Albiziae Cortex

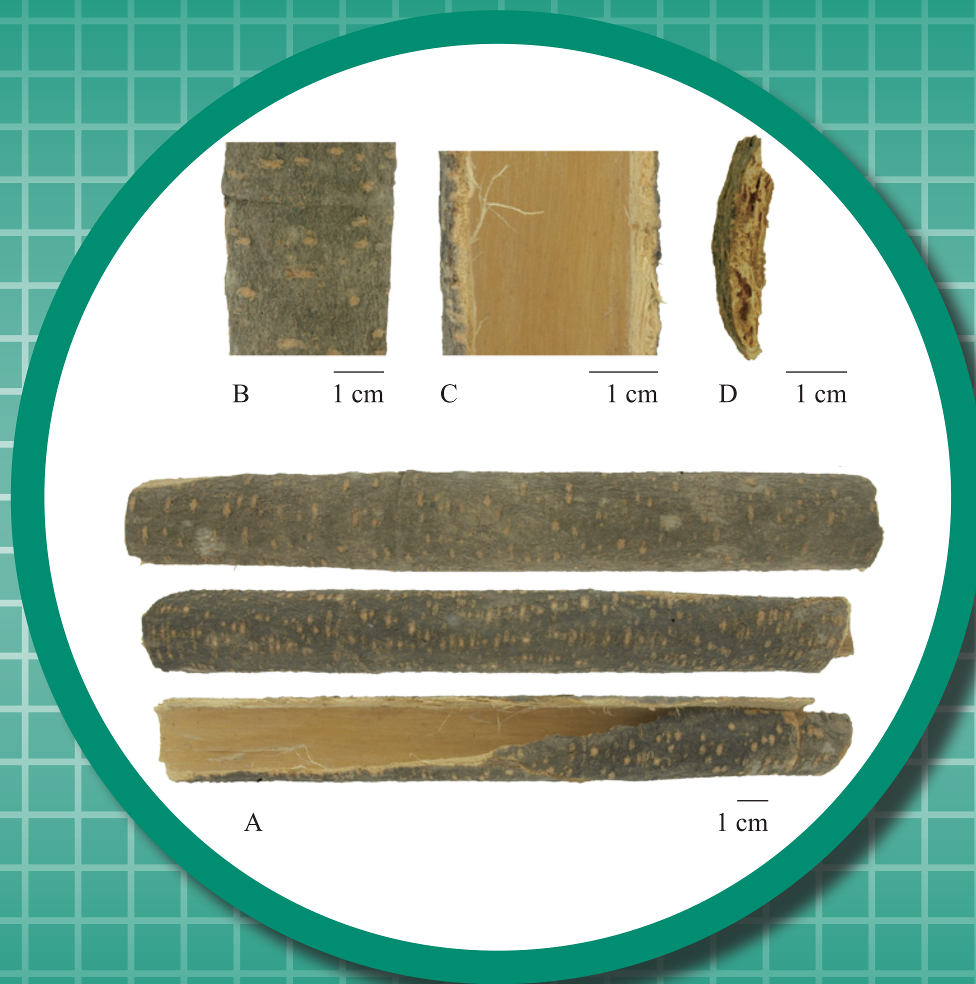

Figure 1 A photograph of Albiziae Cortex

A. Albiziae Cortex B. Outer surface of stem bark

C. Inner surface of stem bark D. Transverse section of stem bark

1. NAMES

Official Name: Albiziae Cortex

Chinese Name: 合歡皮

Chinese Phonetic Name: Hehuanpi

2. SOURCE

Albiziae Cortex is the dried stem bark of *Albizia julibrissin* Durazz. (Fabaceae). The stem bark is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Albiziae Cortex.

3. DESCRIPTION

Quilled or semi-quilled pieces, 40-80 cm long, 1-3 mm thick. Externally greyish-brown, sometimes longitudinally wrinkled or shallowly fissured, with distinct transverse elliptical lenticels, brown to reddish-brown, occasionally showing protuberant transverse ridges or relatively large rounded branch scars, and usually covered with patches of lichens. The inner surface pale yellowish-brown to yellowish-white, smooth, with fine and dense longitudinal striations. Texture hard and fragile, easily broken. Fracture laminate-fibrous, pale yellowish-brown to yellowish-white. Odour slightly aromatic; taste slightly astringent and irritating to the tongue, followed by the discomfort of throat (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork cells varying in layers, up to more than 20 layers. Cortex consists of tangentially prolonged cells, scattered with groups of stone cells and prisms of calcium oxalate; most of stone cells in groups. A band of stone cell groups intermittently located around pericycle position. The cells contain prisms of calcium oxalate densely distributed around stone cells and fibre bundles. Phloem rays 1-4 rows of cells wide, slightly curved. Stone cells scattered in outer part of phloem. Phloem fibres in bundles, arranged in more than 10 rows discontinuously, surrounded by parenchymatous cells which contain prisms of calcium oxalate, forming crystal fibres (Fig. 2).

Powder

Colour pale yellow. Fibres usually in bundles, slender, 7-22 µm in diameter, surrounding by parenchymatous cells which contain prisms of calcium oxalate, forming crystal fibres; polychromatic under the polarized microscope. Phloem parenchymatous cells subrectangular or irregular, walls thickened or beaded-thickened, pits visible. Prisms of calcium oxalate up to 18 µm in diameter; bright white or polychromatic under the polarized microscope. Stone cells suboblong, subrounded, rectangular or irregular, 16-58 µm in diameter, walls relatively thickened, pit canals distinct, some branched; bright white under the polarized microscope. Cork cells pale yellowish-brown, polygonal in surface view (Fig. 3).

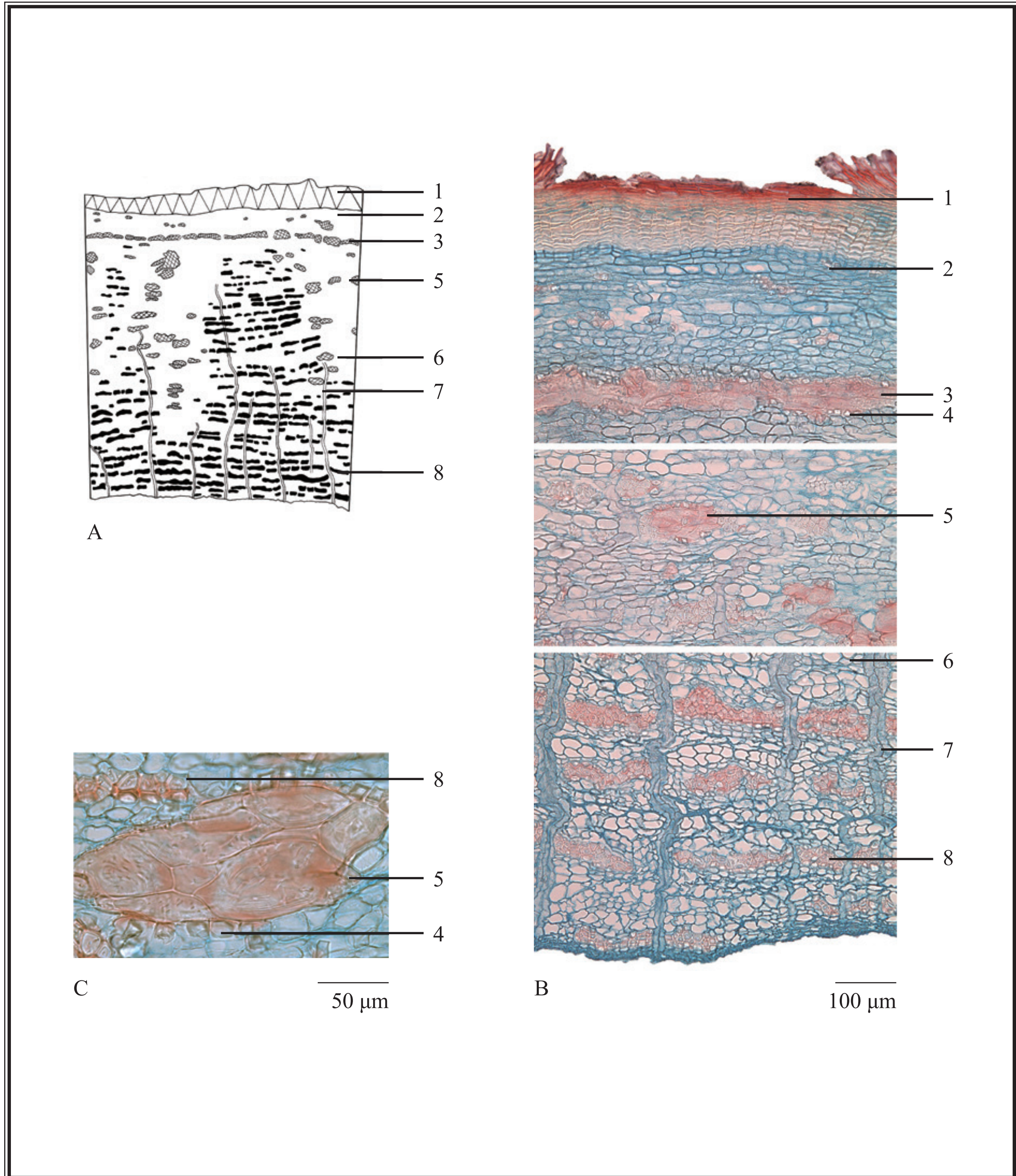

Figure 2 Microscopic features of transverse section of Albiziae Cortex

A. Sketch B. Section illustration C. Stone cells, prisms of calcium oxalate and fibres

1. Cork 2. Cortex 3. Stone cell group band 4. Prisms of calcium oxalate
5. Stone cells 6. Phloem 7. Phloem rays 8. Fibre bundles

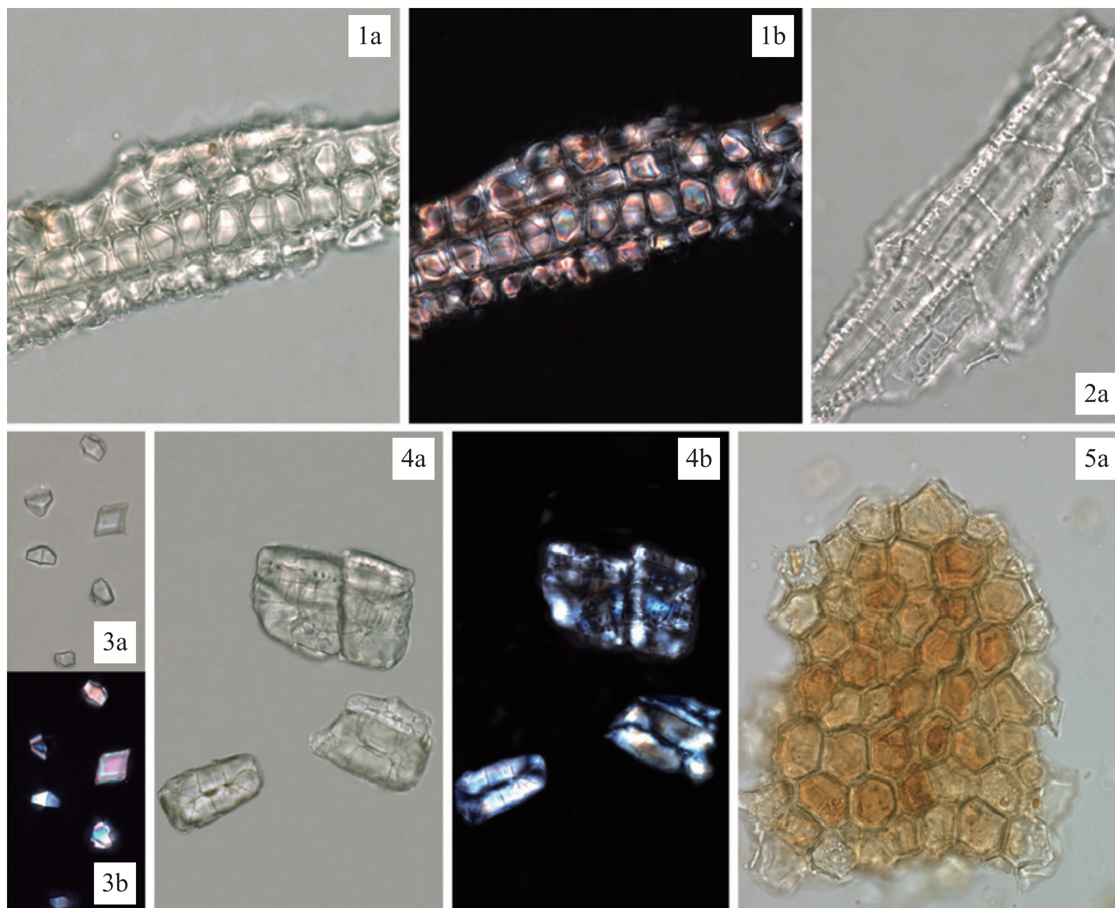

Figure 3 Microscopic features of powder of Albiziae Cortex

1. Crystal fibres 2. Phloem parenchymatous cells 3. Prisms of calcium oxalate 4. Stone cells
5. Cork cells (in surface view)

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

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

Standard solution

(-)-Syringaresinol-4-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside standard solution

Weigh 0.5 mg of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside CRS (Fig. 4) and dissolve in 1 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (7:3:1, v/v). Keep in a refrigerator at a temperature below 10ºC for at least 10 h. Mix 10 mL of the lower layer with 0.1 mL of formic acid.

Spray reagent

Weigh 2.5 g of phosphomolybdic acid hydrate and dissolve in 50 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (50%). Sonicate (270 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 0.5 mL of ethanol (50%).

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 (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside standard solution (1 μL) and the test solution (2 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. Spray the plate evenly with the spray reagent and heat at about 110ºC until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

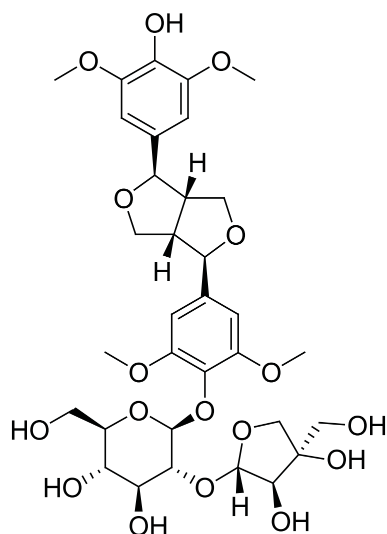

Figure 4 Chemical structure of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside

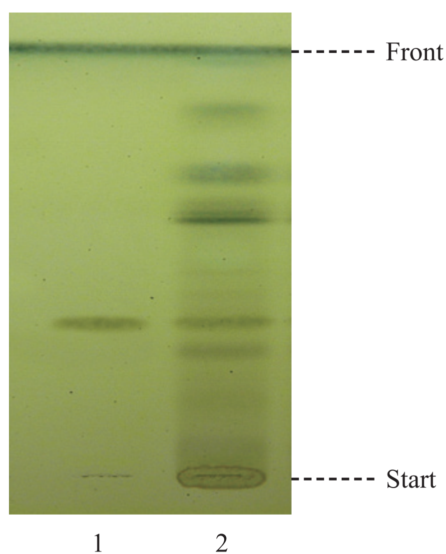

Figure 5 A reference HPTLC chromatogram of Albiziae Cortex extract observed under visible light after staining

1. (-)-Syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside 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 (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside (Fig. 5).

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

Standard solution

(-)-Syringaresinol-4-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 2.5 mg of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside CRS and dissolve in 50 mL of methanol (50%).

Test solution

Weigh 2.0 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 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 methanol (50%). Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (204 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 0.8 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 – 40	82 → 60	18 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside should not be more than 5.0%; the RSD of the retention time of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside peak should not be more than 2.0%; the column efficiency determined from (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside peak should not be less than 20000 theoretical plates.

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

Procedure

Separately inject (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside peak in the chromatogram of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-FP. The retention times of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside 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 three characteristic peaks of Albiziae Cortex extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Albiziae Cortex extract

Peak No.	RRT	Acceptable Range
1	0.56	± 0.03
2 [marker, (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside]	1.00	-
3	1.92	± 0.05

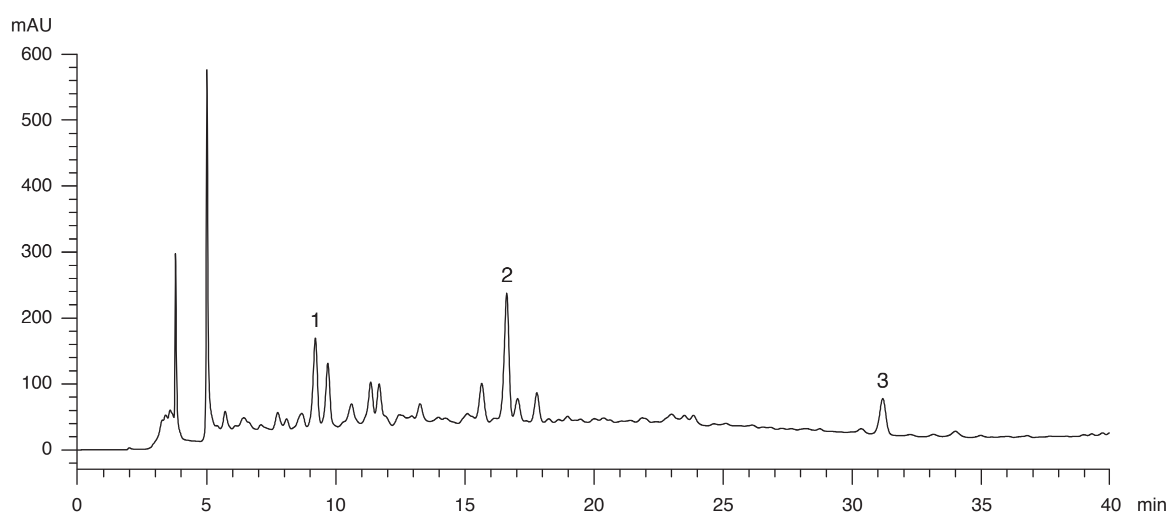

Figure 6 A reference fingerprint chromatogram of Albiziae Cortex extract

For positive identification, the sample must give the above three 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 6.0%.
Acid-insoluble ash: not more than 1.0%.

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 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

Standard solution

(-)-Syringaresinol-4-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside CRS and dissolve in 5 mL of methanol (50%).

(-)-Syringaresinol-4-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside standard solution for assay, Std-AS

Measure accurately the volume of the (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-Stock, dilute with methanol (50%) to produce a series of solutions of 5, 10, 25, 50, 70 mg/L for (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside.

Test solution

Weigh accurately 2.0 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 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 methanol (50%). Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (204 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 0.8 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 – 40	82 → 60	18 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-AS (25 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside should not be more than 5.0%; the RSD of the retention time of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside peak should not be more than 2.0%; the column efficiency determined from (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside peak should not be less than 20000 theoretical plates.

The *R* value between (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside 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 (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside against the corresponding concentrations of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside 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 (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside Std-AS. The retention times of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside 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 (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside in the test solution, and calculate the percentage content of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.030% of (-)-syringaresinol-4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside ($C_{33}H_{44}O_{17}$), calculated with reference to the dried substance.