Cynanchi Paniculati Radix et Rhizoma

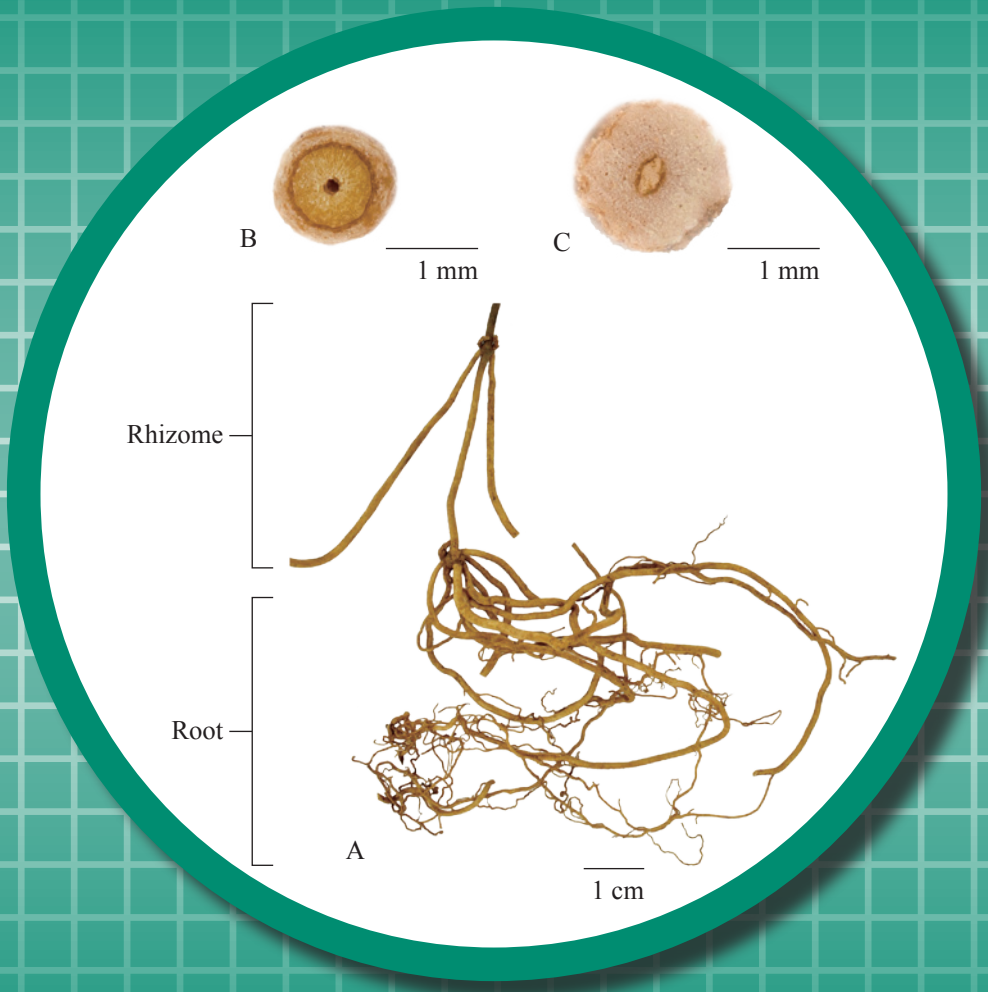

Figure 1 A photograph of Cynanchi Paniculati Radix et Rhizoma

A. Cynanchi Paniculati Radix et Rhizoma B. Magnified transverse section of rhizome
C. Magnified transverse section of root

1. NAMES

Official Name: Cynanchi Paniculati Radix et Rhizoma

Chinese Name: 徐長卿

Chinese Phonetic Name: Xuchangqing

2. SOURCE

Cynanchi Paniculati Radix et Rhizoma is the dried root and rhizome of *Cynanchum paniculatum* (Bge.) Kitag. (Asclepiadaceae). The root and rhizome is collected in autumn, foreign matter removed, then dried in a shaded area to obtain Cynanchi Paniculati Radix et Rhizoma.

3. DESCRIPTION

Roots slender-cylindrical, curved, 4-26 cm long, 0.5-2 mm in diameter. Externally pale yellowish-white to pale brownish-yellow or brown; with fine longitudinal wrinkles and fibrous rootlets. Rhizomes irregularly cylindrical, knotty, 0.5-3 cm long, 0.5-1.5 mm in diameter. Some of the rhizomes with remnants of stem at the apex, stem slender-cylindrical, fracture hollow; nodes of rhizomes surrounded by numerous roots. Texture fragile, easily broken. Fracture starchy, bark whitish to yellowish-white, cambium ring pale brown, wood small. Odour aromatic; taste slightly pungent and cool (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Epidermis consists of 1 layer of cells, outer cell walls thickened. Cortex broad, consisting of parenchymatous cells, comprising about 2/3 of the root, containing clusters of calcium oxalate and numerous of starch granules. Endodermis scatterd with distinct casparian dots. Phloem narrow. Cambium indistinct. Xylem broad, cells lignified [Fig. 2 (i)].

Rhizome: Epidermis consists of 1 layer of cells, covered with cuticle. Cortex broad, consisting of parenchymatous cells, containing clusters of calcium oxalate and numerous of starch granules. Vascular bundles amphiphloic siphonostele; phloem narrow, cells always compressed; cambium consists of 1-2 layers of cells; xylem broad, cells lignified. Pith always hollow [Fig. 2 (ii)].

Powder

Colour pale greyish-brown to pale brown. Epidermal cells polygonal in surface view, anticlinal walls slightly sinuous, lignified subsquare cells distribute among epidermal cells; subrectangular in lateral view, some with thickened fine stripes in radial walls. Parenchymatous cells mostly fragmented, subrounded to polygonal, containing numerous starch granules; pale white under the polarized microscope. Fibres colourless, slender. Vessels bordered-pitted, mostly in bundles. Clusters of calcium oxalate 10-55 μm in diameter; polychromatic under the polarized microscope. Starch granules numerous, simple starch granules subrounded or subrectangular, 3-11 μm in diameter, hilum dotted or indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-6 units (Fig. 3).

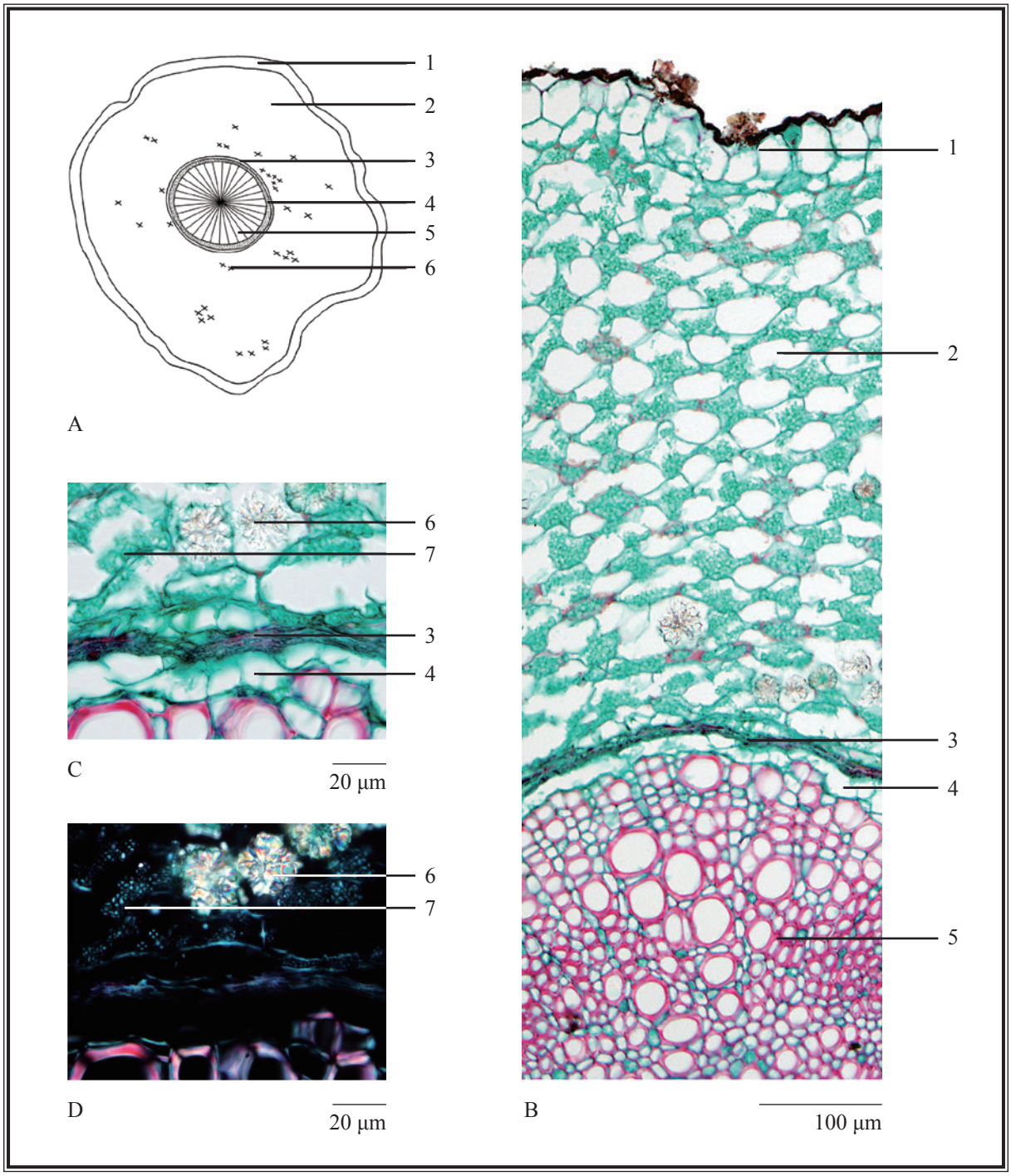

Figure 2 (i) Microscopic features of transverse section of root of Cynanchi Paniculati Radix et Rhizoma

A. Sketch B. Section illustration C. Section magnified
D. Section magnified (under the polarized microscope)

1. Epidermis 2. Cortex 3. Endodermis 4. Phloem 5. Xylem
6. Clusters of calcium oxalate 7. Starch granules

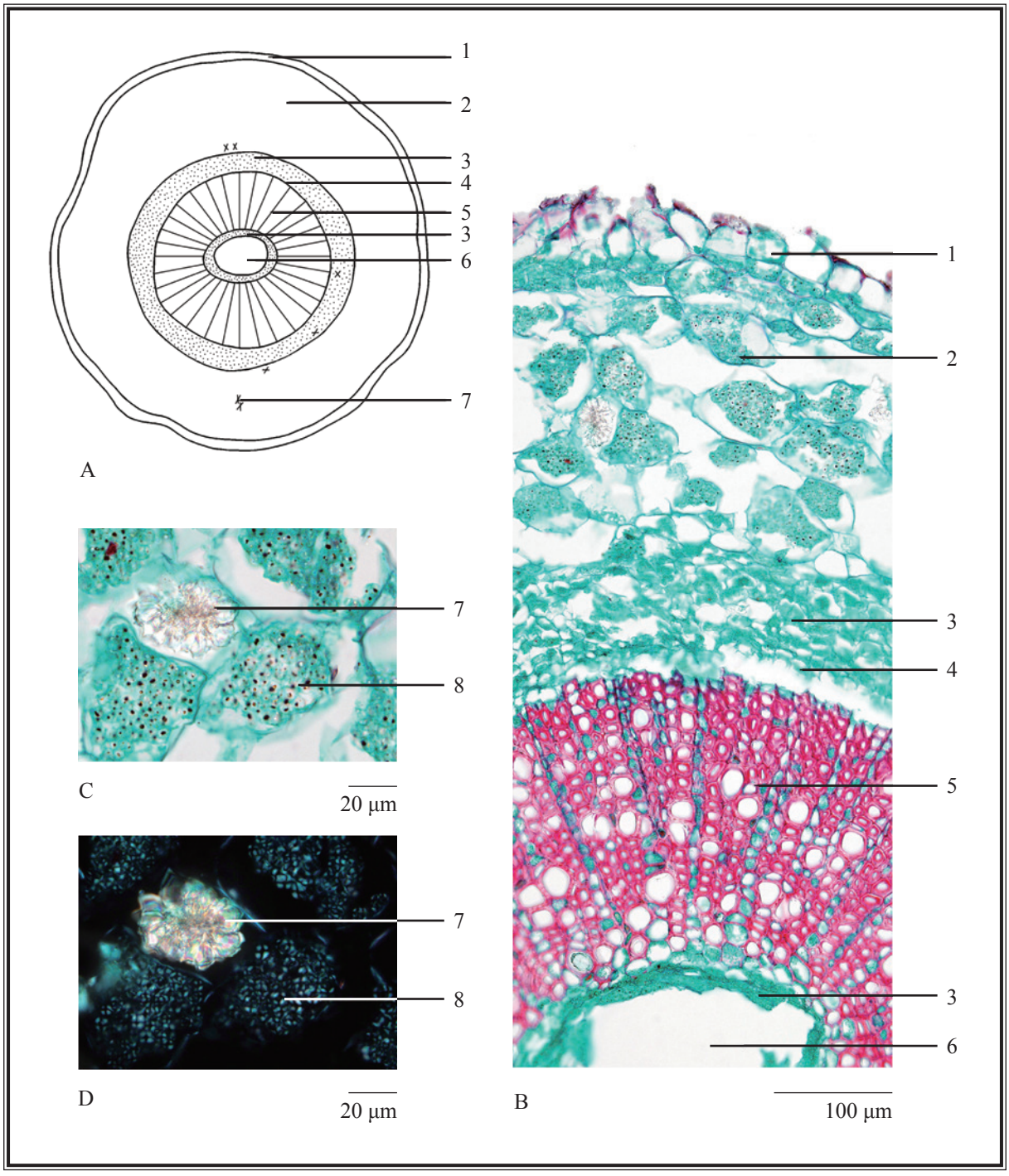

Figure 2 (ii) Microscopic features of transverse section of rhizome of Cynanchi Paniculati Radix et Rhizoma

A. Sketch B. Section illustration C. Section magnified
D. Section magnified (under the polarized microscope)

1. Epidermis 2. Cortex 3. Phloem 4. Cambium 5. Xylem
6. Pith 7. Clusters of calcium oxalate 8. Starch granules

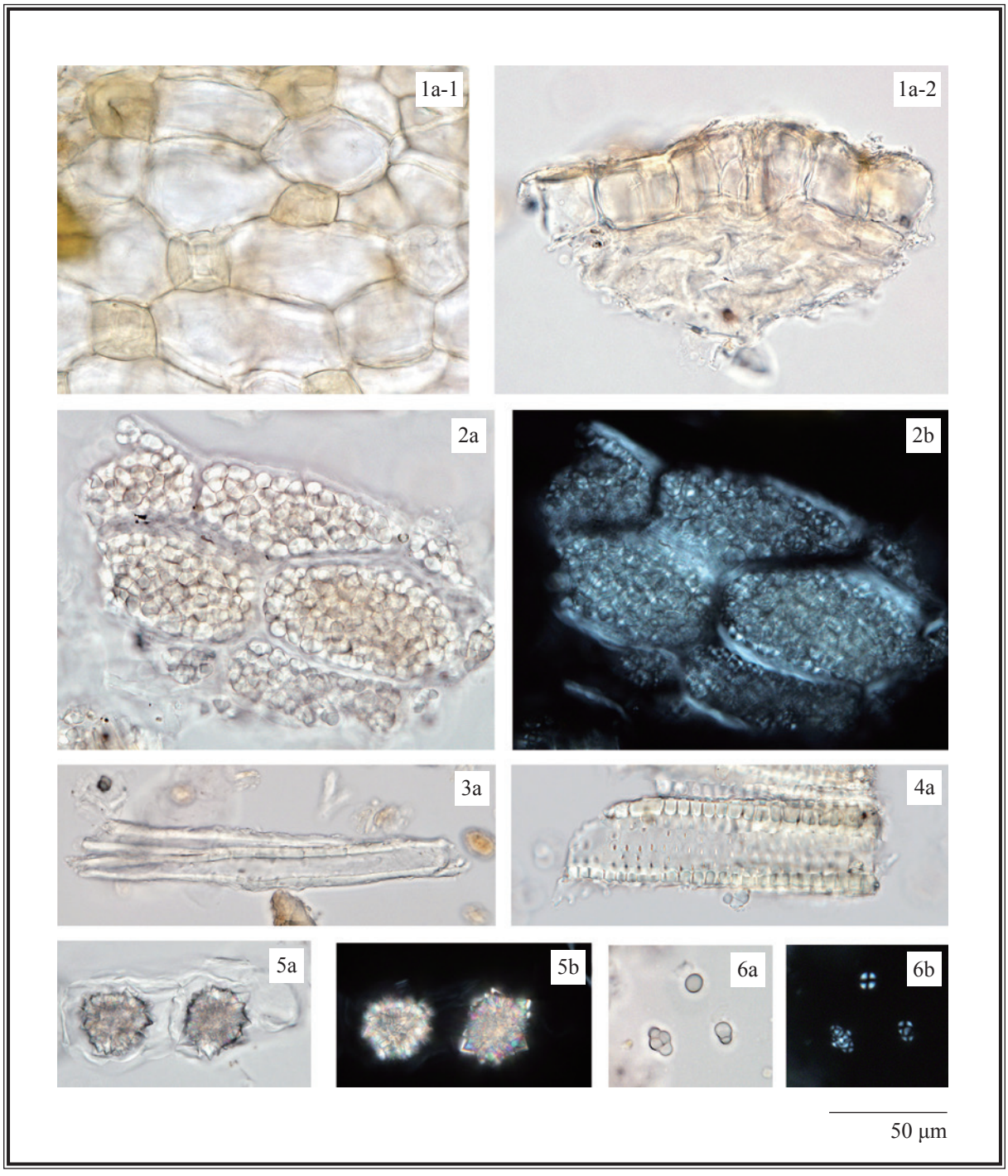

Figure 3 Microscopic features of powder of Cynanchi Paniculati Radix et Rhizoma

1. Epidermal cells (1-1 in surface view, 1-2 in lateral view)
2. Parenchymatous cells with starch granules 3. Fibres 4. Bordered-pitted vessel
5. Clusters of calcium oxalate 6. Starch granules

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

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

Standard solution

Paeonol standard solution

Weigh 1.0 mg of paeonol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

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

Spray reagent

Weigh 2.5 g of ferric trichloride and dissolve in 50 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately paeonol standard solution (3 μL) and the test solution (6 μL) to the plate. 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. Spray the plate evenly with the spray reagent and dry in air. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

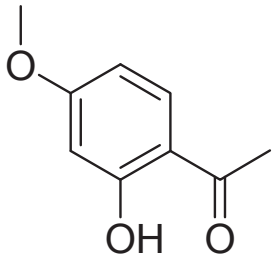

Figure 4 Chemical structure of paeonol

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

1. Paeonol standard solution 2. Test solution

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 paeonol (Fig. 5).

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

Standard solution

Paeonol standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of paeonol CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.2 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	85	15	isocratic
20 – 30	85 → 75	15 → 25	linear gradient
30 – 60	75 → 30	25 → 70	linear gradient
60 – 90	30 → 0	70 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of paeonol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of paeonol should not be more than 5.0%; the RSD of the retention time of paeonol peak should not be more than 2.0%; the column efficiency determined from paeonol peak should not be less than 200000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Cynanchi Paniculati Radix et Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.38	± 0.03
2	0.47	± 0.03
3	0.92	± 0.03
4 (marker, paeonol)	1.00	-
5	1.33	± 0.03

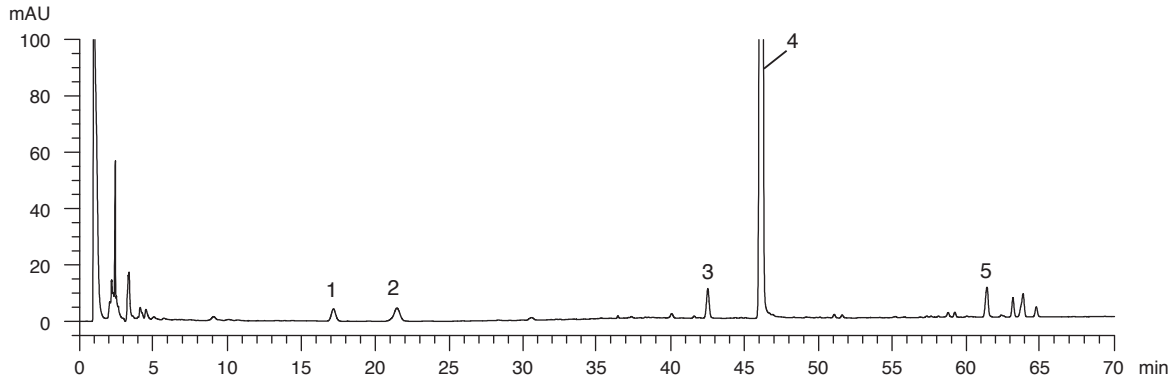

Figure 6 A reference fingerprint chromatogram of Cynanchi Paniculati Radix et Rhizoma extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 6.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.0%.

Acid-insoluble ash: not more than 5.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 16.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 18.0%.
Ethanol-soluble extractives (cold extraction method): not less than 16.0%.

7. ASSAY

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

Standard solution

Paeonol standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of paeonol CRS and dissolve in 5 mL of methanol.

Paeonol standard solution for assay, Std-AS

Measure accurately the volume of the paeonol Std-Stock, dilute with methanol to produce a series of solutions of 5, 10, 30, 50, 100 mg/L for paeonol.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for two more times. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (275 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 water and acetonitrile (55:45, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of paeonol Std-AS (30 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of paeonol should not be more than 5.0%; the RSD of the retention time of paeonol peak should not be more than 2.0%; the column efficiency determined from paeonol peak should not be less than 10000 theoretical plates.

The *R* value between paeonol 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 paeonol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of paeonol against the corresponding concentrations of paeonol Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify paeonol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of paeonol Std-AS. The retention times of paeonol 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 paeonol in the test solution, and calculate the percentage content of paeonol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.3% of paeonol ($C_9H_{10}O_3$), calculated with reference to the dried substance.