Rhizoma Cimicifugae

Figure 1 A photograph of Rhizoma Cimicifugae

1. NAMES

Official Name: Rhizoma Cimicifugae

Chinese Name: 升麻

Chinese Phonetic Name: Shengma

2. SOURCE

Rhizoma Cimicifugae is the dried rhizome of *Cimicifuga heracleifolia* Kom. (Ranunculaceae). The rhizome is collected in the autumn, after removal of soil and fibrous root, it is dried under the sun to obtain Rhizoma Cimicifugae.

3. DESCRIPTION

Irregular long pieces, frequently branched, nodular, 6-30 cm long, 12-40 mm in diameter. Externally blackish-brown or brown, rough, showing remains of many wiry fibrous roots; the upper part shows several round and hollow remains of stems, the inner wall with reticulate furrows, the lower part lumpy with fibrous root scars. Texture light and hard, not easily broken; fracture uneven, cracked, fibrous, yellowish-green or pale yellowish-white. Odour slight; taste slightly bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Part of outer metaderm cells walls thickened by suberization. Sometimes the outer and anticlinal wall of some metaderm cells nipple-shaped thickened, and stretched into the cell cavities. Cortex mainly consists of parenchyma cells. Phloem fibre bundles crescent-shaped, 5-110 fibres in one bundle. Vascular bundles up to 60, arranged in a ring. Pith broad (Fig. 2).

Powder

Colour yellowish-brown or brown. Metaderm cells yellowish-brown, rectangular or subpolygonal,

wall unevenly thickened; some cells have nipple-shaped, thickened wall stretched into the cell cavities. Vessels mainly bordered-pitted and reticulate. Most of the xylem fibres are fibre-tracheids, either singles or in bundles, slightly shuttle-shaped, the end elongated, sharp or blunt, some branched, with oblique, crisscross or V-shaped pits. Phloem fibres mostly in bundles, narrow and elongated in shape, the end blunt or slightly sharp, lignified, and with distinct pit-canals. Xylem parenchyma cells single or in groups, rectangular or subround in shape, with round or shortly slit-shaped pits (Fig. 3).

4.2 Physicochemical Identification

Procedure

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of dichloromethane. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer 0.5 mL of the supernatant to a test tube. Cautiously add about 0.5 mL of sulphuric acid along the inner wall of the test tube. Allow to stand for 20 min. A reddish-brown or yellowish-brown ring is observed at the interface of the two solvent layers.

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

Standard solution

Isoferulic acid standard solution

Weigh 1.0 mg of isoferulic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of toluene, dichloromethane and glacial acetic acid (6:1:0.5, v/v).

Test solution

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 50 mL of ethanol. Reflux the mixture for 1 h. Cool down to room temperature. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

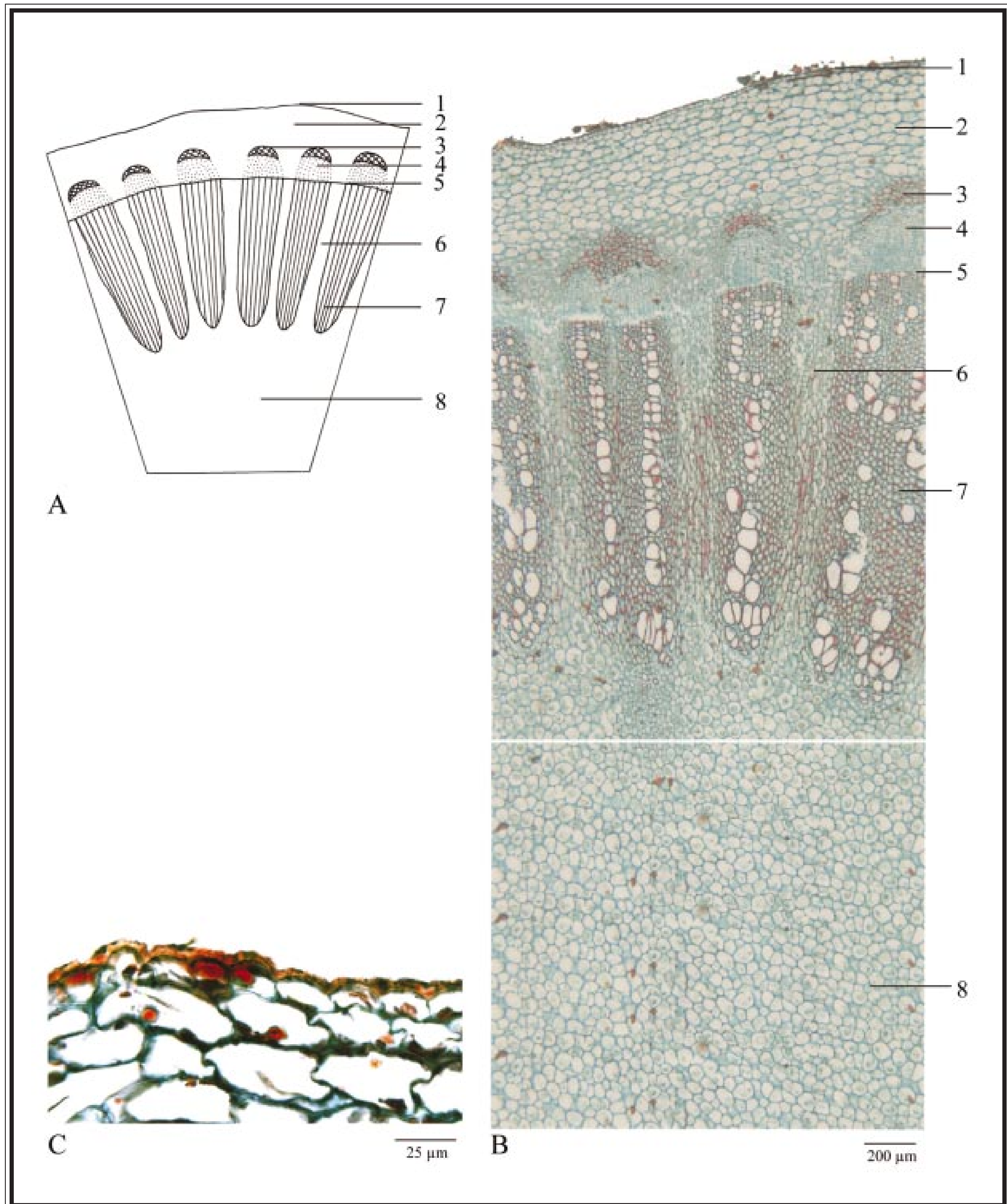

Figure 2 Microscopic features of transverse section of Rhizoma Cimicifugae

A. Sketch B. Section illustration C. Metaderm (phellem)

1. Metaderm 2. Cortex 3. Phloem fibre bundles 4. Phloem 5. Cambium 6.Xylem rays 7. Xylem 8. Pith

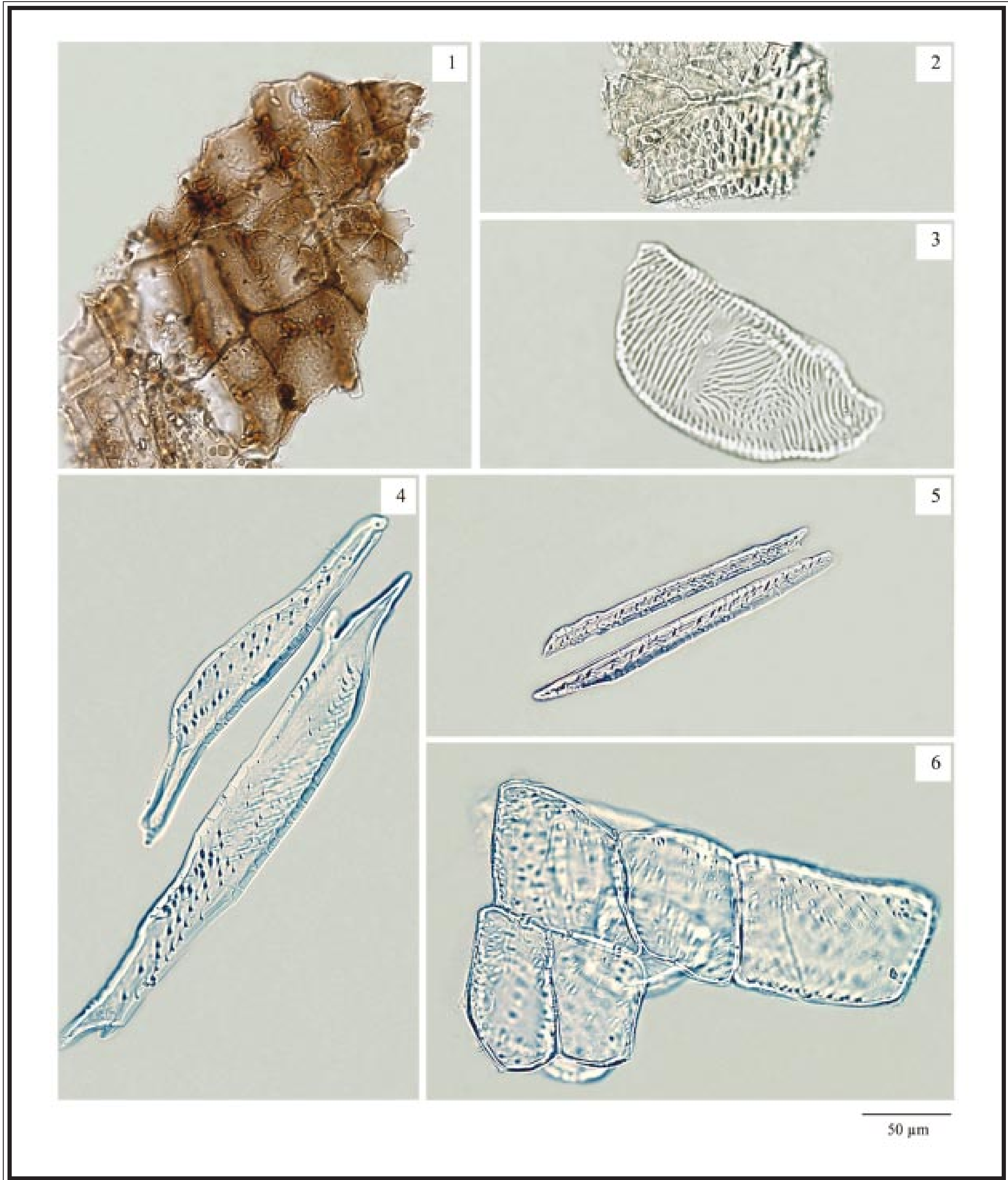

Figure 3 Microscopic features of powder of Rhizoma Cimicifugae (under the light microscope)

1. Metaderm cells   2. Bordered-pitted vessels   3. Reticulate vessel   4. Xylem fibres   5. Phloem fibres
6. Xylem parenchyma cells

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 isoferulic acid standard solution and the test solution (5 µL each) 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. Examine the plate under UV light (254 nm). 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 isoferulic acid.

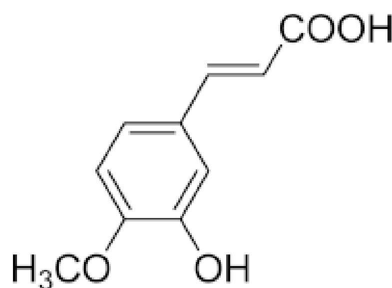

Figure 4 Chemical structure of isoferulic acid

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

Standard solution

Isoferulic acid standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of isoferulic acid CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 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 × *g* for 5 min. Filter the supernatant through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (325 nm) and a column (3.9 × 300 mm) packed with ODS bonded silica gel (4 µm particle size). The flow rate is about 0.9 mL/min. Programme the chromatographic system as follows –

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	95	5	isocratic
5 – 50	95 → 75	5 → 25	linear gradient
50 – 70	75 → 50	25 → 50	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 µL of isoferulic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of isoferulic acid should not be more than 5.0%; the RSD of the retention time of isoferulic acid peak should not be more than 2.0%; the column efficiency determined from isoferulic acid peak should not be less than 50000 theoretical plates.

The *R* value between peak 4 and peak 5 in the chromatogram of test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject isoferulic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of isoferulic acid peak in the chromatogram of isoferulic acid Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify isoferulic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isoferulic acid Std-FP. The retention times of isoferulic acid 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 six characteristic peaks of Rhizoma Cimicifugae extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the six characteristic peaks of Rhizoma Cimicifugae extract

Peak No.	RRT	Acceptable Range
1 (ferulic acid)	0.93	±0.03
2 (marker, isoferulic acid)	1.00	-
3	1.42	±0.03
4	1.52	±0.03
5	1.55	±0.03
6	1.90	±0.03

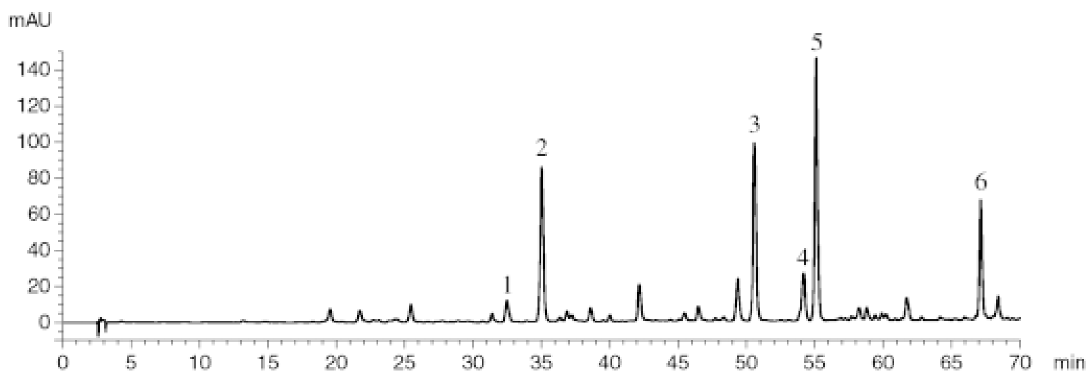

Figure 5 A reference fingerprint chromatogram of Rhizoma Cimicifugae extract

For positive identification, the sample must give the above six 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)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.5%.
Acid-insoluble ash: not more than 5.5%.

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

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 19.0%.
Ethanol-soluble extractives (hot extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

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

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

Isoferulic acid standard solution for assay, Std-AS

Measure accurately the volume of the isoferulic acid Std-Stock, dilute with methanol to produce a series of solutions of 10, 20, 30, 40, 50 mg/L for isoferulic acid.

Test solution

Weigh accurately 0.4 g of the powdered sample and put into a 50-mL centrifugal tube, then add 30 mL of methanol. Incubate in a water bath (40 rpm) at 60°C for 30 min with shaking. Centrifuge at about 3000 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter. Transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction thrice each with 10 mL of methanol. 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 (300 nm) and a column (3.9 × 300 mm) packed with ODS bonded silica gel (4 μm particle size). The flow rate is about 0.9 mL/min. Programme the chromatographic system as follows –

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	90 → 85	10 → 15	linear gradient
5 – 25	85 → 80	15 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of isoferulic acid Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of isoferulic acid should not be more than 5.0%; the RSD of the retention time of isoferulic acid peak should not be more than 2.0%; the column efficiency determined from isoferulic acid peak should not be less than 15000 theoretical plates.

The *R* value between isoferulic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.3.

Calibration curve

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

Limits

The sample contains not less than 0.040% of isoferulic acid ($C_{10}H_{10}O_4$), calculated with reference to the dried substance.