Saxifragae Herba

Figure 1 A photograph of Saxifragae Herba

A. Saxifragae Herba B. Magnified image of upper surface of leaf
C. Magnified image of lower surface of leaf
D. Magnified image of stolons E. Magnified image of root

1. NAMES

Official name: Saxifragae Herba

Chinese name: 虎耳草

Chinese phonetic name: Huercao

2. SOURCE

Saxifragae Herba is the dried whole plant of *Saxifraga stolonifera* Curtis (Saxifragaceae). The whole plant is collected all year round, foreign matter removed, then dried under the sun to obtain Saxifragae Herba.

3. DESCRIPTION

Basal leaves with petiole 3-15 cm; leaf blade subcordate or reniform to subrounded, 2-8 cm long, 3-10 cm wide, apex obtuse, base rounded to cordate, margin irregularly dentate or undulated. Upper surface of the leaf greenish-brown to brown, lower surface pale greenish-brown to brown. Stolons densely crisped. Root fibrous. Odour slightly pungent; taste acrid (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Epidermis consists of 1 layer of thin and flat cells, covered with cuticle. Cortex broad, consisting of irregularly shaped cells, sinuous walls. Clusters of calcium oxalate numerous, in rosette aggregate, scattered in the cortex and pith. Endodermis distinct. Stele cambium visible, in interrupted ring within the vascular cylinder. Pith consists of parenchymatous cells, clusters of calcium oxalate numerous [Fig. 2(i)].

Stolon: Epidermis consists of 1 layer of cells, cells sub-rectangular to polygonal. Cortex broad, parenchymatous cells irregular in shape, walls sinuous. Endodermis distinct. Vascular bundle closed collateral. Pith consists of parenchymatous cells [Fig. 2(ii)].

Leaf: Unicellular or multicellular non-glandular hair found on the upper epidermis, maybe broken or fallen off. Upper epidermis consists of 1 layer of cells, cells subrectangular. Palisade tissue consists of 3-4 layers of cells, cells oval in shape. Vascular bundle amphicribral. Cells of spongy tissue loosely arranged, irregular in shape, tile-like, sinuous walls. Clusters of calcium oxalate scattered in mesophyll, in rosette aggregate. Cells of lower epidermis polygonal, walls thin, flattened. Multicellular non-glandular hair found on the lower epidermis, broken or fallen off [Fig. 2(iii)].

Powder

Colour brownish. Upper epidermal cells of leaf tile-like, flat with non-glandular multicellular hair or non-glandular unicellular hairs. Lower epidermal cells with anisocytic stomata, 3 unequal subsidiary walls. Multicellular non-glandular hairs occasionally found, fragmented. Simple starch granules spherical; black and cruciate-shaped under the polarized microscope. Epidermal cells of stolons with wavy, undulated cell wall. Clusters of calcium oxalate in rosette aggregate, 20-50 μm in diameter; polychromatic under the polarized microscope. Unicellular non-glandular hairs occasionally found. Fibres appears singly or in bundles. Spiral vessels occasionally found, 8-87 μm in diameter (Fig. 3).

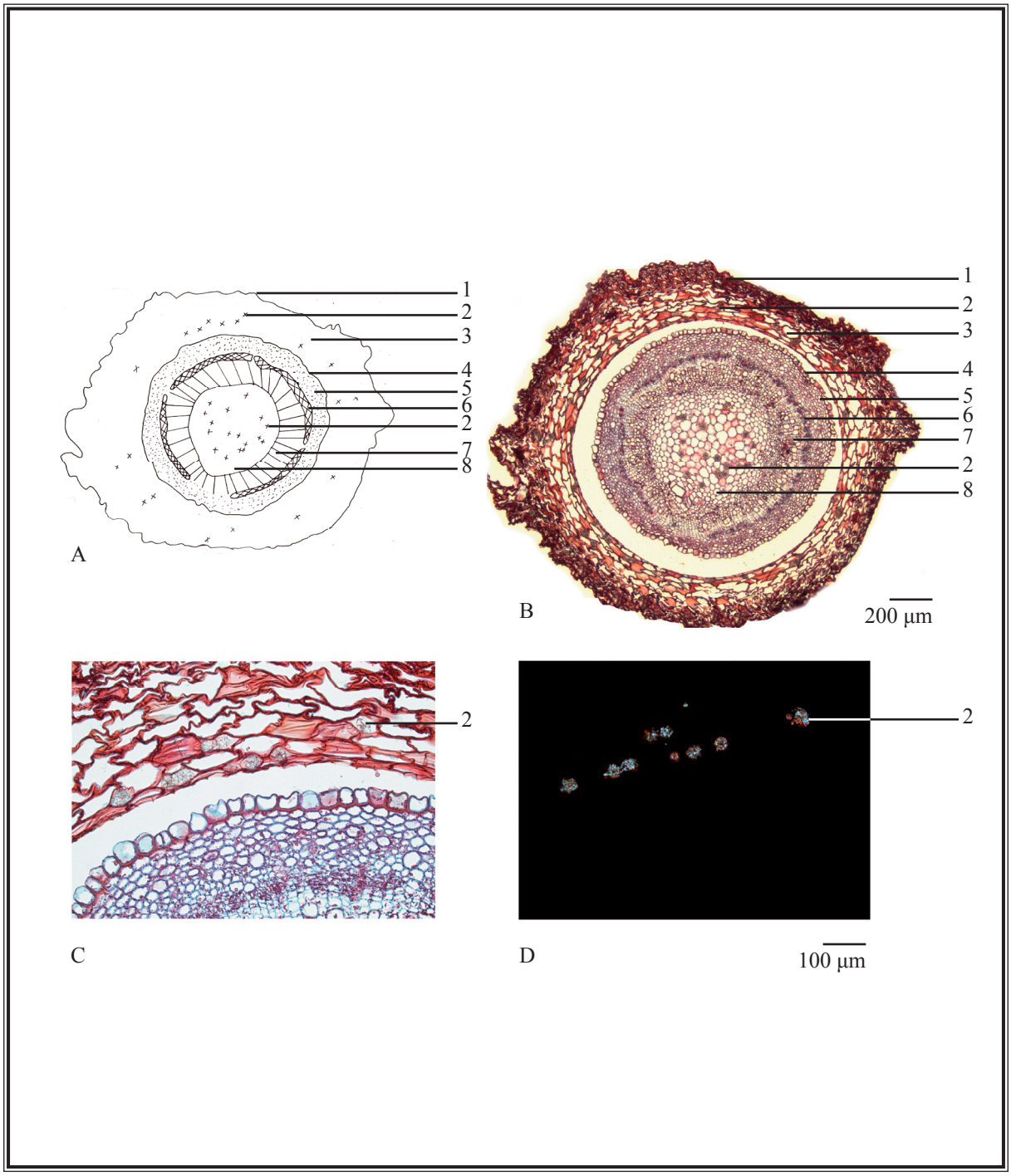

Figure 2 (i) Microscopic features of transverse section of root of Saxifragae Herba

A. Sketch B. Section illustration

C. Clusters of calcium oxalate in cortex (under the light microscope)

D. Clusters of calcium oxalate in cortex (under the polarized microscope)

1. Epidermis 2. Cluster of calcium oxalate 3. Cortex 4. Endodermis

5. Phloem 6. Cambium 7. Xylem 8. Pith

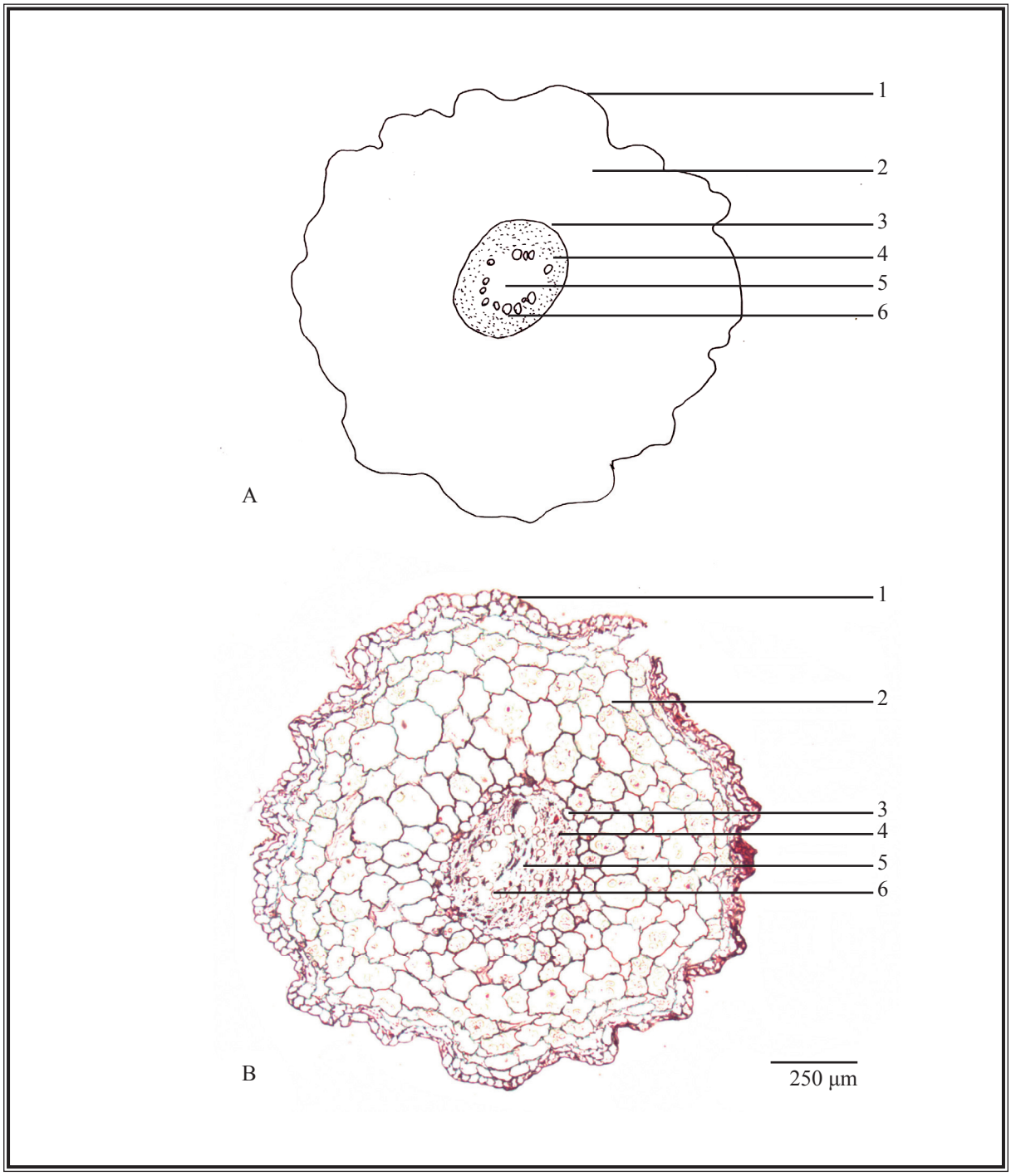

Figure 2 (ii) Microscopic features of transverse section of stolon of Saxifragae Herba

A. Sketch B. Section illustration

1. Epidermis 2. Cortex 3. Endodermis 4. Phloem 5. Pith 6. Xylem

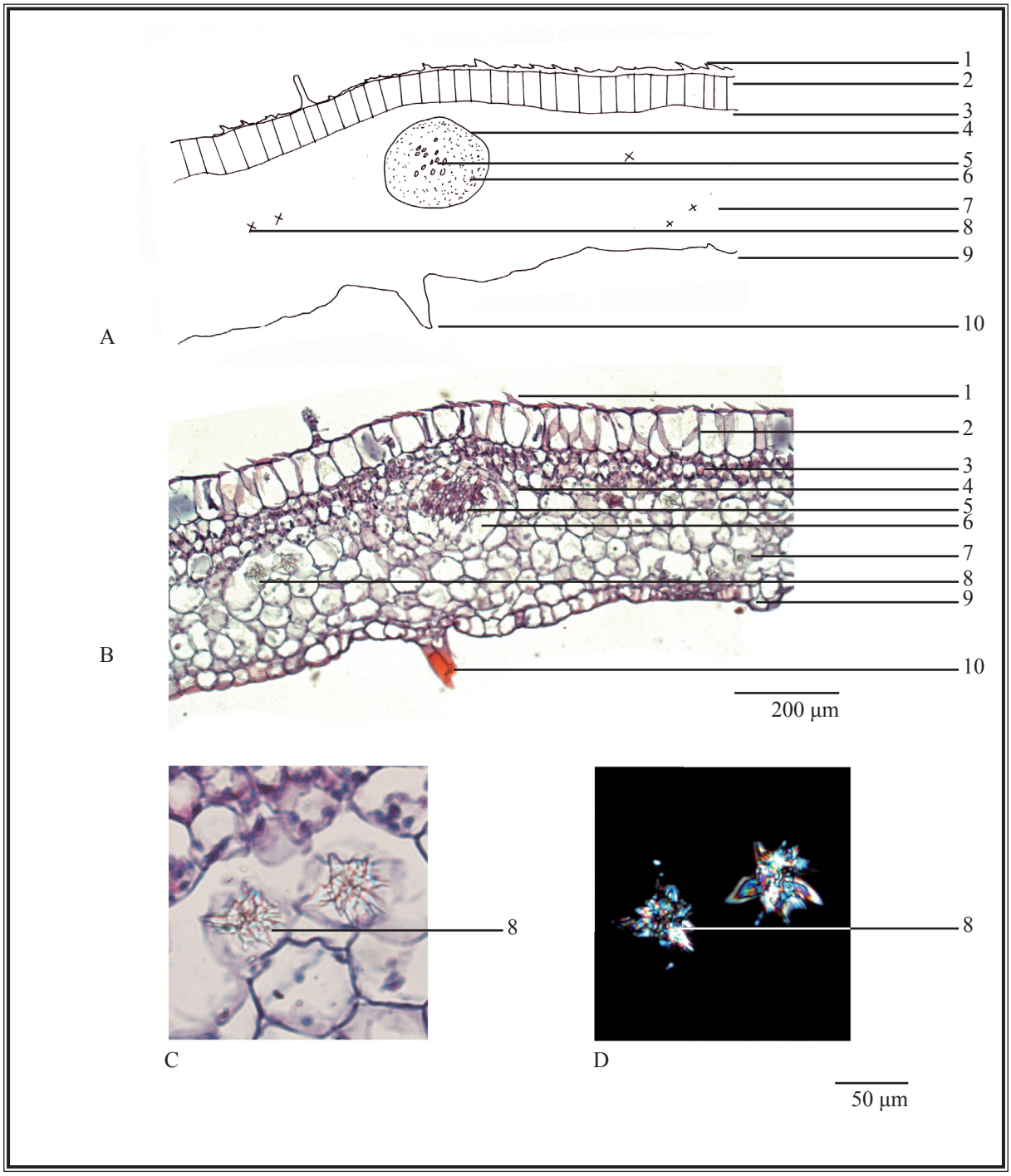

Figure 2 (iii) Microscopic features of transverse section of leaf of Saxifragae Herba

A. Sketch B. Section illustration

C. Clusters of calcium oxalate (under the light microscope)

D. Clusters of calcium oxalate (under the polarized microscope)

1. Unicellular non-glandular hair 2. Upper epidermis 3. Palisade tissue

4. Vascular bundle 5. Xylem 6. Phloem 7. Spongy tissue

8. Cluster of calcium oxalate 9. Lower epidermis 10. Multicellular non-glandular hair

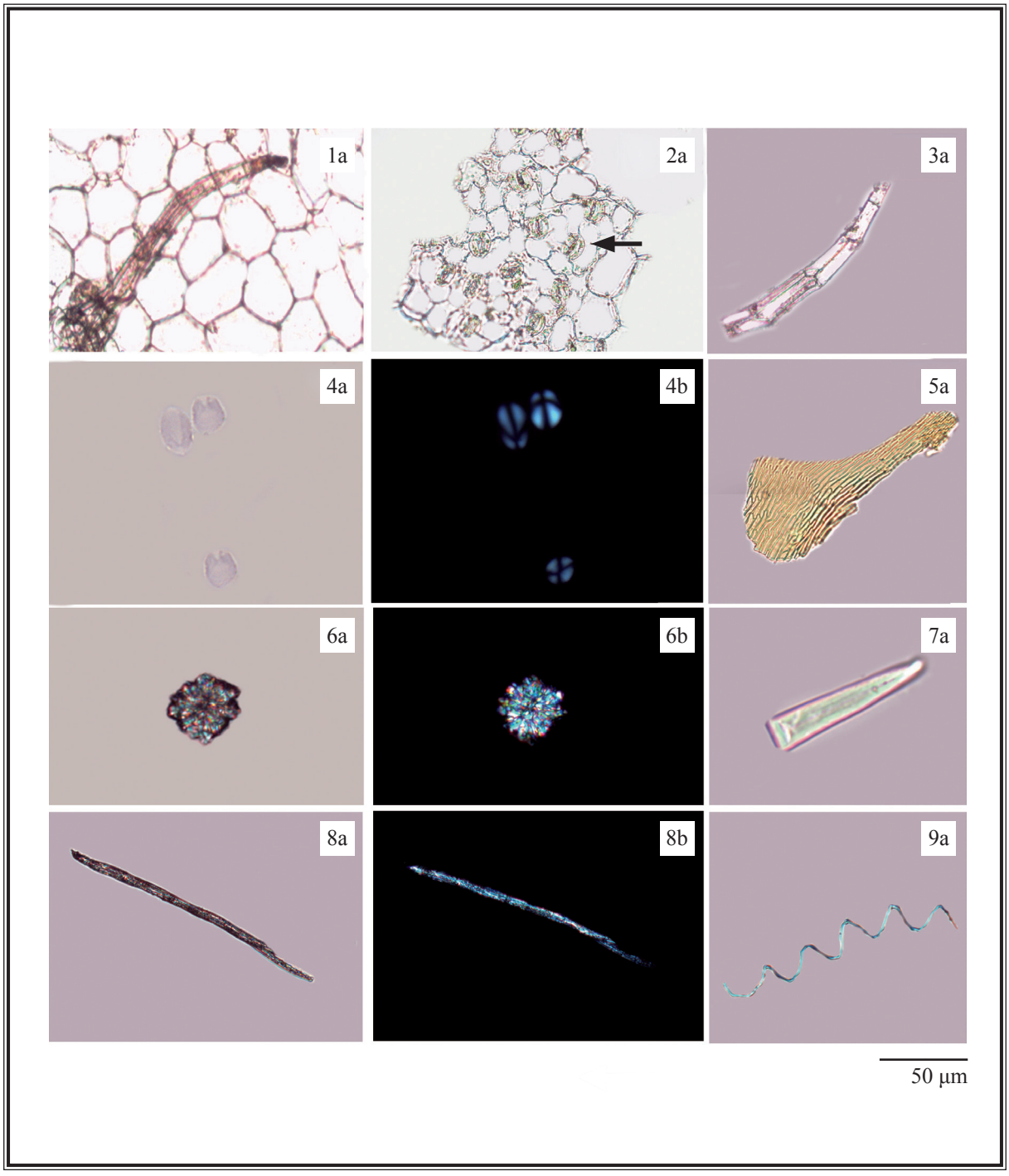

Figure 3 Microscopic features of powder of Saxifragae Herba

1. Upper epidermal cells of leaf with non-glandular hair
2. Lower epidermal cells with anisocytic stomata (⟶)
3. Multi-cellular non-glandular hair 4. Starch granules 5. Epidermal cells of stolon
6. Cluster of calcium oxalate 7. Unicellular non-glandular hair 8. Fibre
9. Spiral vessel

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

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

Standard solution

Bergenin standard solution

Weigh 1.0 mg of bergenin CRS (Fig. 4) and dissolve in 1 mL of ethanol (30%).

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate, isopropanol, formic acid and methanol (5:1.5:1:0.5:0.3, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of ethanol (30%). Sonicate (200 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

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 bergenin standard solution (2 μL) and the test solution (6 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 7 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).

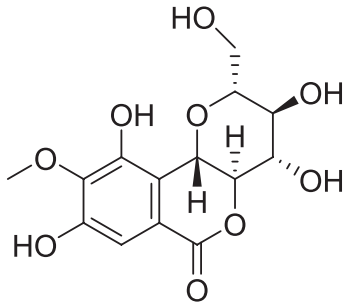

Figure 4 Chemical structure of bergenin

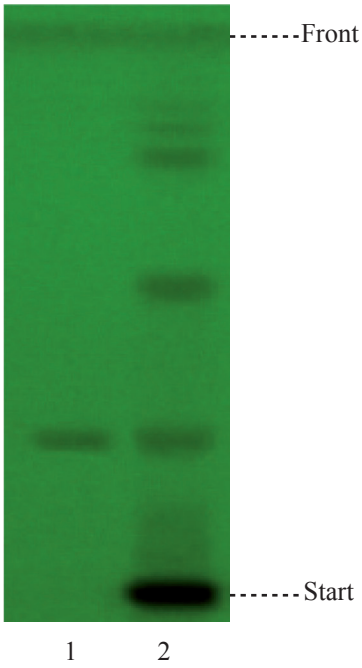

Figure 5 A reference HPTLC chromatogram of Saxifragae Herba extract observed under UV light (254 nm)

1. Bergenin 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 bergenin (Fig. 5).

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

Standard solution

Bergenin standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 3.0 mg of bergenin CRS and dissolve in 50 mL of ethanol (30%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (30%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.05% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 20	100 → 90	0 → 10	linear gradient
20 – 45	90 → 70	10 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of bergenin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of bergenin should not be more than 5.0%; the RSD of the retention time of bergenin peak should not be more than 2.0%; the column efficiency determined from bergenin peak should not be less than 120000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject bergenin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of bergenin peak in the chromatogram of bergenin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify bergenin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of bergenin Std-FP. The retention times of bergenin 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 Saxifragae Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Saxifragae Herba extract

Peak No.	RRT	Acceptable Range
1 (gallic acid)	0.35	± 0.03
2 (protocatechuic acid)	0.57	± 0.03
3	0.74	± 0.03
4 (marker, bergenin)	1.00	-

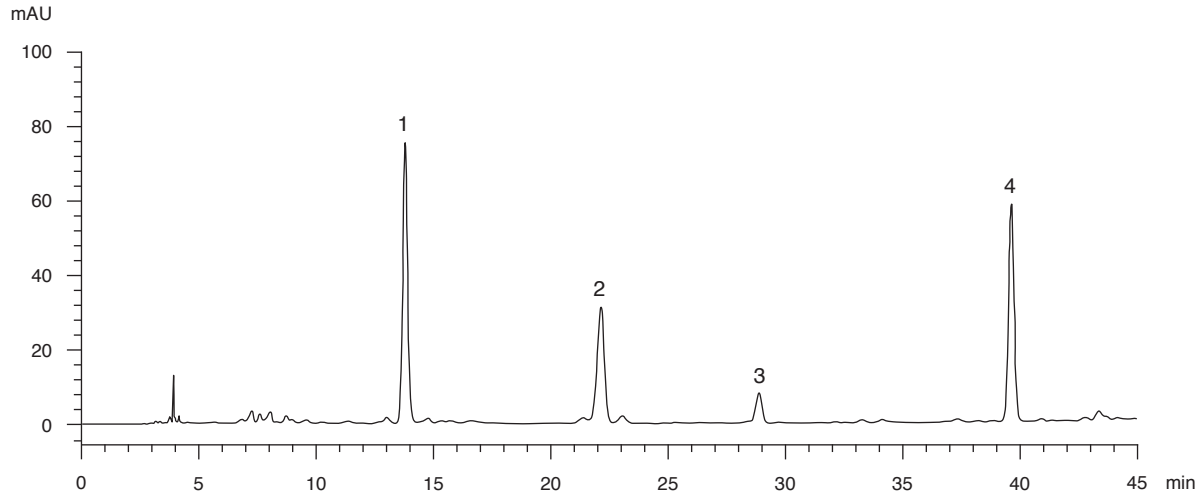

Figure 6 A reference fingerprint chromatogram of Saxifragae Herba extract

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. 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 XVI)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 15.5%.
Acid-insoluble ash: not more than 4.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 5.0 mg of bergenin CRS and dissolve in 5 mL of ethanol (30%).

Bergenin standard solution for assay, Std-AS

Measure accurately the volume of the bergenin Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 5, 10, 20, 40, 80 mg/L for bergenin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (30%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction two more times. Wash the residue with 25 mL of ethanol (30%). Centrifuge at about 4000 × *g* for 10 min. Combine the supernatants and make up to the mark with ethanol (30%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.05% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 20	100 → 90	0 → 10	linear gradient
20 – 45	90 → 70	10 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of bergenin Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of bergenin should not be more than 5.0%; the RSD of the retention time of bergenin peak should not be more than 2.0%; the column efficiency determined from bergenin peak should not be less than 120000 theoretical plates.

The *R* value between bergenin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of bergenin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of bergenin against the corresponding concentrations of bergenin 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 bergenin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of bergenin Std-AS. The retention times of bergenin 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 bergenin in the test solution, and calculate the percentage content of bergenin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.13% of bergenin ($C_{14}H_{16}O_9$), calculated with reference to the dried substance.

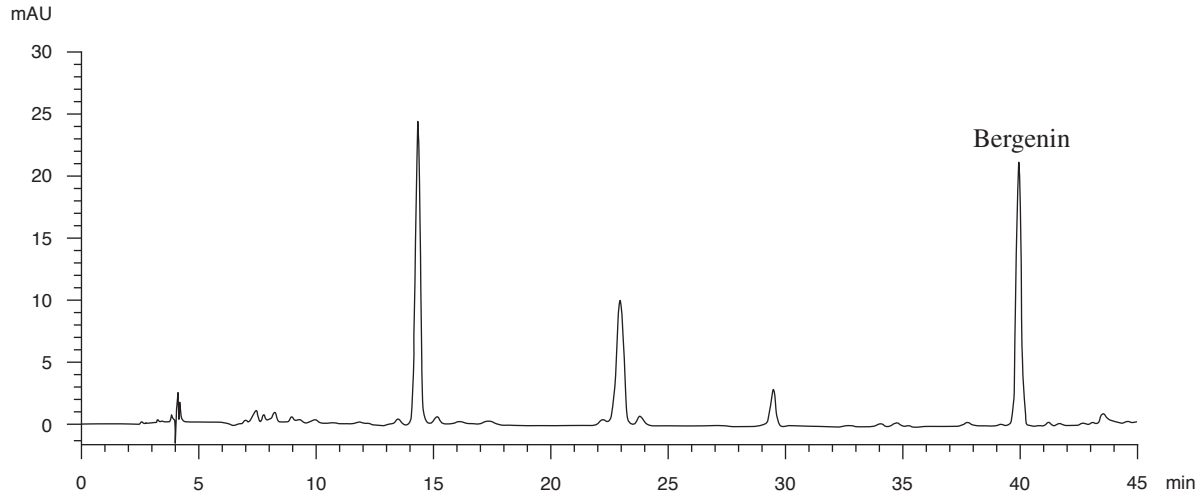

Figure 7 A reference assay chromatogram of Saxifragae Herba extract