deoxypodophyllotoxin and podophyllotoxin (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

**Standard solutions**

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

Weigh 5.0 mg of deoxypodophyllotoxin CRS and place it in a 50-mL volumetric flask, then add 40 mL of methanol. Sonicate (500 W) the mixture for 30 min and make up to the mark with methanol. Pipette 0.4 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol (50%). Keep at about 4ºC.

**Podophyllotoxin standard solution for fingerprinting, Std-FP (200 mg/L)**

Weigh 10.0 mg of podophyllotoxin CRS and place it in a 10-mL volumetric flask, then add 8 mL of methanol. Sonicate (500 W) the mixture for 30 min and make up to the mark with methanol. Pipette 0.4 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol (50%). Keep at about 4ºC.

**Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (500 W) the mixture at below 25ºC for 30 min. Centrifuge at about $2800 \times g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about $2800 \times g$ for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size, 120 Å pore size, 17% carbon loading and with endcapping). The test sample solution is maintained at a temperature of 4ºC. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

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**Figure 4** Chemical structures of (i) deoxypodophyllotoxin and (ii) podophyllotoxin

**Figure 5** A reference HPTLC chromatogram of Sinopodophyli Radix et Rhizoma extract observed under visible light after staining

1. Podophyllotoxin standard solution  
2. Deoxypodophyllotoxin standard solution  
3. Test solution
For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the $R_f$ values, corresponding to those of deoxypodophyllotoxin and podophyllotoxin (Fig. 5).

### 4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

**Standard solutions**

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*Podophyllotoxin standard solution for fingerprinting, Std-FP (200 mg/L)*

Weigh 10.0 mg of podophyllotoxin CRS and place it in a 10-mL volumetric flask, then add 8 mL of methanol. Sonicate (500 W) the mixture for 30 min and make up to the mark with methanol. Pipette 0.4 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol (50%). Keep at about 4°C.

**Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (500 W) the mixture at below 25°C for 30 min. Centrifuge at about 2800 × $g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about 2800 × $g$ for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 120 Å pore size, 17% carbon loading and with endcapping). The test sample solution is maintained at a temperature of 4°C. 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>0.5% Phosphoric acid (% v/v)</th>
<th>Acetonitrile: Methanol (5:3, % v/v)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 8</td>
<td>85 → 65</td>
<td>15 → 35</td>
<td>linear gradient</td>
</tr>
<tr>
<td>8 – 20</td>
<td>65 → 55</td>
<td>35 → 45</td>
<td>linear gradient</td>
</tr>
<tr>
<td>20 – 30</td>
<td>55</td>
<td>45</td>
<td>isocratic</td>
</tr>
<tr>
<td>30 – 50</td>
<td>55 → 38</td>
<td>45 → 62</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

**System suitability requirements**

Perform at least five replicate injections, each using 10 µL of deoxypodophyllotoxin Std-FP and podophyllotoxin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of deoxypodophyllotoxin and podophyllotoxin should not be more than 5.0%; the RSD of the retention times of deoxypodophyllotoxin and podophyllotoxin peaks should not be more than 2.0%; the column efficiencies determined from deoxypodophyllotoxin and podophyllotoxin peaks should not be less than 100000 and 40000 theoretical plates respectively.

The $R$ value between peak 2 and the closest peak; and the $R$ value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

**Procedure**

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

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.73</td>
<td>± 0.03</td>
</tr>
<tr>
<td>2 (marker, podophyllotoxin)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.04</td>
<td>± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>1.42</td>
<td>± 0.03</td>
</tr>
<tr>
<td>5 (deoxypodophyllotoxin)</td>
<td>1.65</td>
<td>± 0.03</td>
</tr>
</tbody>
</table>

Figure 6  A reference fingerprint chromatogram of Sinopodophylli Radix et Rhizoma 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. 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 4.0%.

5.6 Ash (Appendix IX)

Total ash: not more than 7.0%.

Acid-insoluble ash: not more than 2.5%.
5.7  **Water Content** *(Appendix X)*

Oven dried method: not more than 11.0%.

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

Water-soluble extractives (hot extraction method): not less than 38.0%.
Ethanol-soluble extractives (hot extraction method): not less than 28.0%.

7.  **ASSAY**

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

**Standard solution**

*Mixed deoxypodophyllotoxin and podophyllotoxin standard stock solution, Std-Stock (50 mg/L for deoxypodophyllotoxin and 500 mg/L for podophyllotoxin)*

Weigh accurately 1.0 mg of deoxypodophyllotoxin CRS and 10.0 mg of podophyllotoxin CRS, and place it in a 10-mL volumetric flask, then add 6 mL of methanol. Sonicate (500 W) the mixture for 30 min and make up to the mark with methanol. Pipette 1 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol (50%). Keep at about 4°C.

*Mixed deoxypodophyllotoxin and podophyllotoxin standard solution for assay, Std-AS*

Measure accurately the volume of the mixed deoxypodophyllotoxin and podophyllotoxin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 2.5, 5, 10, 20, 40 mg/L for deoxypodophyllotoxin and 25, 50, 100, 200, 400 mg/L for podophyllotoxin. Keep at about 4°C.

**Test solution**

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (500 W) the mixture at below 25°C for 30 min. Centrifuge at about 2800 × g for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about 2800 × g for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.
Chromatographic system

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size, 120 Å pore size, 17% carbon loading and with endcapping). The test sample solution is maintained at a temperature of 4ºC. 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>0.5% Phosphoric acid (% v/v)</th>
<th>Acetonitrile: Methanol (5:3, % v/v)</th>
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</tr>
</tbody>
</table>

**System suitability requirements**

Perform at least five replicate injections, each using 10 µL of the mixed deoxypodophyllotoxin and podophyllotoxin Std-AS (10 mg/L for deoxypodophyllotoxin and 100 mg/L for podophyllotoxin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of deoxypodophyllotoxin and podophyllotoxin should not be more than 5.0%; the RSD of the retention times of deoxypodophyllotoxin and podophyllotoxin peaks should not be more than 2.0%; the column efficiencies determined from deoxypodophyllotoxin and podophyllotoxin peaks should not be less than 100000 and 40000 theoretical plates respectively.

The *R* value between deoxypodophyllotoxin peak and the closest peak; and the *R* value between podophyllotoxin 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 deoxypodophyllotoxin and podophyllotoxin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of deoxypodophyllotoxin and podophyllotoxin against the corresponding concentrations of the mixed deoxypodophyllotoxin and podophyllotoxin 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 deoxypodophyllotoxin and podophyllotoxin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed deoxypodophyllotoxin and podophyllotoxin Std-AS. The retention times of deoxypodophyllotoxin and podophyllotoxin peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of deoxypodophyllotoxin and podophyllotoxin in the sample by using the equations as indicated in Appendix IV (B).

**Limits**

The sample contains not less than 2.0% of the total content of deoxypodophyllotoxin (C\textsubscript{22}H\textsubscript{22}O\textsubscript{7}) and podophyllotoxin (C\textsubscript{22}H\textsubscript{22}O\textsubscript{8}), calculated with reference to the dried substance.

**8. CAUTION**

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.