

Radix Paeoniae Alba



Figure 1 A photograph of Radix Paeoniae Alba

1. NAMES

Official Name: Radix Paeoniae Alba

Chinese Name: 白芍

Chinese Phonetic Name: Baishao

2. SOURCE

Radix Paeoniae Alba is the dried processed root of *Paeonia lactiflora* Pall. (Ranunculaceae). The root is collected in the summer and autumn; after washing it, the two ends and the rootlets are removed. The rootbark is peeled either before or after boiling the root in water, followed by drying the root under the sun to obtain Radix Paeoniae Alba.

3. DESCRIPTION

Cylindrical, straight or slightly curved, the two ends truncate, 5-27 cm long, 8-34 mm in diameter. Externally whitish or pale reddish-brown, smooth and glossy or with longitudinal wrinkles, with rootlet scars and occasional remains of brown cork. Texture compact, not easily broken; fracture relatively even, whitish or pale brownish-red; cambium ring distinct, the rays radial. Odour slight; taste slightly bitter and sour (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Parenchyma cells in the cortex elongated tangentially. Cambium in a ring. Xylem rays consist of 6 to more than 30 rows of cells. Vessels arranged radially. Cluster crystals of calcium oxalate and masses of gelatinized starch grains are found in parenchyma cells (Fig. 2).

Powder

Colour greyish-white to pale brown. Masses of gelatinized starch grains fairly abundant. Cluster

crystals of calcium oxalate 8-37 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. Bordered-pitted or reticulate vessels 13-66 μm in diameter. Xylem fibres long-fusiform with thickened and slightly lignified wall, which is large round-pitted or oblique-pitted (Fig. 3).

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.

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.

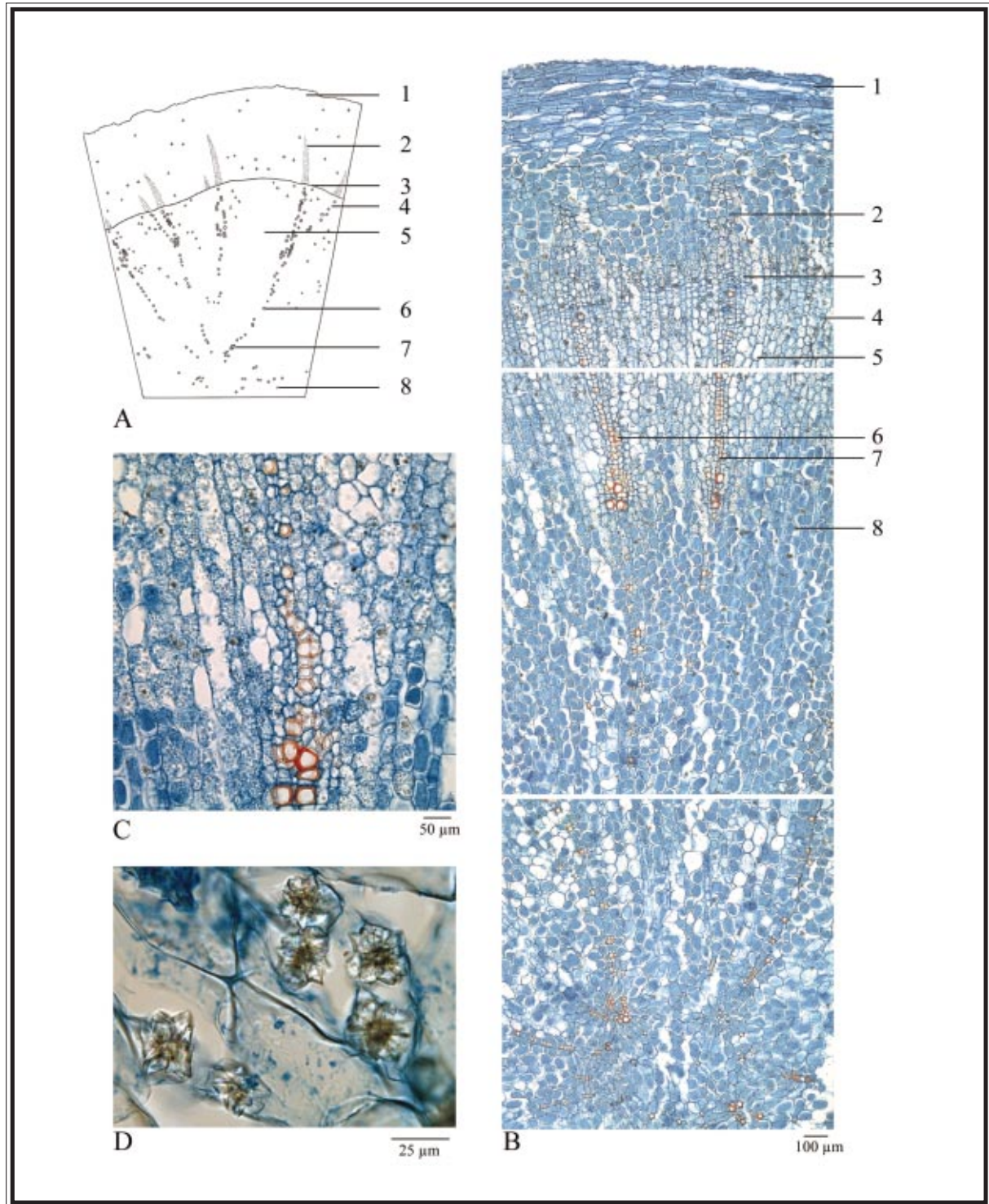


Figure 2 Microscopic features of transverse section of Radix Paeoniae Alba

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

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

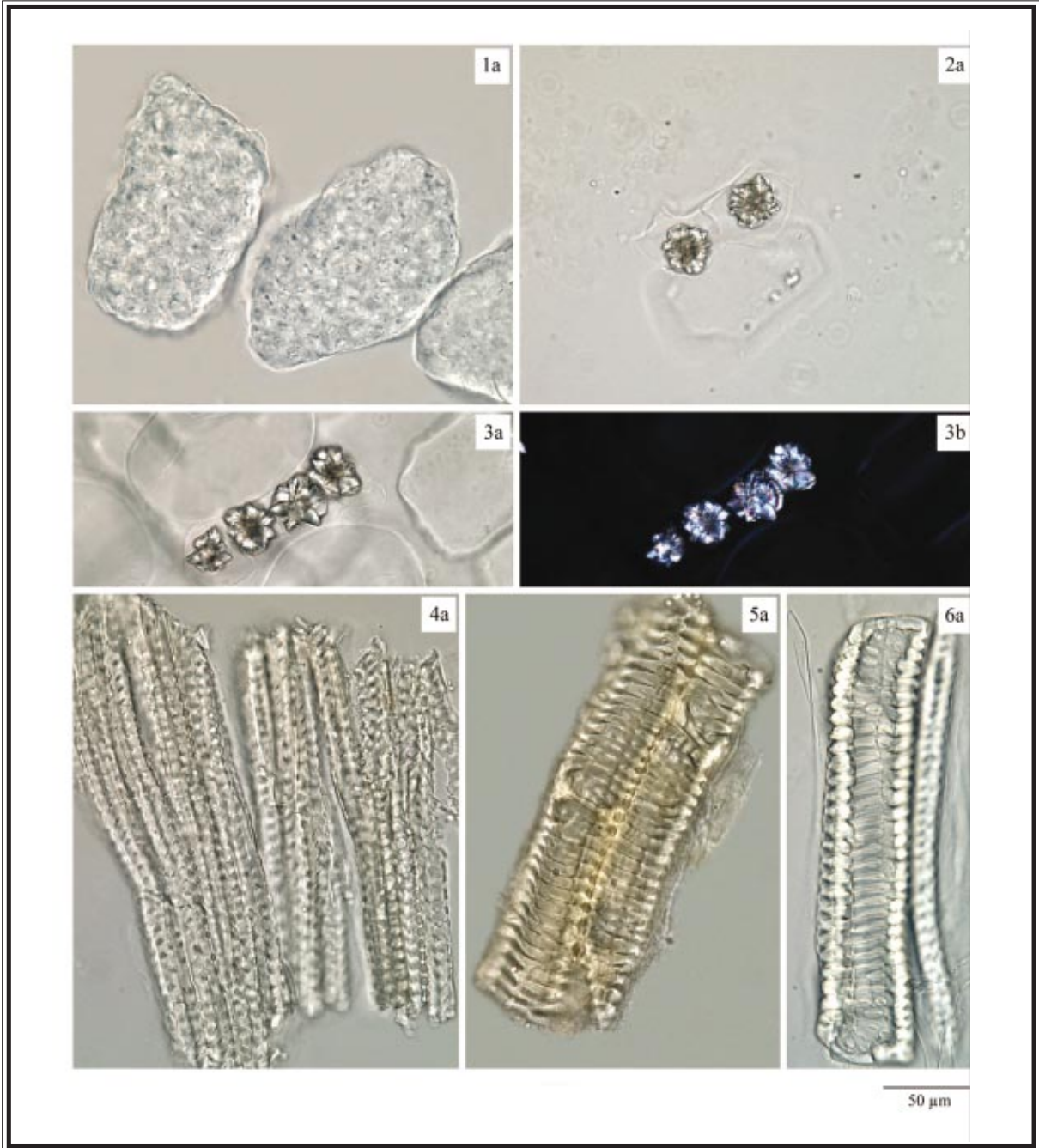


Figure 3 Microscopic features of powder of Radix Paeoniae Alba

- 1. Starch masses 2. Cluster crystals of calcium oxalate 3. Cluster crystals of calcium oxalate
- 4. Xylem fibres 5. Reticulate vessels 6. Bordered-pitted vessel
- a. Features under the light microscope b. Features under the polarized microscope

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 (1 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.

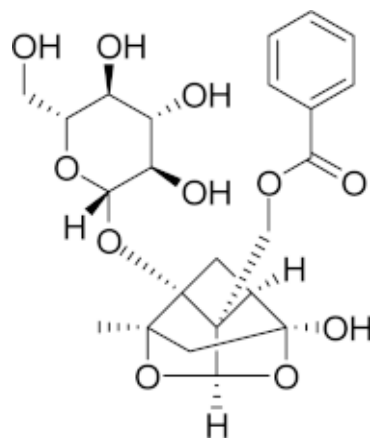


Figure 4 Chemical structure of paeoniflorin

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 0.5 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-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (273 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 –

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

System suitability requirements

Perform at least five replicate injections each with 10 µL 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.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject paeoniflorin Std-FP and the test solution (10 µL 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) 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 Radix Paeoniae Alba extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Radix Paeoniae Alba extract

Peak No.	RRT	Acceptable Range
1	0.78	±0.03
2	0.91	±0.03
3 (marker, paeoniflorin)	1.00	-
4	1.36	±0.03

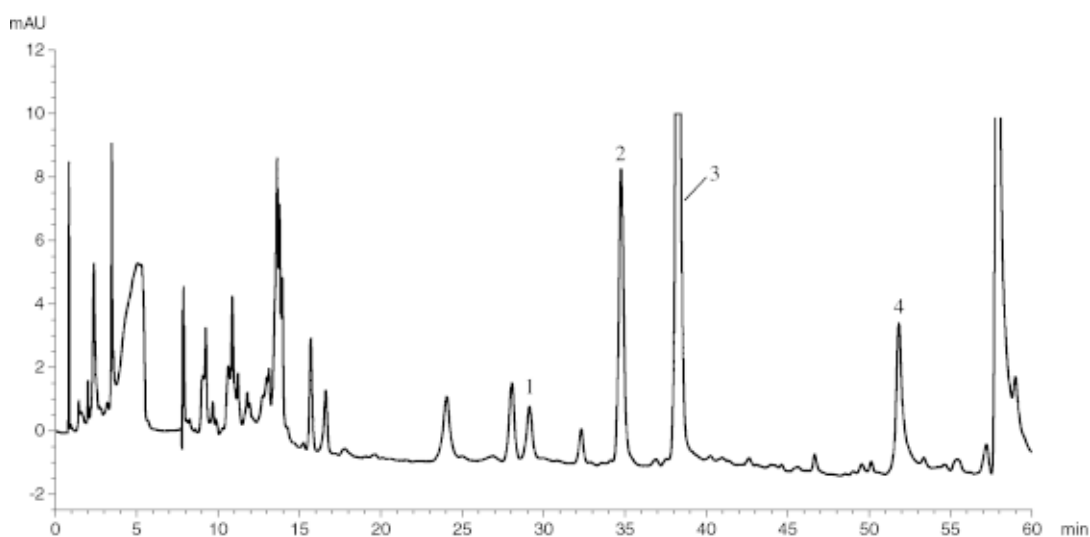


Figure 5 A reference fingerprint chromatogram of Radix Paeoniae Alba extract

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. 5).

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*): not more than 400 mg/kg.
- 5.5 **Foreign Matter** (*Appendix VIII*): not more than 1.0%.

5.6 Ash (Appendix IX)

Total ash: not more than 4.0%.

Acid-insoluble ash: not more than 1.0%.

5.7 Water Content (Appendix X): not more than 14.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (cold extraction method): not less than 21.0%.

Ethanol-soluble extractives (hot extraction method): not less than 16.0%.

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 ethanol (50%).

Paeoniflorin standard solution for assay, Std-AS

Measure accurately the volume of the paeoniflorin Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 1, 50, 100, 150, 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 ethanol (50%). 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 ethanol (50%). Transfer the solution to a 10-mL volumetric flask and make up to the mark with ethanol (50%). 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 → 20	18 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 µL of paeoniflorin Std-AS (50 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 3.0%; 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 1.9% of paeoniflorin (C₂₃H₂₈O₁₁), calculated with reference to the dried substance.