

Radix Paeoniae Rubra



Figure 1(i) A photograph of dried root of *Paeonia lactiflora* Pall.



Figure 1(ii) A photograph of dried root of *Paeonia veitchii* Lynch

1. NAMES

Official Name: Radix Paeoniae Rubra

Chinese Name: 赤芍

Chinese Phonetic Name: Chishao

2. SOURCE

Radix Paeoniae Rubra is the dried root of *Paeonia lactiflora* Pall. or *Paeonia veitchii* Lynch (Ranunculaceae). The root is collected in the spring and autumn; after removal of the rhizomes, rootlets and soil, the root is dried under the sun to obtain Radix Paeoniae Rubra.

3. DESCRIPTION

Cylindrical, slightly curved, 4-40 cm long, 5-30 mm in diameter. Externally brown or reddish-brown, rough, longitudinally furrowed and wrinkled, and showing rootlet scars and transversely prominent lenticels, sometimes the outer bark easily exfoliated. Texture hard and fragile, easily broken; fracture chalk-white or pink, bark narrow, wood with distinct radial striations, sometimes with clefts. Odour slightly fragrant; taste slightly bitter, sour and astringent [Fig. 1(i) and (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

***Paeonia lactiflora* Pall. :** The cork consisting of several layers of brown cells. Parenchyma cells in the cortex elongated tangentially. Phloem narrow. Cambium in a ring. Xylem rays consist of 7 to more than 30 rows of cells; vessels, xylem fibres and parenchyma cells tangentially and alternately arranged; central xylem mostly appearing as diarch to polyarch. Parenchyma cells contain cluster crystals of calcium oxalate and starch grains [Fig. 2(i)].

***Paeonia veitchii* Lynch :** Cork consisting of several layers of brown cells. Parenchyma cells in the cortex elongated tangentially. Cambium in a ring. Xylem rays consist of 3-21 rows of cells;

vessels arranged radially, accompanied by fewer xylem fibres. Parenchyma cells contain cluster crystals of calcium oxalate and starch grains [Fig. 2(ii)].

Powder

Colour greyish-white to pale brown. Simple starch granules subglobular or ellipsoid, showing a black, cross shape when examined under the polarized microscope. Cluster crystals of calcium oxalate 5-46 μm in diameter, crystal cells sometimes joined, arranged in rows or one to several cluster crystals in one cell, showing polychrome when examined under the polarized microscope. Xylem fibres long-fusiform with thickened and slightly lignified wall, with oblique or cross pits. Bordered-pitted or reticulate vessels 11-80 μm in diameter. Cork cells brown or reddish-brown, striped, rectangular or polygonal, the wall slightly thickened, straight or slightly sinuous [Fig. 3(i) and (ii)].

4.2 Physicochemical Identification

Procedure

Weigh 0.5 g of the powdered sample and put into a 25-mL conical flask, then add 10 mL of water. Heat the mixture to boil, then cool down to room temperature. Transfer the mixture to a 15-mL centrifuge tube and centrifuge at about $3000 \times g$ for 5 min. Transfer 1 mL of the supernatant to a test tube. Add 1 drop of iron (III) chloride solution (5%, w/v) to the mixture and mix well. A dark blue or dark green solution is observed (make appropriate dilution with water to observe where necessary).

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

Standard solution

Paeoniflorin standard solution

Weigh 2.0 mg of paeoniflorin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate, methanol and formic acid (250:25:50:1, 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.

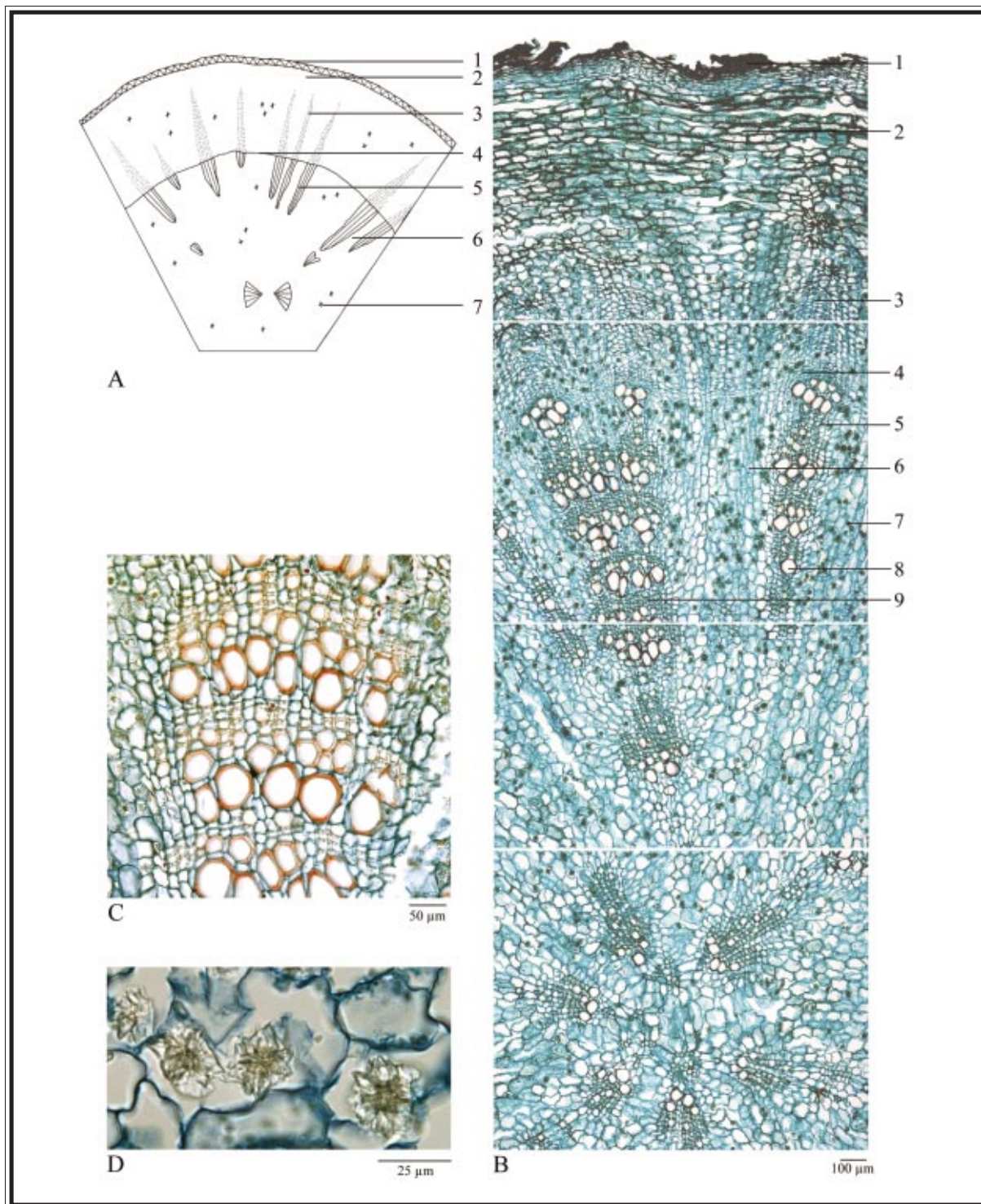


Figure 2(i) Microscopic features of transverse section of dried root of *Paeonia lactiflora* Pall.

A. Sketch B. Section illustration C. Part of xylem D. Cluster crystals of calcium oxalate

- 1. Cork 2. Cortex 3. Phloem 4. Cambium 5. Xylem 6. Rays 7. Cluster crystals of calcium oxalate
- 8. Vessels 9. Xylem fibres

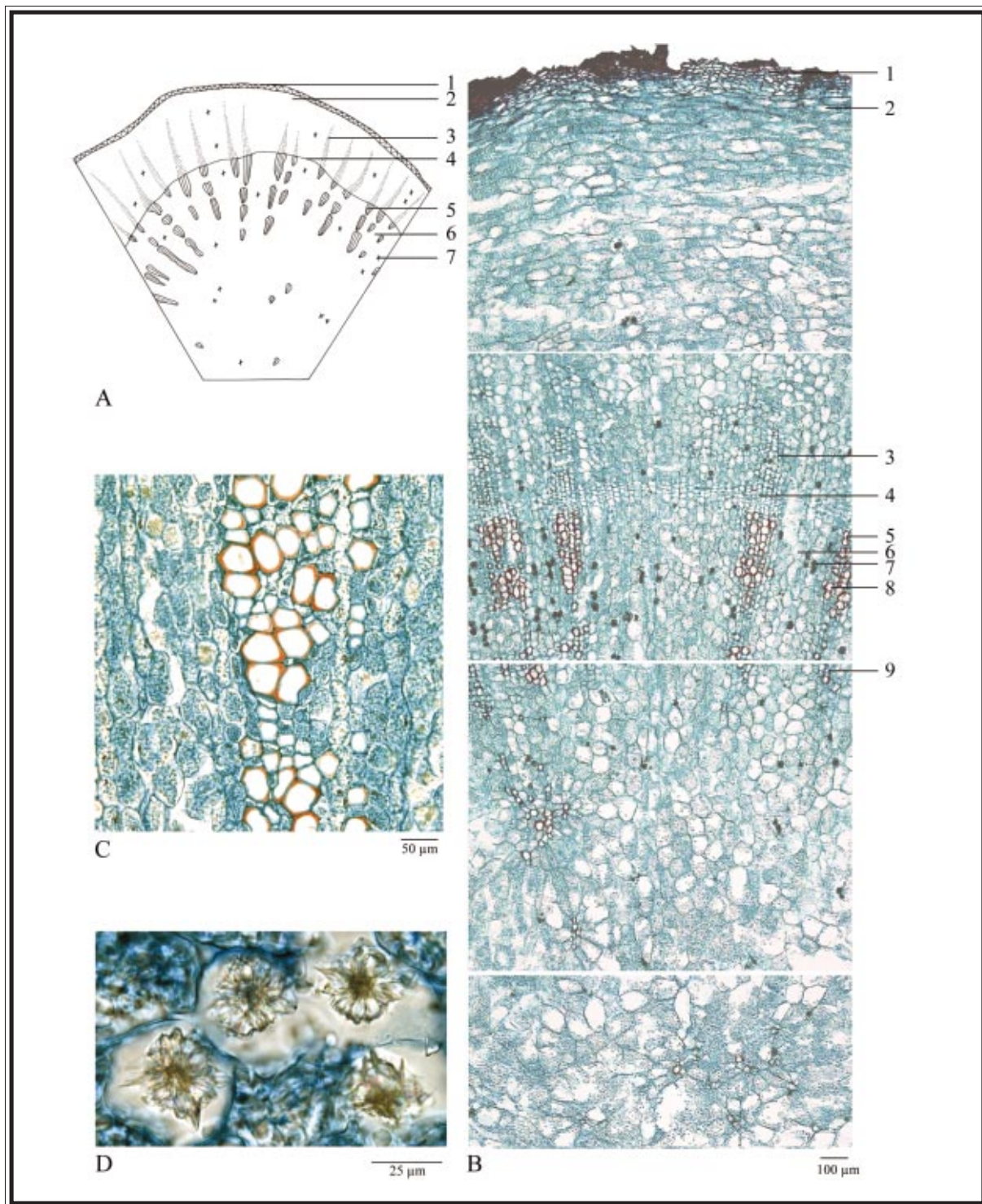


Figure 2(ii) Microscopic features of transverse section of dried root of *Paeonia veitchii* Lynch

A. Sketch B. Section illustration C. Part of xylem D. Cluster crystals of calcium oxalate

1. Cork
2. Cortex
3. Phloem
4. Cambium
5. Xylem
6. Rays
7. Cluster crystals of calcium oxalate
8. Vessels
9. Xylem fibres

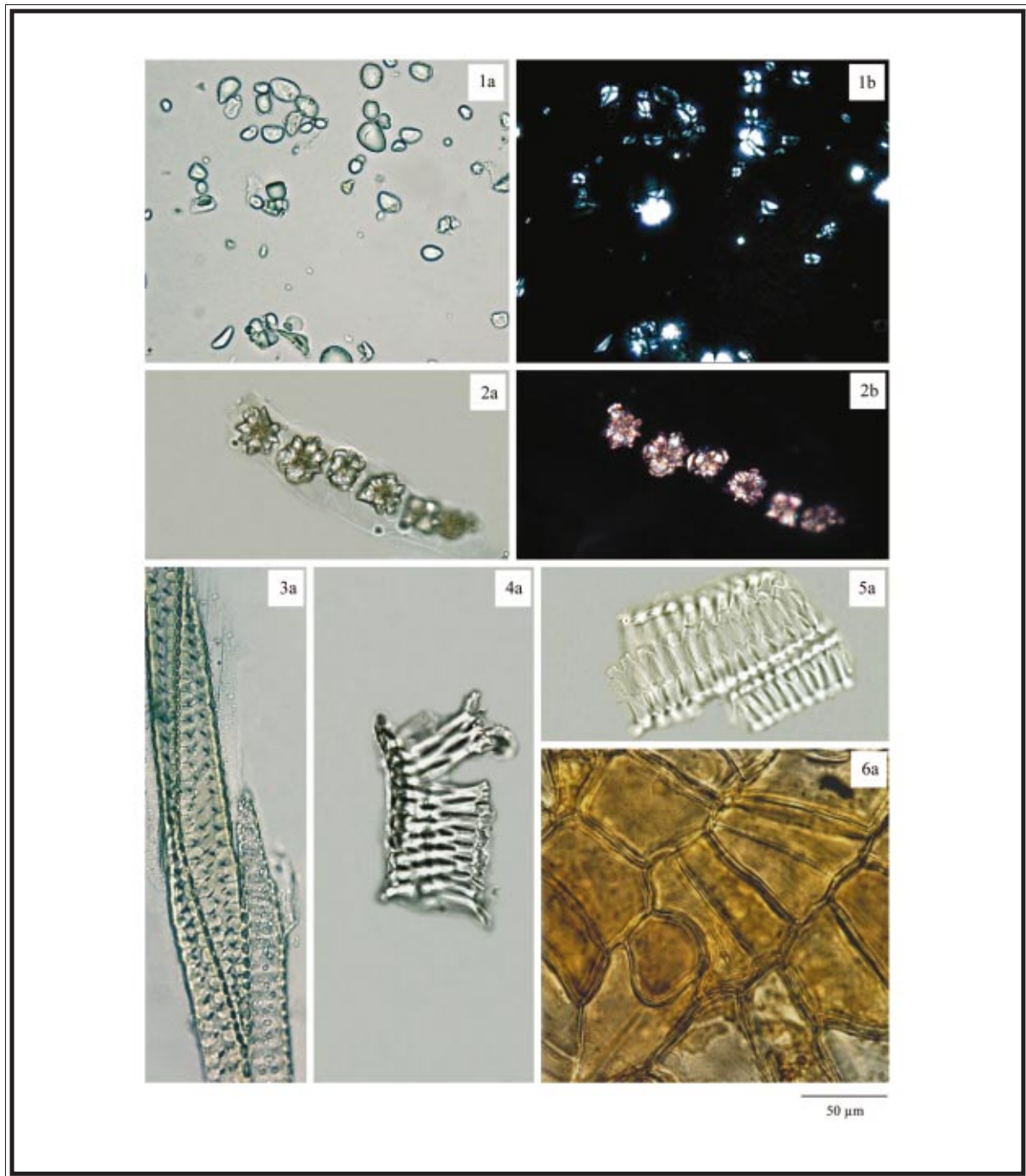


Figure 3(i) Microscopic features of powder of dried root of *Paeonia lactiflora* Pall.

1. Starch grains
2. Cluster crystals of calcium oxalate
3. Xylem fibres
4. Reticulate vessels
5. Bordered-pitted vessel
6. Cork cells

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

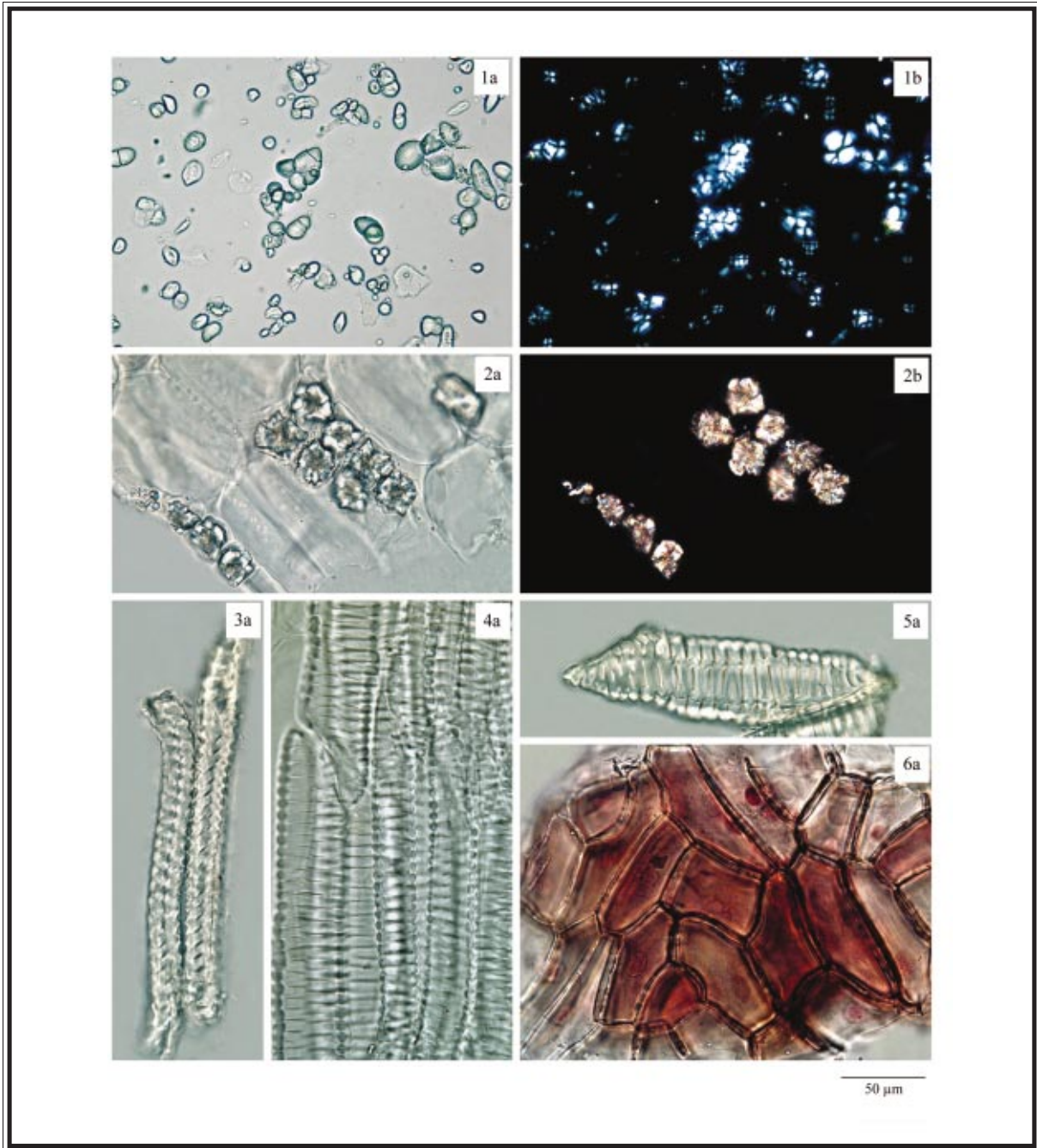


Figure 3(ii) Microscopic features of powder of dried root of *Paeonia veitchii* Lynch

- 1. Starch grains 2. Cluster crystals of calcium oxalate 3. Xylem fibres 4. Reticulate vessels
- 5. Bordered-pitted vessel 6. Cork cells

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

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 (560 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate and a freshly prepared developing solvent system as described above. Apply separately paeoniflorin standard solution (3 μL) and the test solution (0.5 μL) to the plate. Develop over a path of about 4 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 110°C until the spots or bands become visible (about 15 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV(A).

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 paeoniflorin.

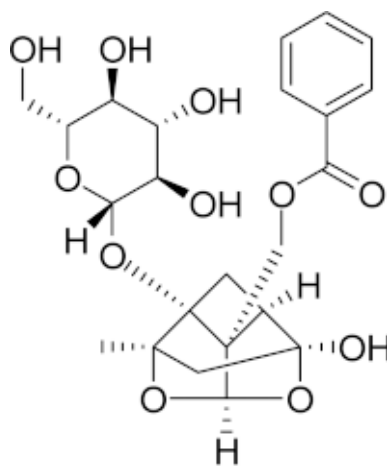


Figure 4 Chemical structure of paeoniflorin

4.4 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

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

Weigh 2.0 mg of paeoniflorin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL test tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter through a 0.45- μ m RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (273 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	100 \rightarrow 90	0 \rightarrow 10	linear gradient
10 – 20	90	10	isocratic
20 – 60	90 \rightarrow 75	10 \rightarrow 25	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μ L of paeoniflorin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of paeoniflorin should not be more than 3.0%; the RSD of the retention time of paeoniflorin peak should not be more than 1.0%; the column efficiency determined from paeoniflorin peak should not be less than 50000 theoretical plates.

Paeonia lactiflora Pall.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 5(i)].

Paeonia veitchii Lynch

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 5(ii)].

Procedure

Separately inject paeoniflorin Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of paeoniflorin peak in the chromatogram of paeoniflorin Std-FP and the retention times of the four characteristic peaks [Fig. 5(i) or (ii)] in the chromatogram of the test solution. Under the same HPLC conditions, identify paeoniflorin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of paeoniflorin Std-FP. The retention times of paeoniflorin 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 four characteristic peaks of dried root of *Paeonia lactiflora* Pall. extract and *Paeonia veitchii* Lynch extract are listed in Table 1(i) and (ii) respectively.

Table 1(i) The RRTs and acceptable ranges of the four characteristic peaks of dried root of *Paeonia lactiflora* Pall. extract

Peak No.	RRT	Acceptable Range
1	0.69	±0.05
2	0.84	±0.03
3 (marker, paeoniflorin)	1.00	-
4	1.46	±0.07

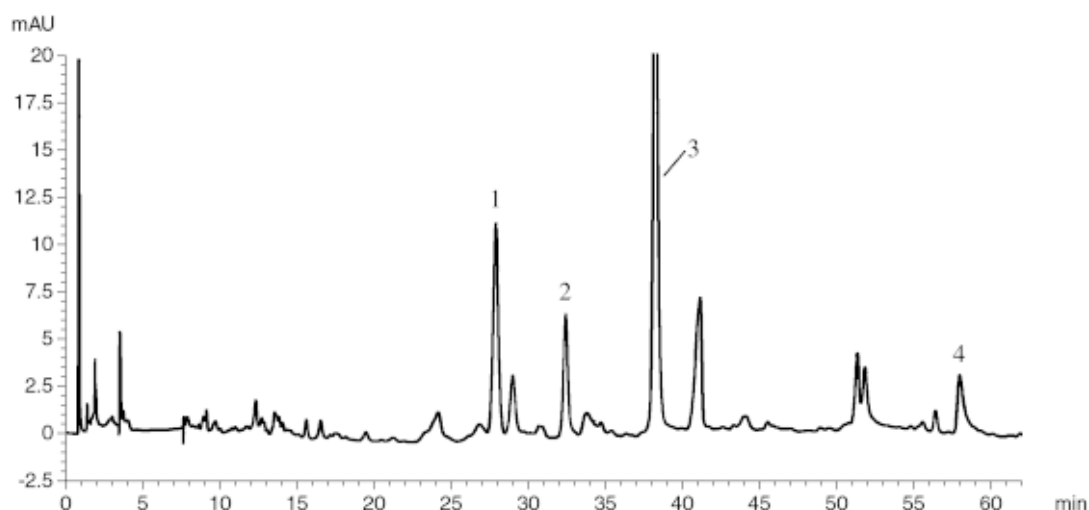


Figure 5(i) A reference fingerprint chromatogram of dried root of *Paeonia lactiflora* Pall. extract

Table 1(ii) The RRTs and acceptable ranges of the four characteristic peaks of dried root of *Paeonia veitchii* Lynch extract

Peak No.	RRT	Acceptable Range
1	0.56	±0.03
2	0.64	±0.03
3 (marker, paeoniflorin)	1.00	-
4	1.46	±0.07

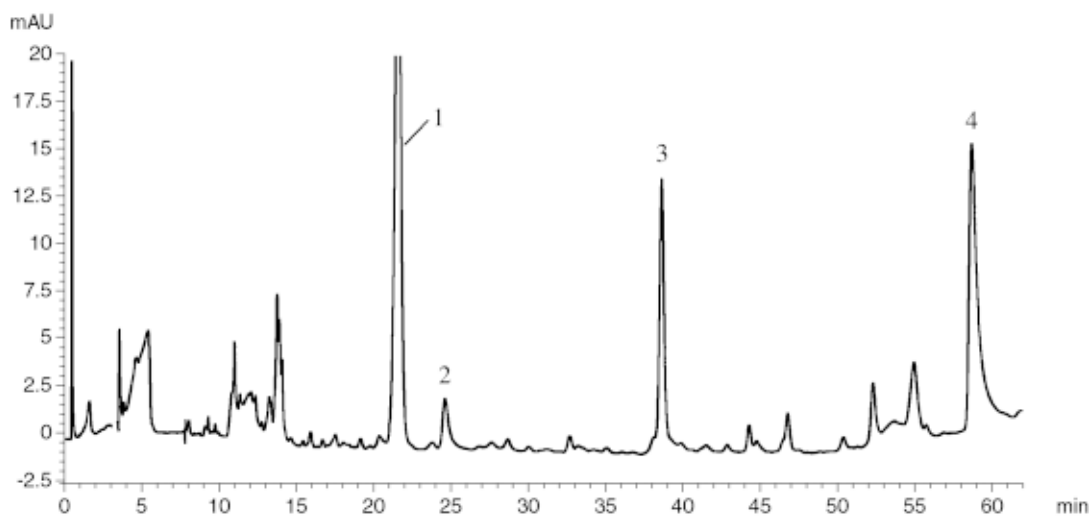


Figure 5(ii) A reference fingerprint chromatogram of dried root of *Paeonia veitchii* Lynch extract

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 chromatograms [Fig. 5(i) or (ii)].

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 2.0%.
- 5.6 Ash** (*Appendix IX*)
- Total ash: not more than 10.0%.
Acid-insoluble ash: not more than 2.5%.
- 5.7 Water Content** (*Appendix X*): not more than 13.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (cold extraction method): not less than 28.0%.

Ethanol-soluble extractives (cold extraction method): not less than 27.0%.

7. ASSAY

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

Standard solution

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

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

Paeoniflorin standard solution for assay, Std-AS

Measure accurately the volume of the paeoniflorin Std-Stock, dilute with methanol to produce a series of solutions of 1, 10, 50, 100, 200 mg/L for paeoniflorin.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 5 min. Filter the supernatant through a 0.45- μm RC filter. Repeat the extraction twice. Combine the filtrate. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (230 nm) and a column (4.6 \times 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	82	18	isocratic
10 – 20	82 \rightarrow 20	18 \rightarrow 80	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of paeoniflorin Std-AS (10 mg/L). The

requirements of the system suitability parameters are as follows: the RSD of the peak area of paeoniflorin should not be more than 5.0%; the RSD of the retention time of paeoniflorin peak should not be more than 2.0%; the column efficiency determined from paeoniflorin peak should not be less than 5000 theoretical plates.

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

Limits

The sample contains not less than 2.8% of paeoniflorin ($C_{23}H_{28}O_{11}$), calculated with reference to the dried substance.