Pseudolaricis Cortex

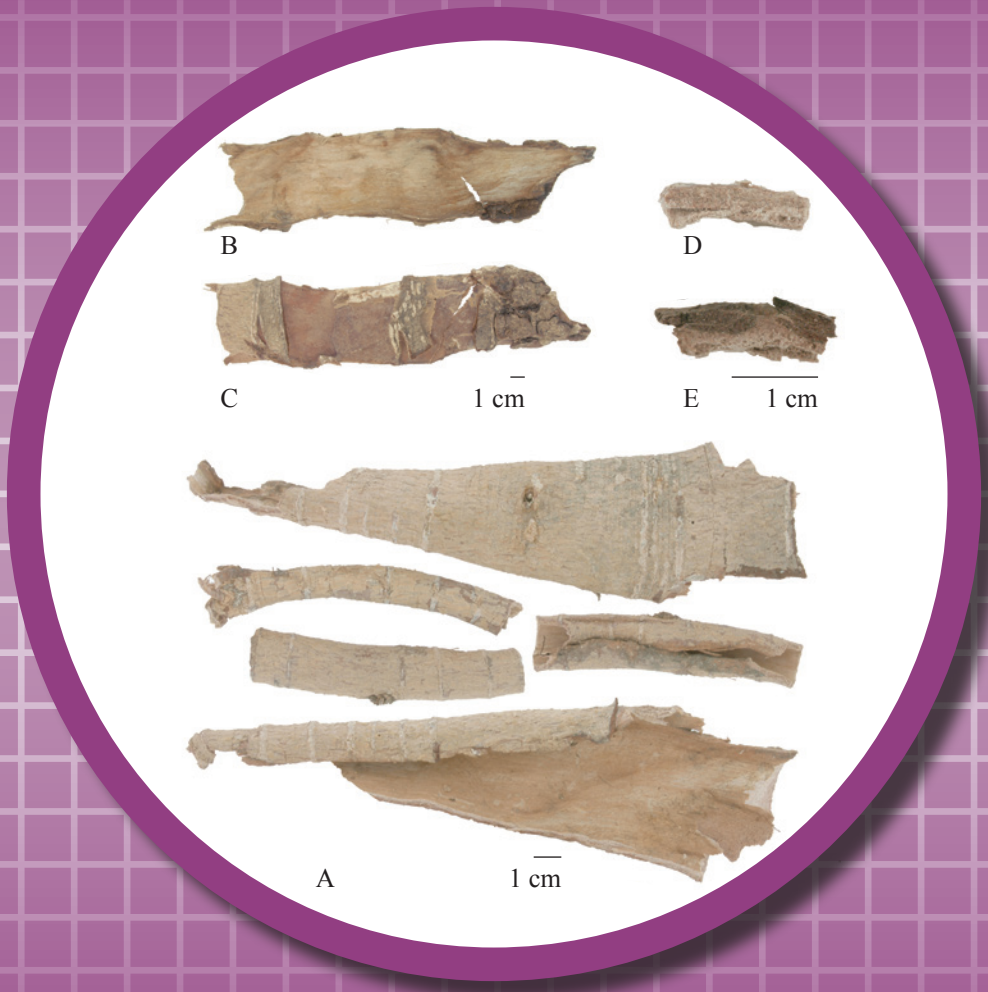

Figure 1 A photograph of Pseudolaricis Cortex

A. Pseudolaricis Cortex
B. Inner surface of stem bark with root bark (root bark near left side end)
C. Outer surface of stem bark with root bark (root bark near left side end)
D. Fracture of root bark E. Fracture of stem bark

1. NAMES

Official Name: Pseudolaricis Cortex

Chinese Name: 土荊皮

Chinese Phonetic Name: Tujingpi

2. SOURCE

Pseudolaricis Cortex is the dried root bark and stem bark just above the root of *Pseudolarix amabilis* (Nelson) Rehd. (Pinaceae). The root bark and stem bark just above the root are collected in summer, foreign matter removed, then dried under the sun to obtain Pseudolaricis Cortex.

3. DESCRIPTION

Root bark: Mostly abundant, irregularly long slat-shaped, twisted and slightly rolled, varying in size, 2-5 mm thick. Outer surface greyish-yellow, rough, wrinkled and greyish-white transversely lenticel-like protuberances, the rugged external tissues frequently scaling off, with reddish-brown exposed surface. Inner surface yellowish-brown to reddish-brown, even, with fine longitudinal striations. Texture tenacious. Fracture splintery, easily detachable in flakes. Odour slight; taste bitter and astringent (Fig. 1).

Stem bark: Occasionally found, usually connected with root bark. Up to about 9 mm thick, the rugged external tissues relatively thick, the outer surface cracked and inner surface relatively rough (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells, up to more than 20 layers, easily fallen off. Cortex cells elongated tangentially. Mucilage cells large, scattered in cortex and phloem. Resin cells with reddish-brown to yellowish-brown resinous substance and prisms of calcium oxalate occasionally found. Stone cells scattered in cortex and phloem. Phloem rays consist of 1 row of cells (Fig. 2).

Powder

Colour pale brown. Stone cells subrectangular, subrounded or irregularly branched, 30-120 μm in diameter, containing yellowish-brown to reddish-brown masses; bright bluish-white or polychromatic under the polarized microscope. Cork cells with slightly thickened walls, sometimes lignified and pitted. Sieve cells mostly in bundles, lateral walls with numerous elliptic sieve areas. Mucilage cells subrounded, 100-300 μm in diameter. Resin cells longitudinally connected into tube-form, containing reddish-brown to yellowish-brown resinous substance, sometimes embedded with prisms of calcium oxalate. Prisms of calcium oxalate visible, 7-28 μm in diameter; polychromatic under the polarized microscope. Starch granules subrounded or elliptic, 6-33 μm in diameter, hilum pointed, cleft-like, stellate or V-shaped; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-3 units (Fig. 3).

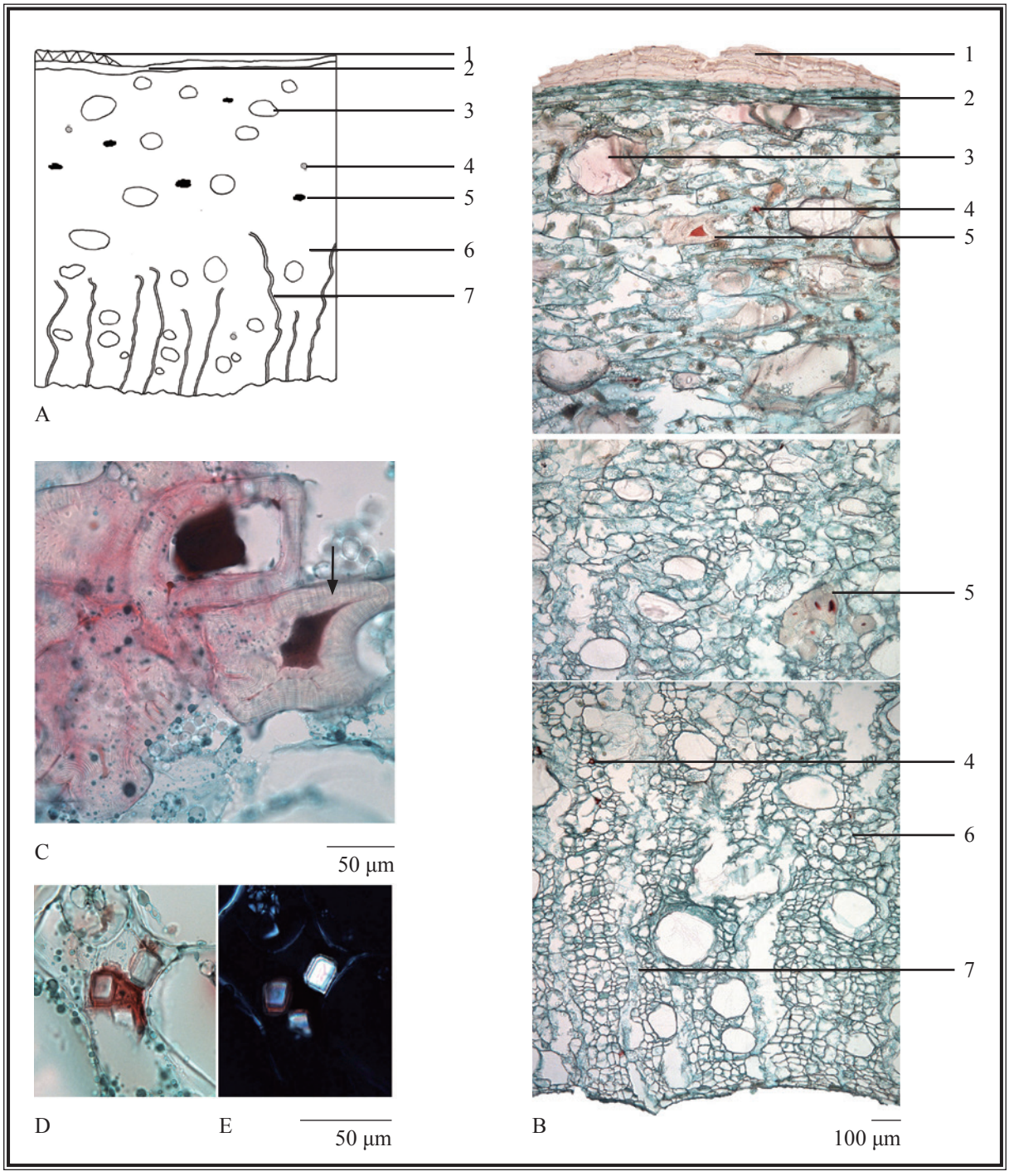

Figure 2 Microscopic features of transverse section of root bark of Pseudolaricis Cortex

A. Sketch B. Section illustration C. Stone cells
D. Resinous cells with reddish-brown substance and prisms of calcium oxalate (under the light microscope)
E. Resinous cells with reddish-brown substance and prisms of calcium oxalate (under the polarized microscope)

1. Cork 2. Cortex 3. Mucilage cells
4. Resinous cells with reddish-brown substance and prisms of calcium oxalate
5. Stone cells 6. Phloem 7. Phloem rays

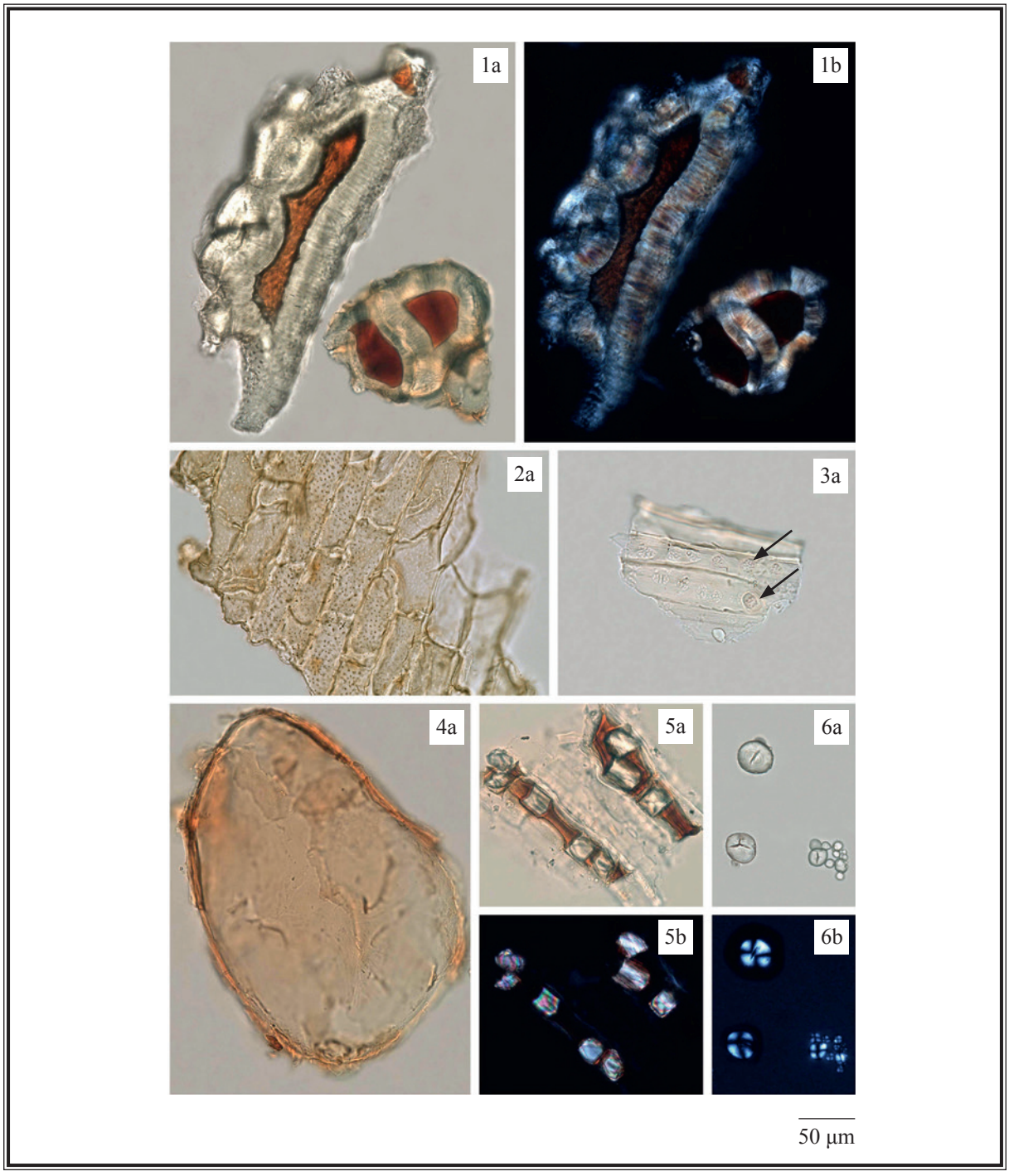

Figure 3 Microscopic features of powder of Pseudolaricis Cortex

1. Stone cells 2. Cork cells (in surface view) 3. Sieve cells (sieve areas ⟶) 4. Mucilage cell
5. Resin cells with reddish-brown substance and prisms 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 solutions

Pseudolaric acid A standard solution

Weigh 1.0 mg of pseudolaric acid A CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Pseudolaric acid B standard solution

Weigh 0.8 mg of pseudolaric acid B CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and formic acid (6:3:0.5, 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 ethanol. Sonicate (270 W) the mixture for 20 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 1 mL of ethanol.

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 pseudolaric acid A standard solution (0.5 µL), pseudolaric acid B standard solution (1 µL) and the test solution (1 µL) to the plate. 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. Spray the plate evenly with the spray reagent and heat at about 105°C (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

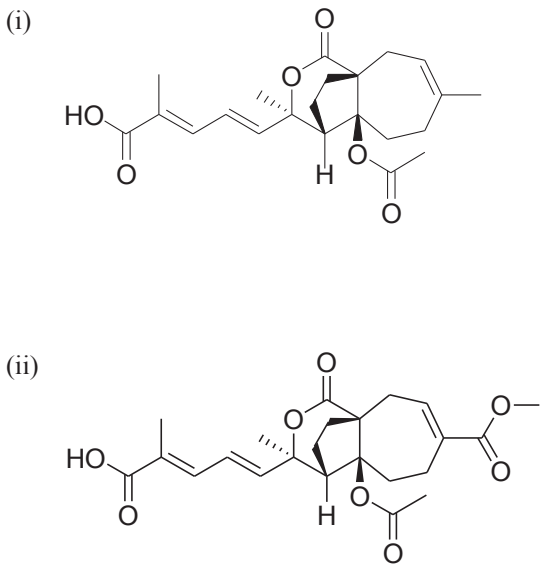

Figure 4 Chemical structures of (i) pseudolaric acid A and (ii) pseudolaric acid B

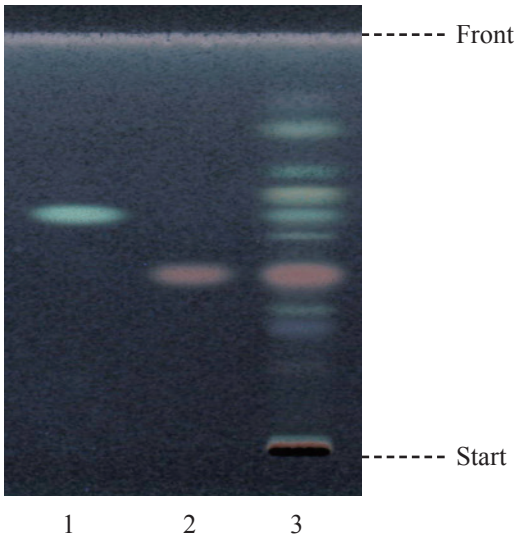

Figure 5 A reference HPTLC chromatogram of Pseudolaricis Cortex extract observed under UV light (366 nm) after staining

1. Pseudolaric acid A standard solution 2. Pseudolaric acid B 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 pseudolaric acid A and pseudolaric acid B (Fig. 5).

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

Standard solutions

Pseudolaric acid A standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.5 mg of pseudolaric acid A CRS and dissolve in 50 mL of ethanol (95%).

Pseudolaric acid B standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 2.5 mg of pseudolaric acid B CRS and dissolve in 50 mL of ethanol (95%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (95%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (95%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (260 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) –

Table 1 Chromatographic system conditions

Time (min)	0.3% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	80 → 10	20 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of pseudolaric acid A Std-FP and pseudolaric acid B Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of pseudolaric acid A and pseudolaric acid B should not be more than 5.0%; the RSD of the retention times of pseudolaric acid A and pseudolaric acid B peaks should not be more than 2.0%; the column efficiencies determined from pseudolaric acid A and pseudolaric acid B peaks should not be less than 130000 and 60000 theoretical plates respectively.

The *R* value between peak 4 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 pseudolaric acid A Std-FP, pseudolaric acid B Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of pseudolaric acid A and pseudolaric acid B peaks in the chromatograms of pseudolaric acid A Std-FP, pseudolaric acid B Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify pseudolaric acid A and pseudolaric acid B peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of pseudolaric acid A Std-FP and pseudolaric acid B Std-FP. The retention times of pseudolaric acid A and pseudolaric acid B 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 Pseudolaricis Cortex extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Pseudolaricis Cortex extract

Peak No.	RRT	Acceptable Range
1	0.66	± 0.04
2	0.70	± 0.03
3	0.89	± 0.03
4 (marker, pseudolaric acid B)	1.00	-
5 (pseudolaric acid A)	1.24	± 0.03

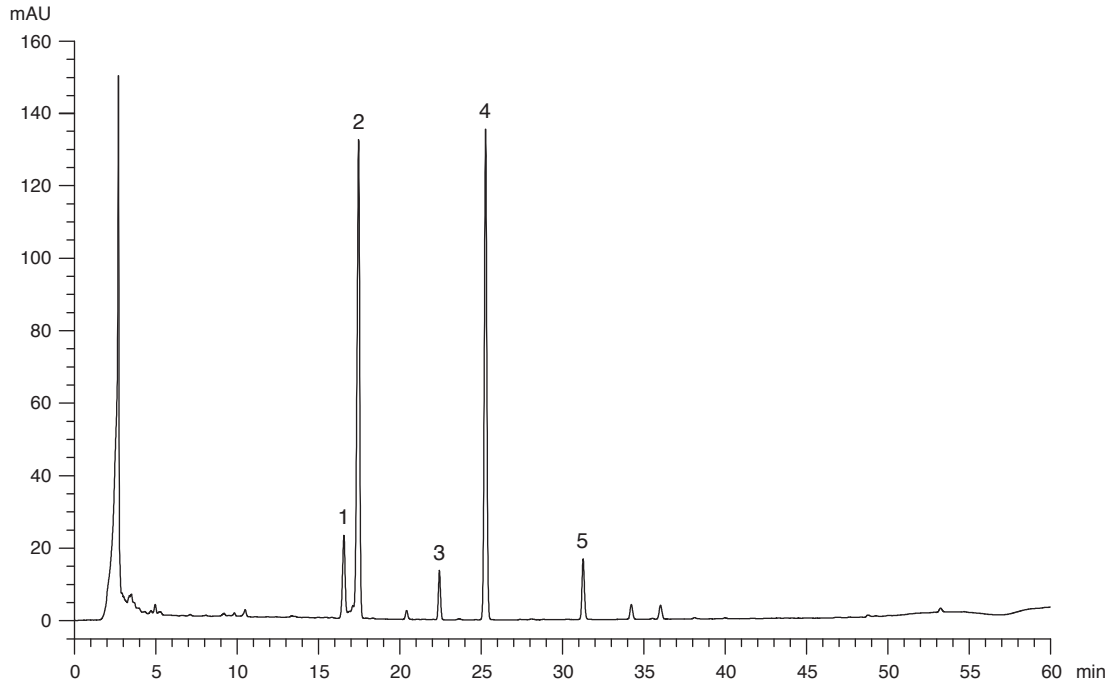

Figure 6 A reference fingerprint chromatogram of Pseudolaricis Cortex extract

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Mixed pseudolaric acid A and pseudolaric acid B standard stock solution, Std-Stock (400 mg/L for pseudolaric acid A and 2000 mg/L for pseudolaric acid B)

Weigh accurately 0.8 mg of pseudolaric acid A CRS and 4.0 mg of pseudolaric acid B CRS, and dissolve in 2 mL of ethanol (95%).

Mixed pseudolaric acid A and pseudolaric acid B standard solution for assay, Std-AS

Measure accurately the volume of the mixed pseudolaric acid A and pseudolaric acid B Std-Stock, dilute with ethanol (95%) to produce a series of solutions of 0.2, 1, 2, 5, 10 mg/L for pseudolaric acid A and 1, 10, 25, 50, 100 mg/L for pseudolaric acid B.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (95%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (95%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (260 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

Time (min)	0.3% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	80 → 10	20 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed pseudolaric acid A and pseudolaric acid B Std-AS (2 mg/L for pseudolaric acid A and 25 mg/L for pseudolaric acid B). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of

pseudolaric acid A and pseudolaric acid B should not be more than 5.0%; the RSD of the retention times of pseudolaric acid A and pseudolaric acid B peaks should not be more than 2.0%; the column efficiencies determined from pseudolaric acid A and pseudolaric acid B peaks should not be less than 130000 and 60000 theoretical plates repectively.

The *R* value between pseudolaric acid A peak and the closest peak; and the *R* value between pseudolaric acid B 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 pseudolaric acid A and pseudolaric acid B Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of pseudolaric acid A and pseudolaric acid B against the corresponding concentrations of the mixed pseudolaric acid A and pseudolaric acid B Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify pseudolaric acid A and pseudolaric acid B peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed pseudolaric acid A and pseudolaric acid B Std-AS. The retention times of pseudolaric acid A and pseudolaric acid B 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 pseudolaric acid A and pseudolaric acid B in the test solution, and calculate the percentage contents of pseudolaric acid A and pseudolaric acid B in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.30% of the total content of pseudolaric acid A ($C_{22}H_{28}O_6$) and pseudolaric acid B ($C_{23}H_{28}O_8$), calculated with reference to the dried substance.