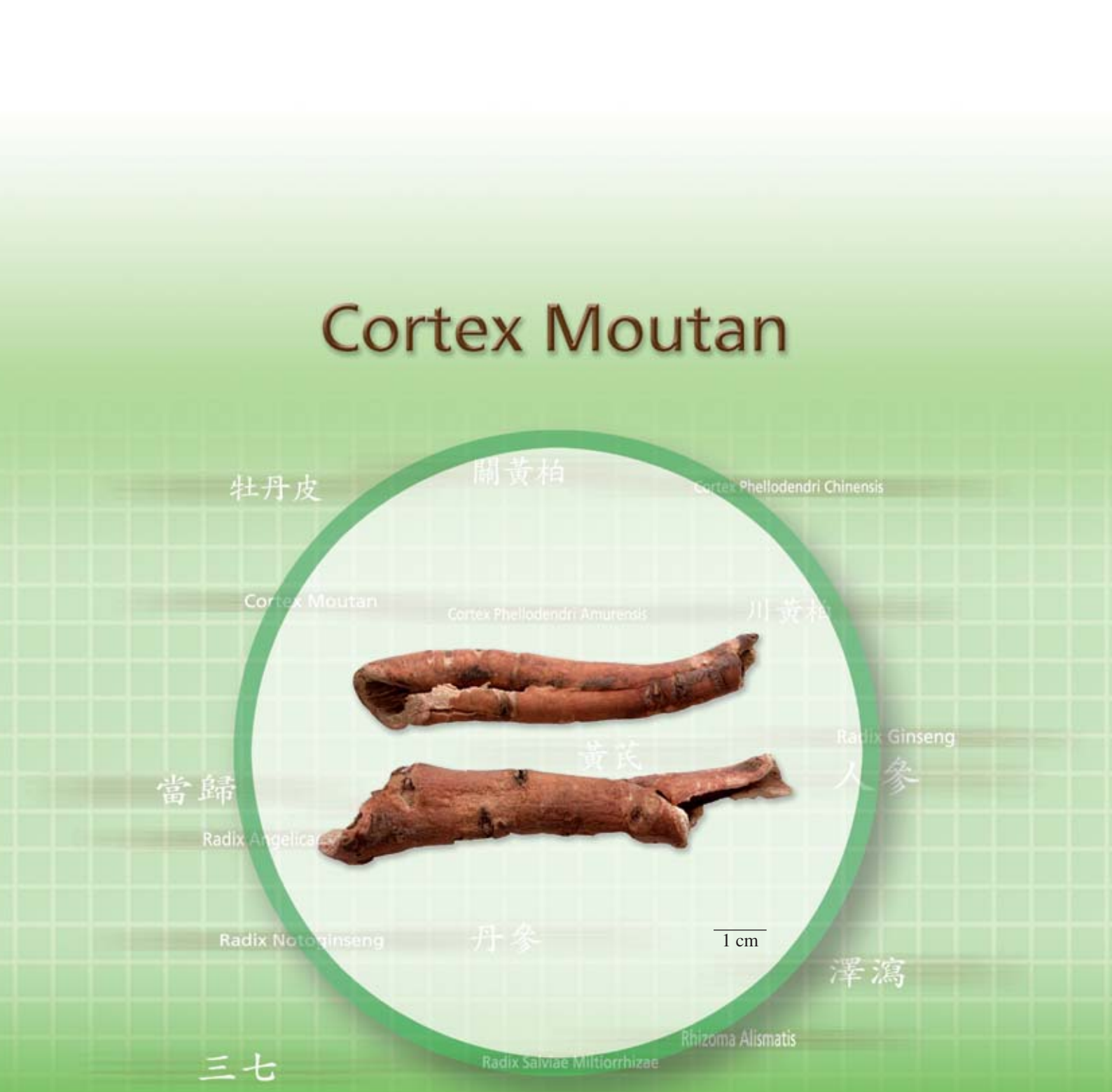

Figure 1　A photograph of Cortex Moutan

1. NAMES

Official Name: Cortex Moutan

Chinese Name: 牡丹皮

Chinese Phonetic Name: Mudanpi

2. SOURCE

Cortex Moutan is the dried root bark of *Paeonia suffruticosa* Andr. (Fam. Ranunculaceae). The root is collected in autumn, the rootlets removed, the bark stripped off, then dried in the sun to obtain Cortex Moutan.

3. DESCRIPTION

Quailed or semi-quailed shape, longitudinally fissured, both edges of the cortex usually curved inwards, varying in size and up to 20 cm in length, 5–16 mm in diameter, 1–4.5 mm in thickness. Externally dark brown or yellowish-brown, showing transverse lenticels, outer surface reddish-brown, pink or yellow-white after the removal of cork; the inner surface pale greyish-yellow or pale brown, with obvious fine longitudinal striation, usually showing bright crystals. Texture hard and fragile, easily broken, fracture relatively even, pale pink or greyish-white, and mealy. Odour, aromatic; taste, slightly bitter and astringent. (Fig. 1)

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The transverse section shows cork consisting of several rows of cells. Cortex consisting of 10–21 rows of parenchyma, which are prolonged tangentially. Pholem broad, occupying up to 80% in radial direction of transverse section; phloem rays 1–3 cells wide. Clusters of calcium oxalate, 6–50 μm in diameter, sometimes crystal cells joined, arranged in rows, or one to several clusters in one cell. (Fig. 2)

Powder

White to greyish-brown. Fairly abundant starch grains present in parenchymatous cells; simple granules suborbicular or polygonal, 3–27 μm in diameter, with dotted, cleft or V-shaped hilum; compound granules composed of 2–6 units, showing a black, cross shape when examined under a polarizing microscope. Clusters of calcium oxalate 6–50 μm in diameter, crystal cells sometimes joined, arranged in rows or one to several clusters in one cell; showing a polychrome when examined under a polarizing microscope. Cork cells rectangular, slightly thick-walled. (Fig. 3)

4.2 Physicochemical Identification

Procedure

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of diethyl ether. Shake the mixture for 2 min. Filter and transfer 5 mL of the filtrate to a test tube. Evaporate to dryness on a water bath at about 60 ºC, then cool to room temperature. Cautiously add about 1 mL of nitric acid to the test tube, a clear greenish-blue solution is observed.

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

Standard solutions

Paeoniflorin standard solution

Weigh 2.0 mg of paeoniflorin and dissolve in 1 mL of methanol.

Paeonol standard solution

Weigh 2.0 mg of paeonol and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of chloroform, ethyl acetate, methanol and formic acid (40:5:10:0.2, v/v).

Spray reagent

Mix 1 mL of dilute sulphuric acid (50%, v/v) and 10 mL of *p*-hydroxybenzaldehyde in methanol (2%, w/v). Freshly prepare the reagent.

Test solution

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of methanol. Sonicate the mixture for 30 min, filter and then evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

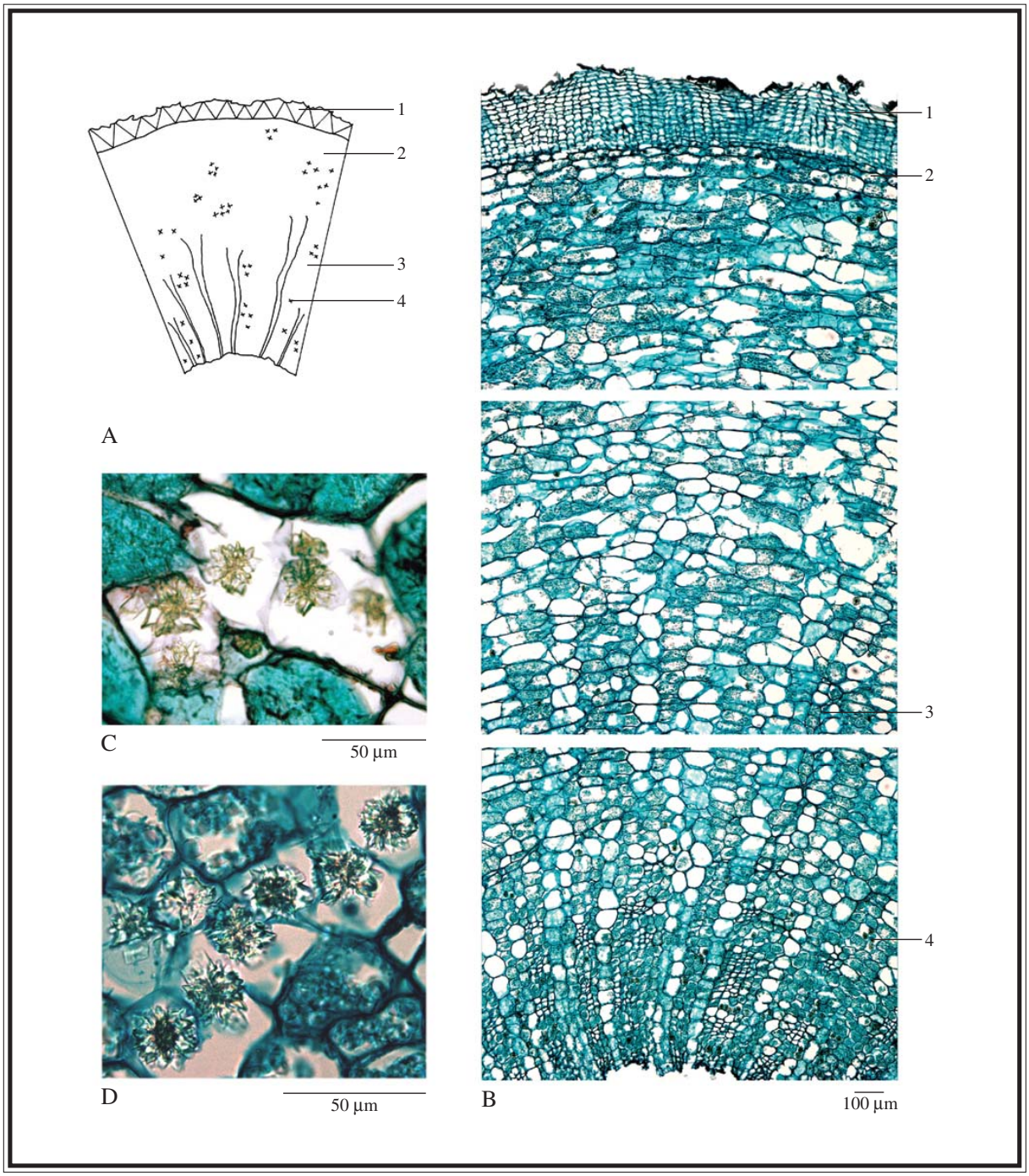

Figure 2 Microscopic features of transverse section of Cortex Moutan

A. Sketch B. Section illustration C. Clusters of calcium oxalate scattered
D. Clusters of calcium oxalate arranged in a row

1. Cork 2. Cortex 3. Phloem 4. Clusters of calcium oxalate

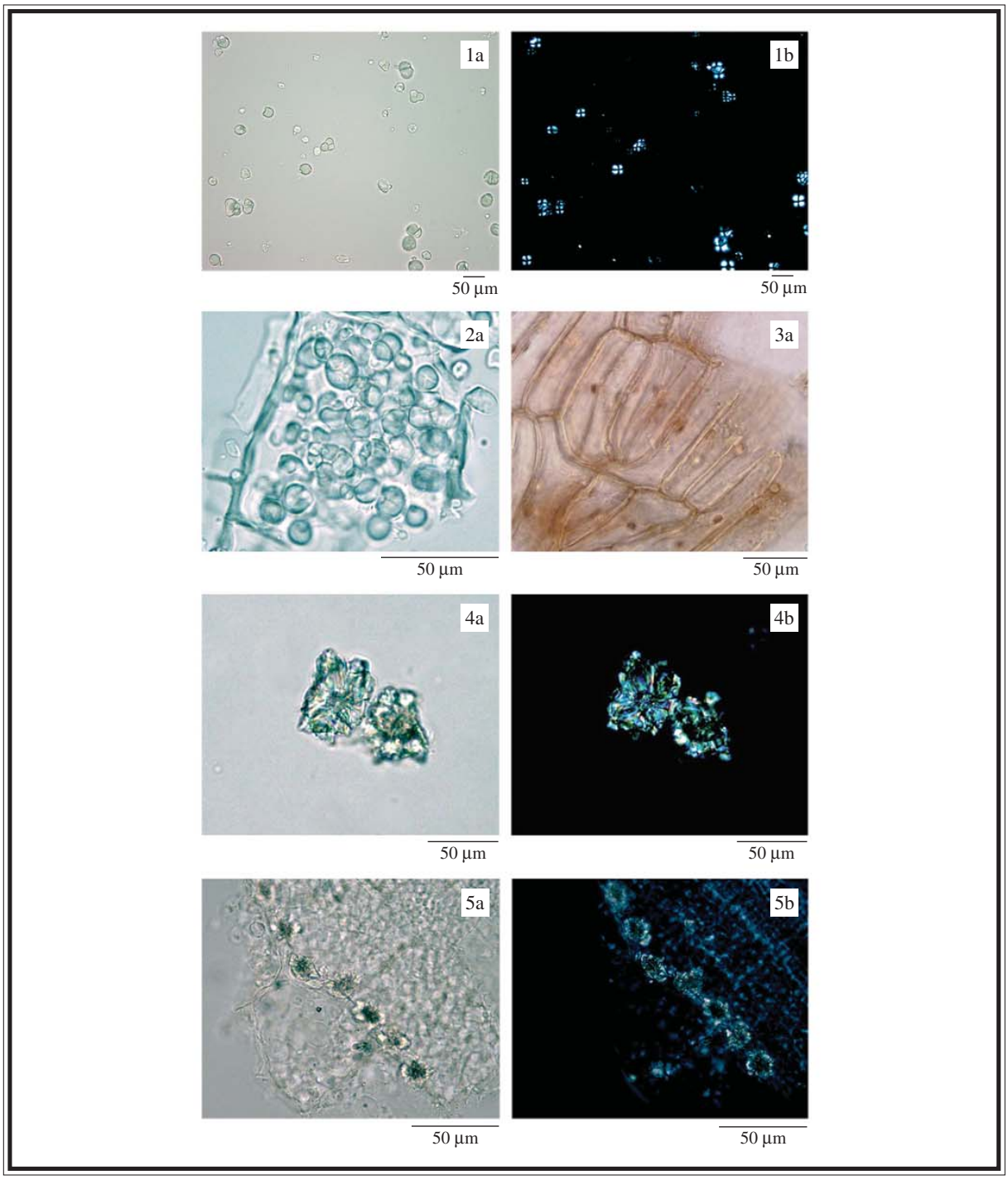

Figure 3 Microscopic features of powder of Cortex Moutan

1. Starch grains 2. Starch grains in parenchymatous cells 3. Cork cells
4. Clusters of calcium oxalate 5. Clusters of calcium oxalate arranged in a row

a. Features under a light microscope b. Features under a polarizing microscope

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Develop over a path of about 4 cm. Apply separately paeoniflorin standard solution (3 µL), paeonol standard solution (5 µL) and the test solution (2 µL) to the plate. 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 125 ºC for about 15 min. Examine the plate in 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 paeoniflorin and paeonol.

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

Standard solution

Paeonol standard stock solution, Std-Stock (2000 mg/L)

Weigh 4.0 mg of paeonol and dissolve in 2 mL of methanol.

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

Pipette 0.5 mL of paeonol Std-Stock to a 5-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol and weigh. Sonicate the mixture for 30 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 3000 x *g* for 5 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (254 nm) and a column (4.6 x 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 –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–20	100 ➔ 80	0 ➔ 20	linear gradient
20–40	80 ➔ 70	20 ➔ 30	linear gradient
40–60	70 ➔ 50	30 ➔ 50	linear gradient

System suitability requirements

Perform at least five replicate injections each with 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 be not more than 5.0%; the RSD of the retention time of paeonol peak should be not more than 2.0%; the column efficiency determined from paeonol peak should be not less than 50,000 theoretical plates.

The *R* value between peaks 3 and 4 (Fig. 4) in the test solution should be not less than 2.0.

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. 4) in the chromatogram of the test solution. Under the same HPLC conditions, identify paeonol peaks 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 Cortex Moutan extract are listed in Table 3.

Table 3 The RRTs and acceptable ranges of the five characteristic peaks of Cortex Moutan extract

Peak No.	RRT	Acceptable Range
1	0.36	±0.03
2 (paeoniflorin)	0.45	±0.03
3	0.71	±0.03
4	0.77	±0.03
5 (marker, paeonol)	1.00	-

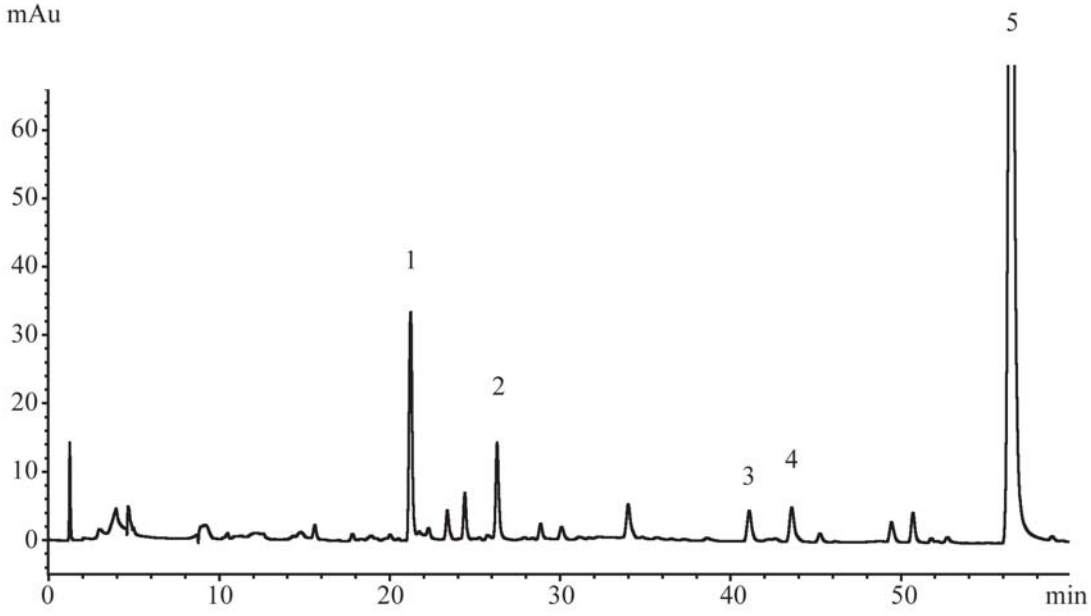

Figure 4 A reference fingerprint chromatogram of Cortex Moutan extract

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. 4).

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 XIV)*: meet the requirements.

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

5.6 **Ash** *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 **Water Content** *(Appendix X)*: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 19.0%.
Ethanol-soluble extractives (cold extraction method): not less than 18.0%.

7. ASSAY

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

Standard solution

Mixed paeoniflorin and paeonol standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 10.0 mg of paeoniflorin and 10.0 mg of paeonol, and dissolve in 10 mL of methanol.

Mixed paeoniflorin and paeonol standard solution for assay, Std-AS

Measure accurately the volume of the mixed paeoniflorin and paeonol Std-Stock, dilute with methanol to produce a series of solutions of 5, 25, 100, 200, 400 mg/L for both paeoniflorin and paeonol.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add accurately 10 mL of methanol and weigh. Sonicate the mixture for 30 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 3000 x *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (230 nm) and a column (4.6 x 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 –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–10	90 → 70	10 → 30	linear gradient
10–20	70 → 20	30 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of the mixed paeoniflorin and paeonol Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of paeoniflorin and paeonol should be not more than 5.0%; the RSD of the retention times of

paeoniflorin peak and paeonol peak should be not more than 2.0%; the column efficiency determined from paeoniflorin peak and paeonol peak should be not less than 50,000 theoretical plates.

The *R* value between paeoniflorin peak and the closest peak in the test solution should be not less than 1.5.

Calibration curves

Inject a series of the mixed paeoniflorin and paeonol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of both paeoniflorin and paeonol against the corresponding concentrations of the mixed paeoniflorin and paeonol 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 paeoniflorin peak and paeonol peak in the chromatogram of the test solution by comparing their retention times with those in chromatogram of the mixed paeoniflorin and paeonol Std-AS. The retention times of paeoniflorin peaks and paeonol peaks in both chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of paeoniflorin and paeonol in the test solution, and calculate the percentage contents of paeoniflorin and paeonol in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.49% of paeoniflorin ($C_{23}H_{28}O_{11}$) and not less than 1.1% of paeonol ($C_9H_{10}O_3$), calculated with reference to the dried substance.