Acanthopanacis Senticosi Radix et Rhizoma seu Caulis

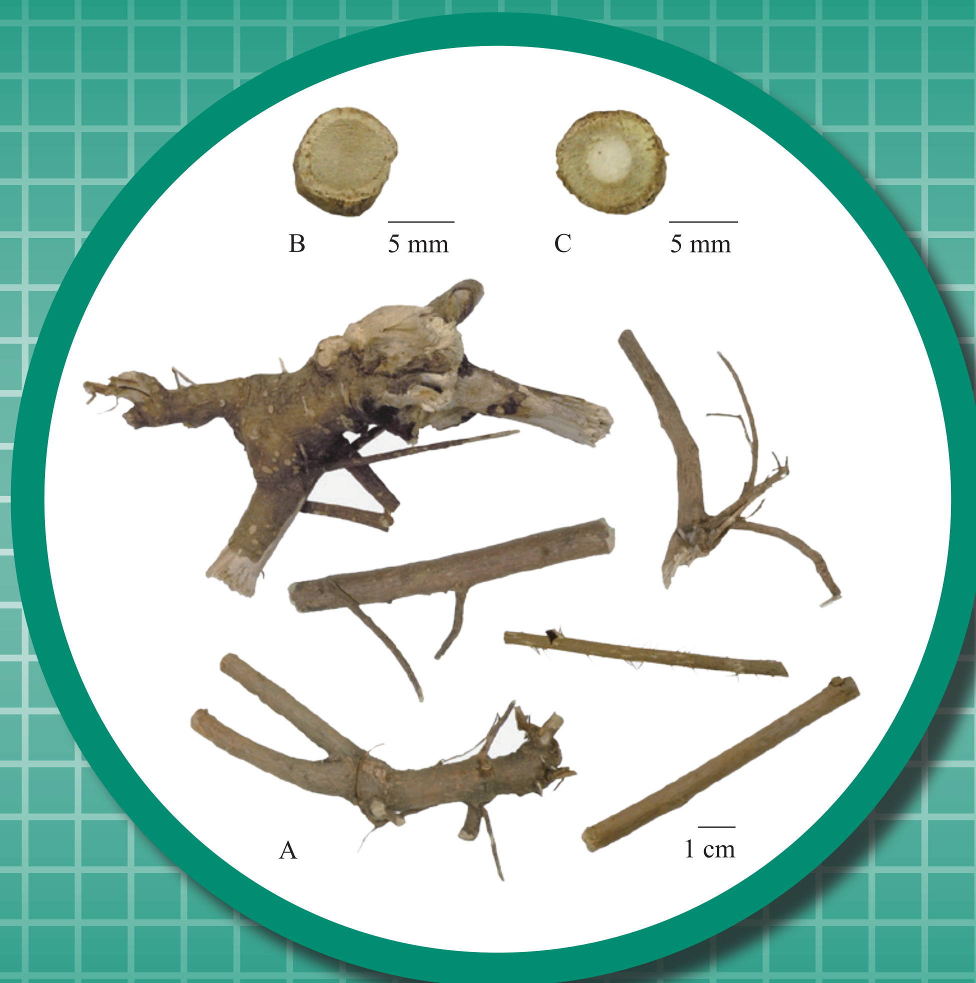

Figure 1 A photograph of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis*

A. Acanthopanacis Senticosi Radix et Rhizoma seu Caulis

B. Magnified transverse section of root C. Magnified transverse section of rhizome

* Remarks: Magnified transverse section of the stem of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis is not shown, as this plant part is found only occasionally in the market samples.

1. NAMES

Official Name: Acanthopanacis Senticosi Radix et Rhizoma seu Caulis

Chinese Name: 刺五加

Chinese Phonetic Name: Ciwujia

2. SOURCE

Acanthopanacis Senticosi Radix et Rhizoma seu Caulis is the dried root and rhizome or stem of *Acanthopanax senticosus* (Rupr. et Maxim.) Harms (Araliaceae). The root, rhizome and stem are collected in spring or autumn, washed clean, dried under the sun or dried by baking to obtain Acanthopanacis Senticosi Radix et Rhizoma seu Caulis.

3. DESCRIPTION

Root cylindrical, sometimes twisted, 2-57 mm in diameter, externally greyish-brown to brown, with longitudinal wrinkles; fracture yellowish-white. Rhizomes knotty, irregularly cylindrical or cylindrical, 4-60 mm in diameter, with pith in the centre. Texture hard, fibrous. Odour distinctively aromatic; taste slightly bitter and astringent. Stem occasionally found, cylindrical, externally yellowish-brown to brown. Old stem inermous; young stem with small prickles. Odour slight; taste slightly pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of several layers of cells. Cortex narrow, scattered with numerous clusters of calcium oxalate. Phloem with fibre bundles scattered at the outer side, some cells contain clusters of calcium oxalate; phloem ray distinct. Cambium in a ring. Xylem occupies the majority of root, consisting of vessels and fibres; xylem rays consist of 1-3 rows of cells. Secretory canals scattered in cortex and phloem, mainly in cortex [Fig. 2 (i)].

Rhizome: Cork consists of several layers of cells. Cortex narrow, scattered with clusters of calcium oxalate. Phloem with fibre bundles scattered at the outer side, some cells contain clusters of calcium oxalate; phloem ray distinct. Cambium in a ring. Xylem consists of vessels and fibres; xylem rays consist of 1-3 rows of cells. Pith consists of parenchymatous cells. Secretory canals scattered in cortex and phloem, mainly in cortex [Fig. 2 (ii)].

Powder

Colour greyish-white. Clusters of calcium oxalate scattered or present in parenchymatous cells, some arranged in a row, 10-74 µm in diameter; polychromatic under the polarized microscope. Phloem fibres usually scattered singly, 9-47 µm in diameter, pit canals distinct; bright yellowish-white under the polarized microscope. Xylem fibres scattered or in bundles, 8-37 µm in diameter, lumens with septum, some with sparse oblique pits; bright yellowish-white under the polarized microscope. Secretory canals mostly broken, filling with yellow or brownish-yellow secretions. Cork cells yellowish-brown, subpolygonal or subrectangular in surface view. Vessels mainly bordered-pitted, often broken, 18-109 µm in diameter (Fig. 3).

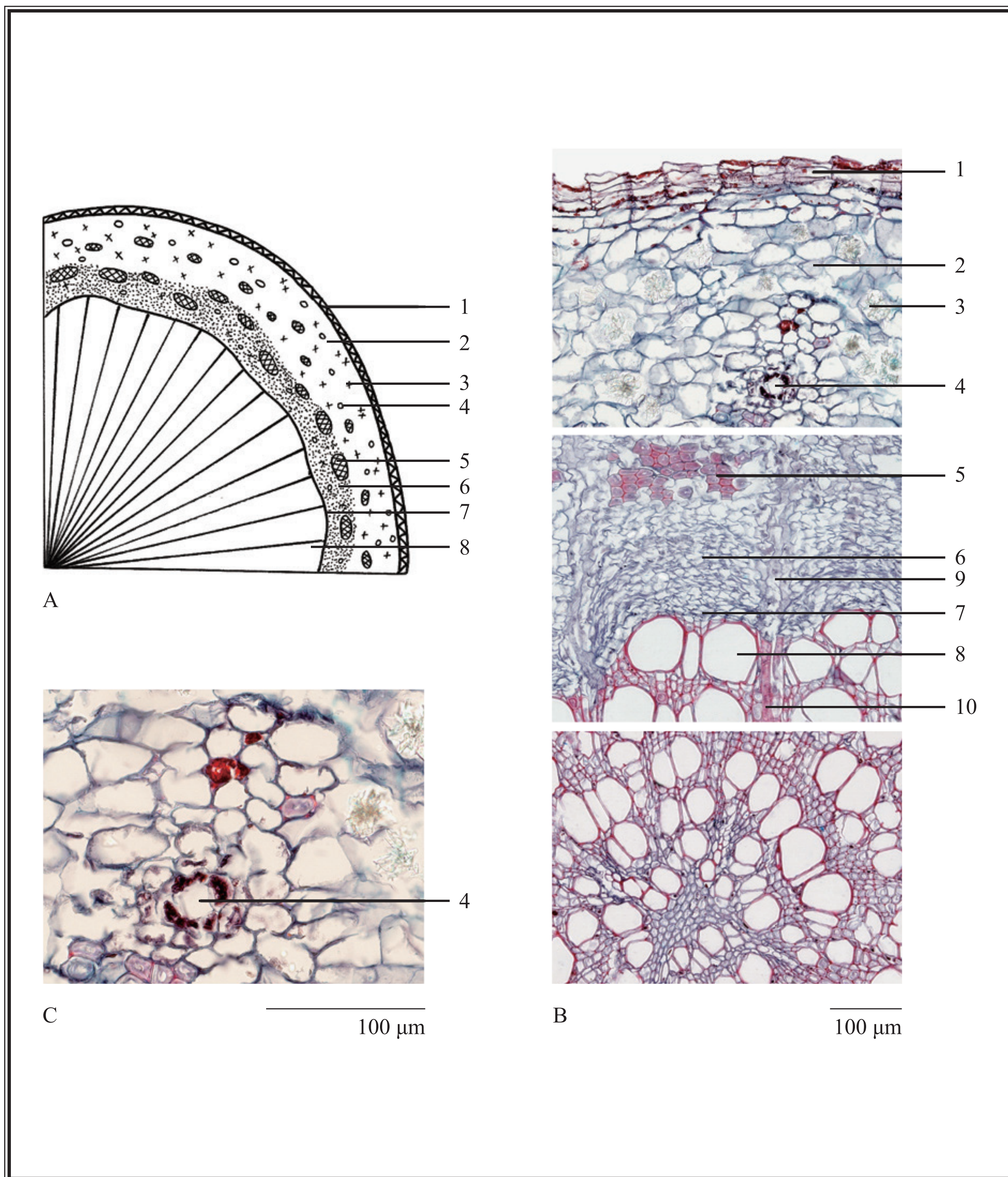

Figure 2 (i) Microscopic features of transverse section of root of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis

A. Sketch B. Section illustration C. Secretory canal

1. Cork 2. Cortex 3. Cluster of calcium oxalate 4. Secretory canal
5. Fibre bundles 6. Phloem 7. Cambium 8. Xylem 9. Phloem ray 10. Xylem ray

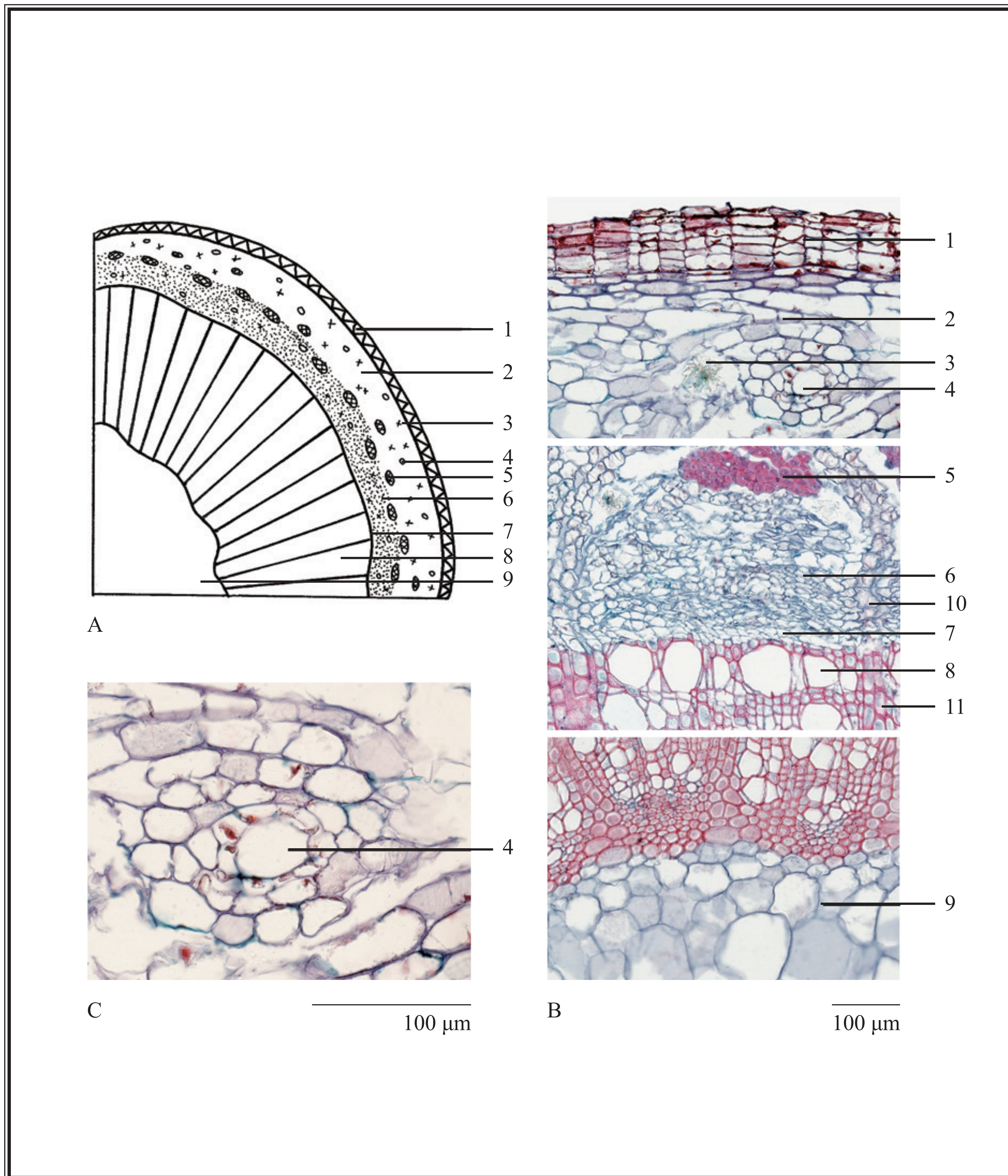

Figure 2 (ii) Microscopic features of transverse section of rhizoma of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis

A. Sketch B. Section illustration C. Secretory canal

1. Cork 2. Cortex 3. Cluster of calcium oxalate 4. Secretory canal
5. Fibre bundles 6. Phloem 7. Cambium 8. Xylem 9. Pith
10. Phloem ray 11. Xylem ray

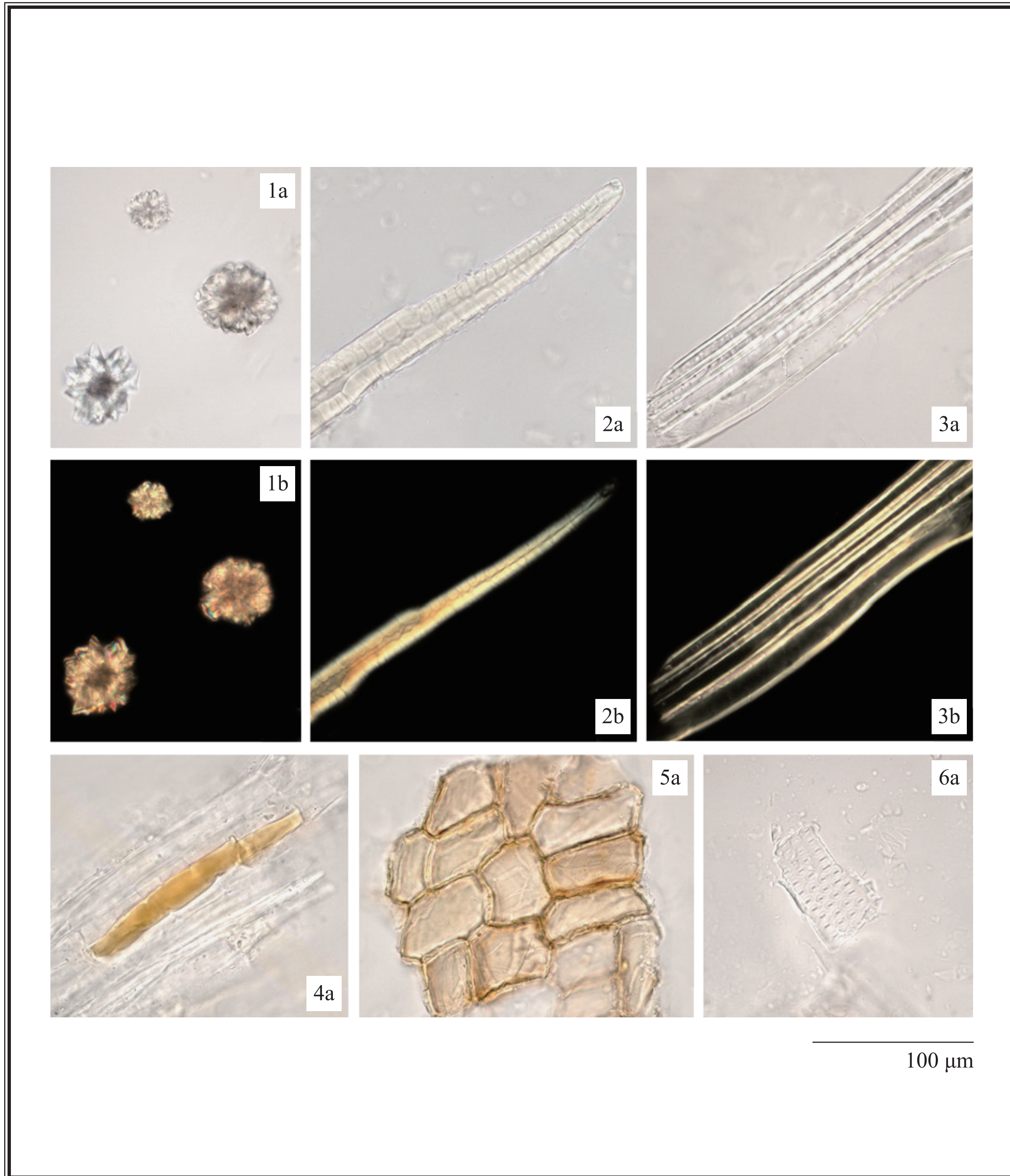

Figure 3 Microscopic features of powder of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis

1. Clusters of calcium oxalate 2. Phloem fibre 3. Xylem fibres
4. Secretory canal 5. Cork cells 6. Vessel

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

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

Standard solutions

Isofraxidin standard solution

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

Liriodendrin (eleutheroside E) standard solution

Weigh 1.0 mg of liriodendrin CRS (Fig. 4) and dissolve in 1 mL of ethanol (50%).

Syringin (eleutheroside B) standard solution

Weigh 1.0 mg of syringin CRS (Fig. 4) and dissolve in 1 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (10:2:0.1, 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 15-mL centrifuge tube, then add 5 mL of ethanol (50%). Sonicate (140 W) the mixture for 10 min. Centrifuge at about 2800 × *g* for 10 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 isofraxidin standard solution (0.5 μL), liriodendrin standard solution (1.5 μL), syringin standard solution (1.5 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 of isofraxidin by using the equation as indicated in Appendix IV (A). Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 3-5 min). Examine the plate under visible light. Calculate the R_f values of liriodendrin and syringin by using the equation as indicated in Appendix IV (A).

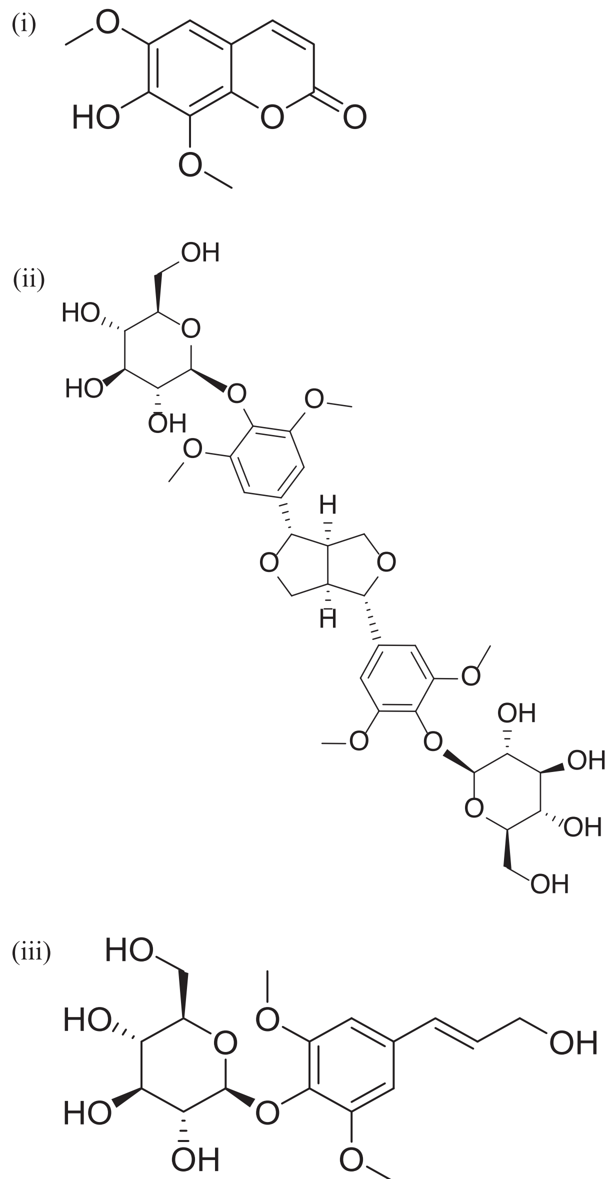

Figure 4 Chemical structures of (i) isofraxidin (ii) liriodendrin (eleutheroside E) and (iii) syringin (eleutheroside B)

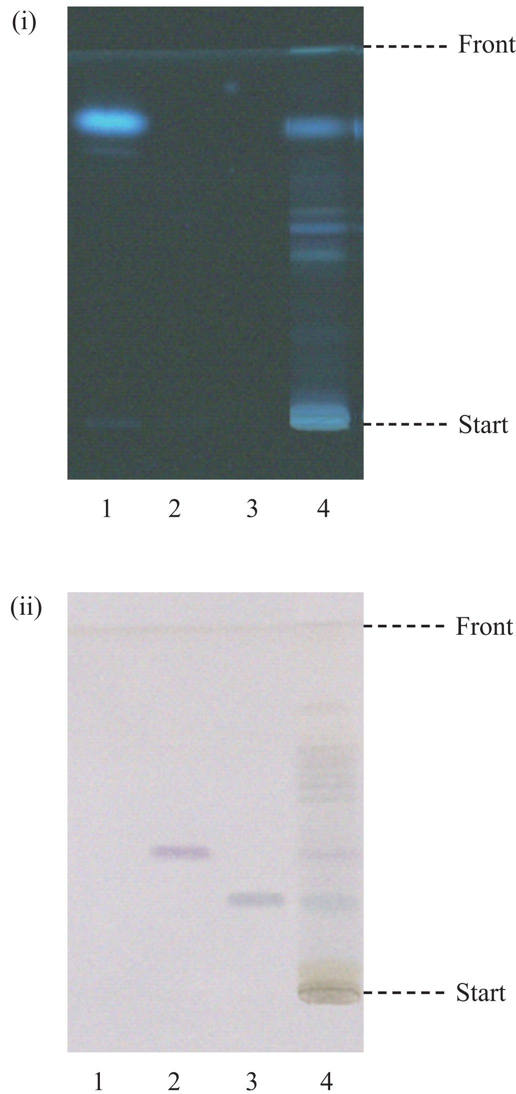

Figure 5 A reference HPTLC chromatogram of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis extract observed (i) under UV light (366 nm) and (ii) under visible light after staining

1. Isofraxidin standard solution 2. Syringin standard solution
3. Liriodendrin standard solution 4. 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 isofraxidin, liriodendrin and syringin (Fig. 5).

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

Standard solutions

Liriodendrin (eleutheroside E) standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of liriodendrin CRS and dissolve in 10 mL of methanol (50%).

Syringin (eleutheroside B) standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of syringin CRS and dissolve in 10 mL of methanol (50%).

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 (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Combine the solutions 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 (220 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 40ºC 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

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	89	11	isocratic
15 – 35	89 → 82	11 → 18	linear gradient
35 – 50	82	18	isocratic
50 – 60	82 → 70	18 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of liriodendrin Std-FP and syringin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of liriodendrin and syringin should not be more than 5.0%; the RSD of the retention times of liriodendrin and syringin peaks should not be more than 2.0%; the column efficiencies determined from liriodendrin and syringin peaks should not be less than 100000 and 10000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject liriodendrin Std-FP, syringin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of liriodendrin and syringin peaks in the chromatograms of liriodendrin Std-FP, syringin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify liriodendrin and syringin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of liriodendrin Std-FP and syringin Std-FP. The retention times of liriodendrin and syringin 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 Acanthopanacis Senticosi Radix et Rhizoma seu Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis extract

Peak No.	RRT	Acceptable Range
1 (syringin)	0.35	± 0.03
2 (chlorogenic acid)	0.39	± 0.03
3 (marker, liriodendrin)	1.00	-
4 (1,5-dicaffeoylquinic acid)	1.49	± 0.03

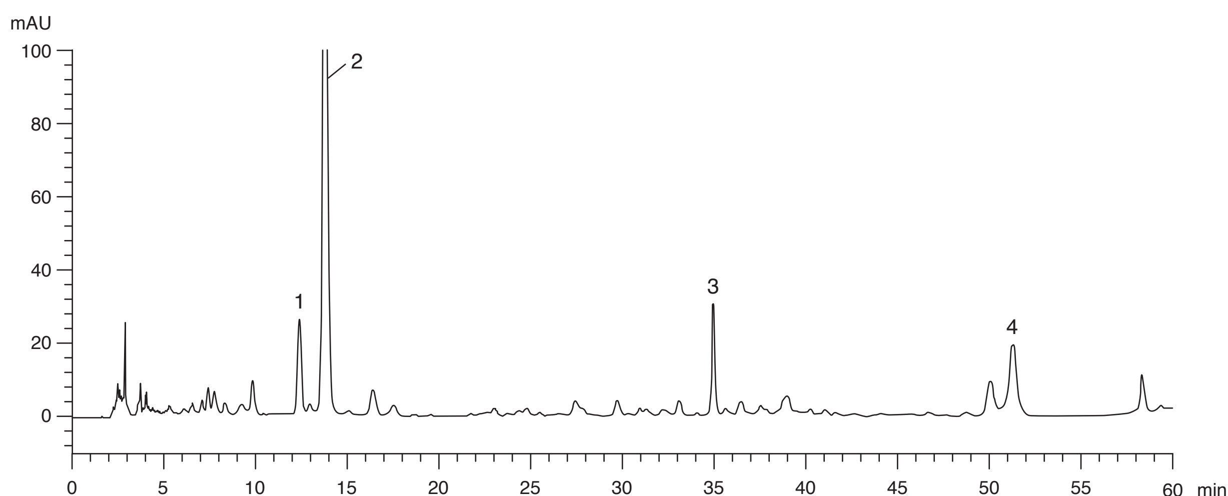

Figure 6 A reference fingerprint chromatogram of Acanthopanacis Senticosi Radix et Rhizoma seu Caulis extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 3.0%.
Ethanol-soluble extractives (cold extraction method): not less than 3.0%.

7. ASSAY

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

Standard solution

Mixed liriodendrin (eleutheroside E) and syringin (eleutheroside B) standard stock solution, Std-Stock (100 mg/L each)

Weigh accurately 1.0 mg of liriodendrin CRS and 1.0 mg of syringin CRS, and dissolve in 10 mL of methanol (50%).

Mixed liriodendrin and syringin standard solution for assay, Std-AS

Measure accurately the volume of the mixed liriodendrin and syringin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 0.5, 1, 2, 10, 50 mg/L for both liriodendrin and syringin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Combine the solutions 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 (206 nm for liriodendrin and 220 nm for syringin) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	89	11	isocratic
15 – 35	89 → 82	11 → 18	linear gradient
35 – 50	82	18	isocratic
50 – 60	82 → 70	18 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed liriodendrin and syringin Std-AS (2 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of liriodendrin and syringin should not be more than 5.0%; the RSD of

the retention times of liriodendrin and syringin peaks should not be more than 2.0%; the column efficiencies determined from liriodendrin and syringin peaks should not be less than 100000 and 10000 theoretical plates respectively.

The *R* value between liriodendrin peak and the closest peak; and the *R* value between syringin 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 liriodendrin and syringin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of liriodendrin and syringin against the corresponding concentrations of the mixed liriodendrin and syringin 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 liriodendrin and syringin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed liriodendrin and syringin Std-AS. The retention times of liriodendrin and syringin 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 liriodendrin and syringin in the test solution, and calculate the percentage contents of liriodendrin and syringin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.024% of liriodendrin ($C_{34}H_{46}O_{18}$) and not less than 0.012% of syringin ($C_{17}H_{24}O_9$), calculated with reference to the dried substance.