Saussureae Involucratae Herba

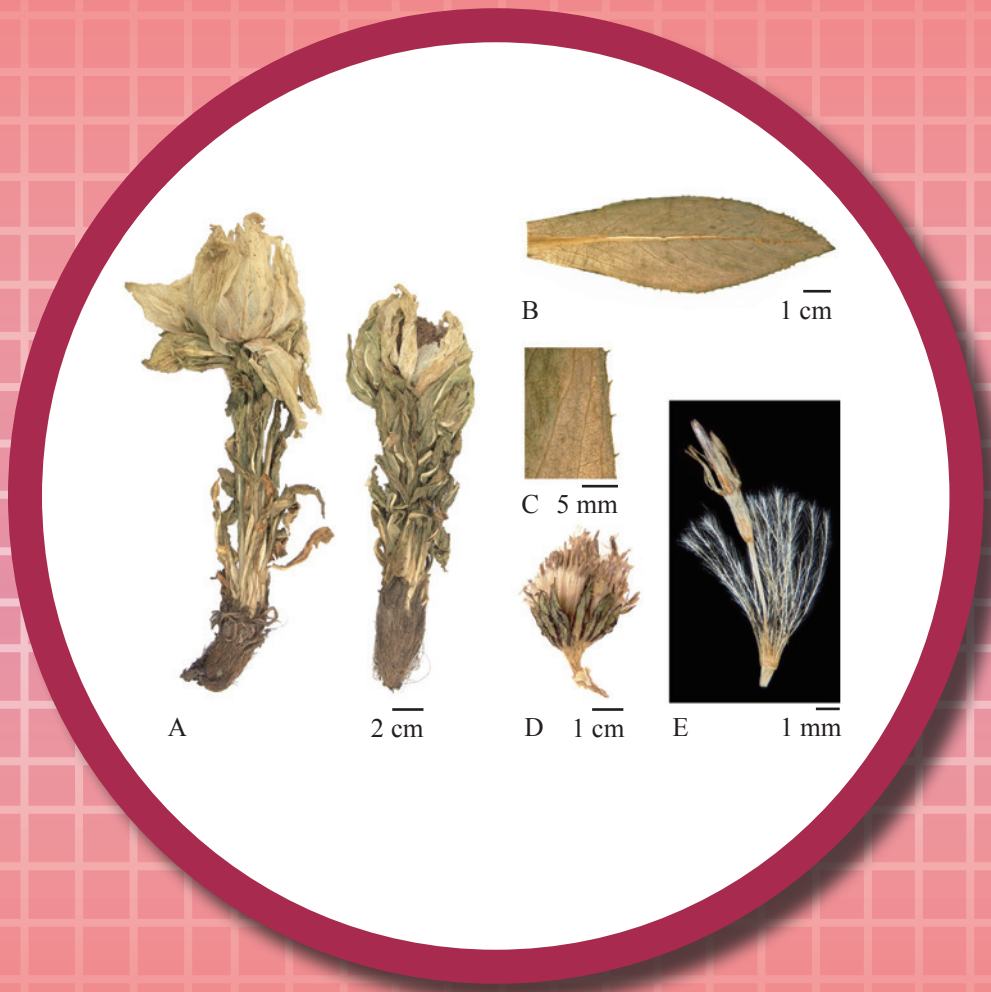

Figure 1 A photograph of Saussureae Involucratae Herba

A. Saussureae Involucratae Herba

B. Magnified image of leaf

C. Magnified image of margin of leaf

D. Magnified image of capitulum

E. Magnified image of tubular floret with pappi

1. NAMES

Official Name: Saussureae Involucratae Herba

Chinese Name: 天山雪蓮

Chinese Phonetic Name: Tianshanxuelian

2. SOURCE

Saussureae Involucratae Herba is the dried aerial part of *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip. (Asteraceae). The aerial part is collected in summer when flowering, foreign matter removed, then dried in shaded area to obtain Saussureae Involucratae Herba.

3. DESCRIPTION

Aerial part with flower, 12-46 cm long. Stem 4-28 mm in diameter, surface with longitudinal ridges, hollowed, bearing remnants of numerous, brown, fibrous leaf bases at base. Leaves sessile, densely arranged, yellowish-green to green, papyraceous, ovate-oblong, oblanceolate, or elliptical when intact, margin spiny, both surfaces puberulent, midvein distinct, yellowish-white. Capitulum terminal, 10-20 densely spherically grouped, 23-121 mm in diameter, enclosed by 2 layers of bracteal leaves; bracteal leaves membranous, pale yellow, broadly-ovate, margins spiny; involucres 3-7 layers, puberulent margin usually dark-purple, lanceolate, apex acute; floret tubular, yellowish-brown, collora lobes linear; stamens fused into a tube, surrounded by yellowish-white feathery pappi. Odour slightly fragrant; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Stem: Epidermis consists of 1 layer of cells, covered with cuticle, sometimes with glandular hairs. Cortex consists of parenchymatous cells. Dozens of vascular bundles arranged in an interrupted ring, each varying in size, collateral, fascicular cambium distinct, with fibre bundles located on the both sides. Pith consists of parenchymatous cells, hollowed in centre [Fig. 2 (i)].

Leaf: Upper epidermis consists of 1 layer of cells, sometimes with glandular hairs. Mesophyll tissue loosely arranged, differentiation of palisade and spongy tissues indistinct. Vascular bundles collateral, several arranged in a row, with sclerenchyma located on the upper and lower sides. Lower epidermis consists of 1 layer of relatively small cells, glandular hairs scattered [Fig. 2 (ii)].

Powder

Colour yellowish-green. Pollen grains numerous, globose-triangular or subglobular, 20-59 μm in diameter, with 3 germinal pores, exine granular and dentate-spinose. Glandular hairs large, mostly broken, both head and stalk consist of 2 rows of cells, intact hair 65-664 μm long; head 6- to 21-celled, 30-93 μm in diameter, some with yellowish-brown secretions; stalk 2- to 16-celled. Pappi colourless or pale yellow, consisting of multiseriate and branched cells, mostly broken, fragment of apex always slender, apex acute, 8-35 μm in diameter. Lower epidermal cells of leaf irregular in shape in surface view, anticlinal walls deeply sinuate, sometimes slightly beaded, stomata abundant, anomocytic. Upper epidermal cells of leaf irregular in shape in surface view, anticlinal walls sinuate, slightly beaded, stomata rare. Epidermal cells of bracteal leaf irregular in shape or subpolygonal in surface view, anticlinal walls relatively straight than that of leaf, sometimes slightly beaded, stomata anomocytic. Epidermal cells of corolla yellowish-brown, long strip-shaped in surface view, anticlinal walls wavy, with distinct cuticular striations on the surface. Epidermal cells of stem subrectangular or long strip-shaped in surface view, with cuticular striations on the surface. Fibres usually in bundles, 3-32 μm in diameter, walls slightly thick or thin, with sparse pits and pit canals. Vessels mainly spiral and reticulate, 4-49 μm in diameter (Fig. 3).

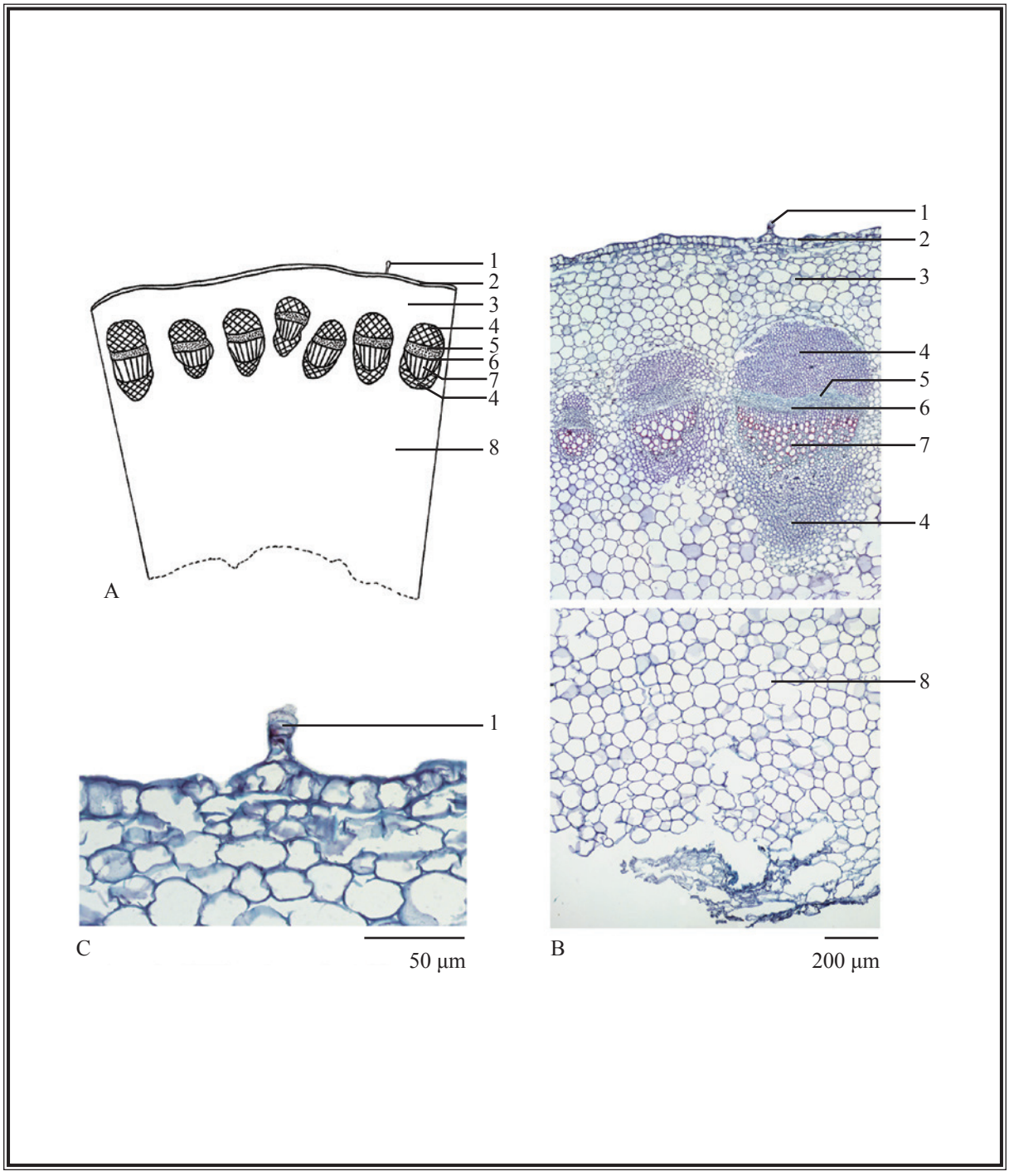

Figure 2 (i) Microscopic features of transverse section of stem of Saussureae Involucratae Herba

A. Sketch B. Section illustration C. Section magnified

1. Glandular hair 2. Epidermis 3. Cortex 4. Fibre bundles 5. Phloem
6. Fascicular cambium 7. Xylem 8. Pith

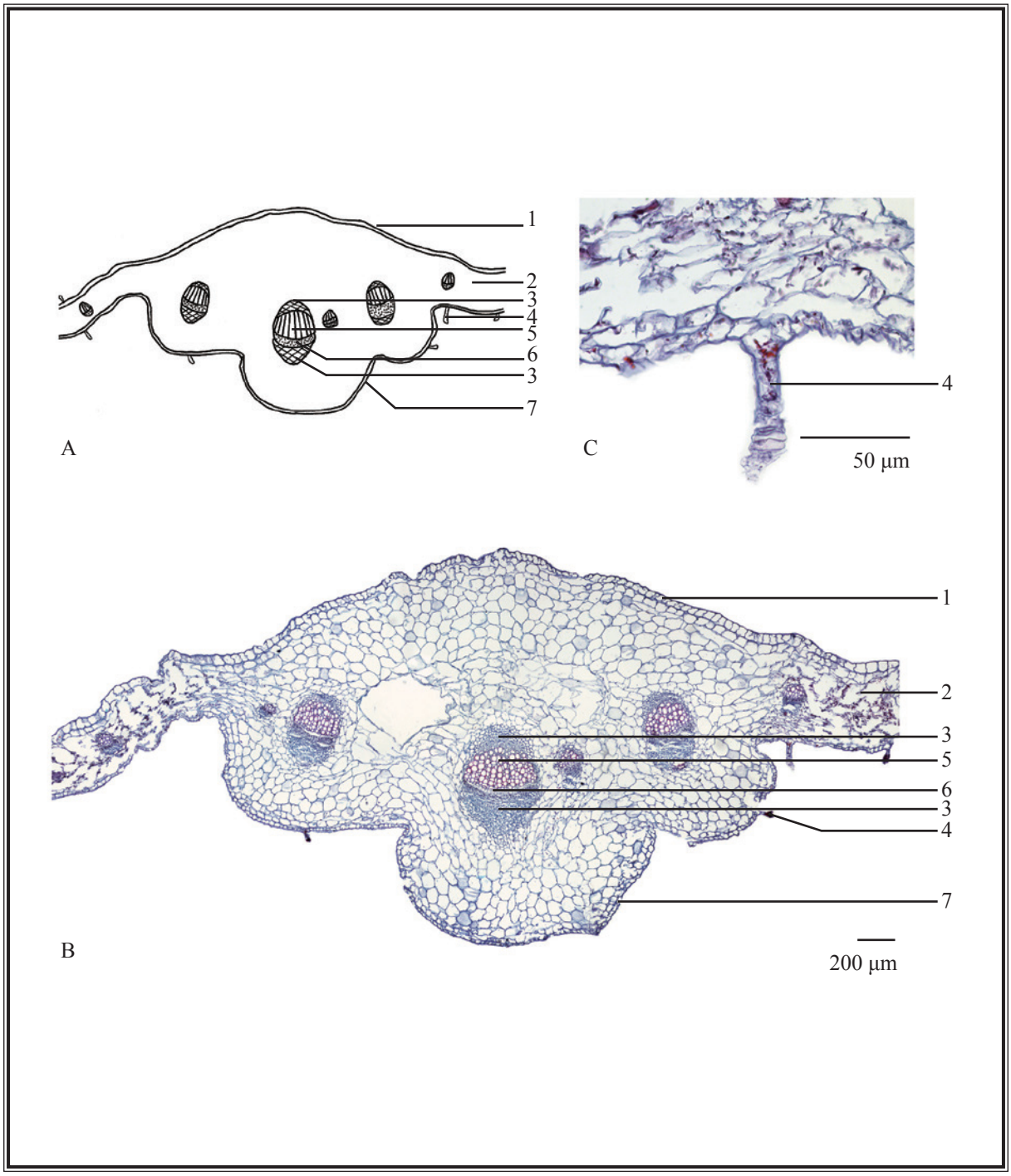

Figure 2 (ii) Microscopic features of transverse section of leaf of Saussureae Involucratae Herba

A. Sketch of midvein B. Section illustration of midvein
C. Section Magnified

1. Upper epidermis 2. Mesophyll 3. Fibre bundle 4. Glandular hair
5. Xylem 6. Phloem 7. Lower epidermis

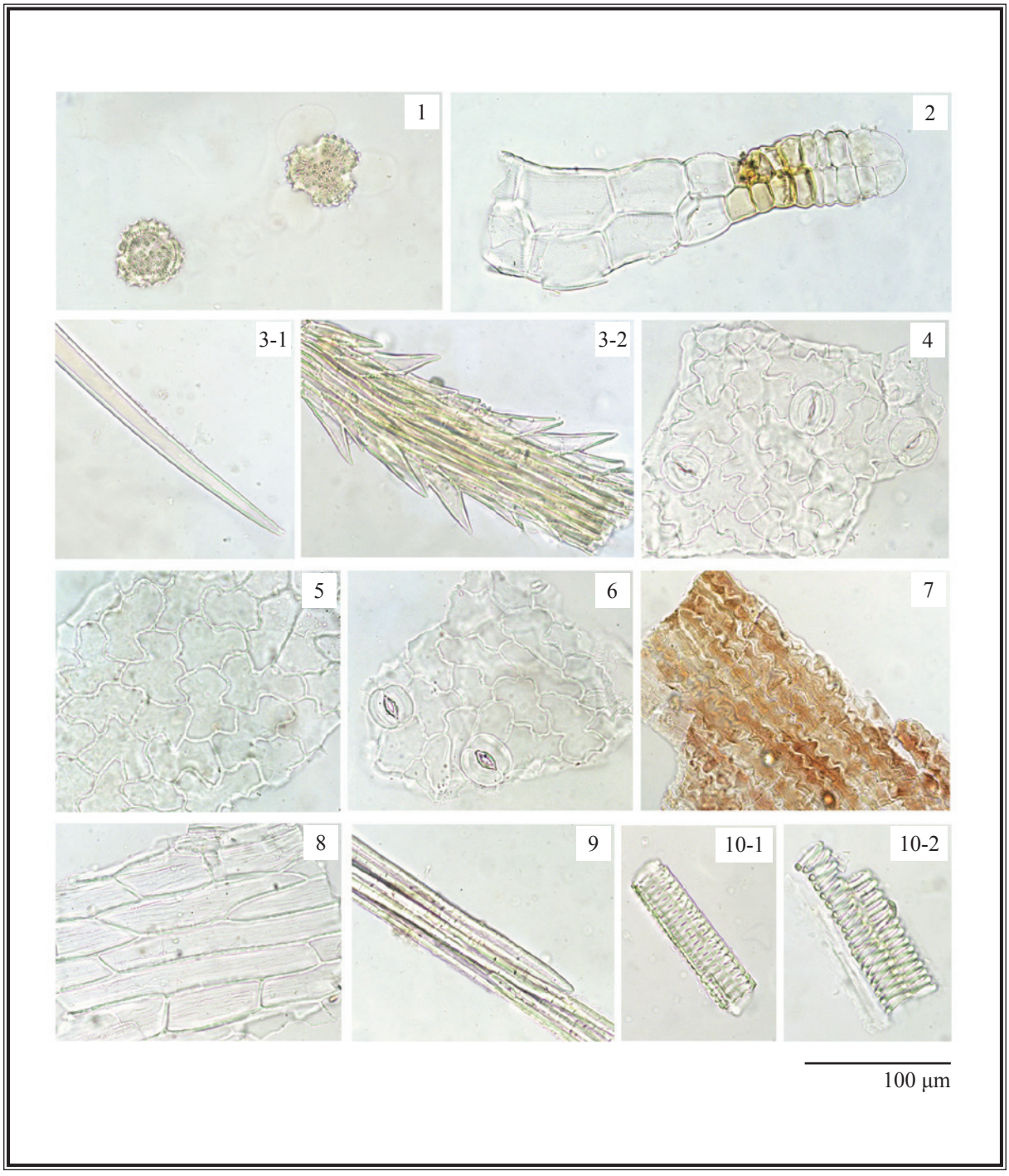

Figure 3 Microscopic features of powder of Saussureae Involucratae Herba (under the light microscope)

1. Pollen grains 2. Glandular hair
3. Pappus (3-1 fragment of apex, 3-2 fragment of base)
4. Lower epidermal cells of leaf 5. Upper epidermal cells of leaf 6. Epidermal cells of bracteal leaf
7. Epidermal cells of corolla 8. Epidermal cells of stem 9. Fibres
10. Vessels (10-1 reticulate vessel, 10-2 spiral vessels)

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

Standard solutions

Chlorogenic acid standard solution

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

Rutin standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, methanol, formic acid and water (10:1:1:1, v/v).

Spray reagent

Weigh 1 g of ferric trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 4 mL of methanol. Filter through a 0.45-μm nylon 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 chlorogenic acid standard solution (2 μL), rutin standard solution (4 μL) and the test solution (10 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 8 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 105ºC until the spots or bands become visible (about 3-5 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

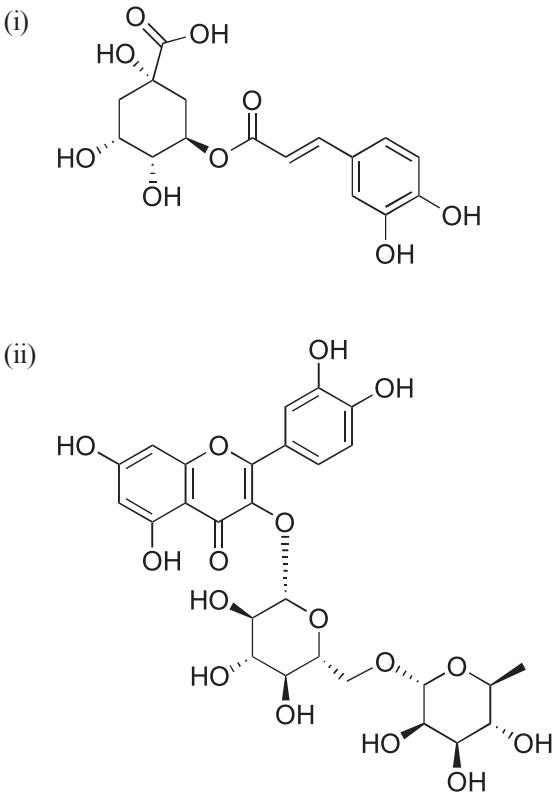

Figure 4 Chemical structures of (i) chlorogenic acid and (ii) rutin

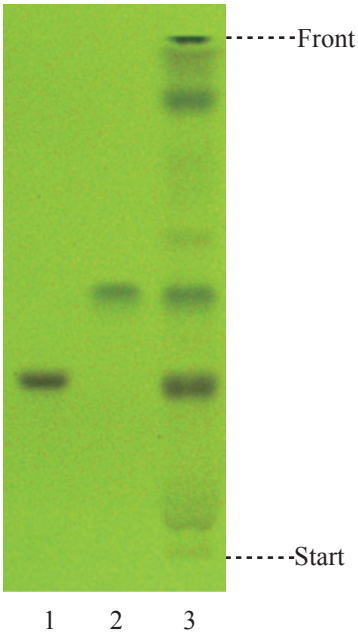

Figure 5 A reference HPTLC chromatogram of Saussureae Involucratae Herba extract observed under visible light after staining

1. Rutin standard solution 2. Chlorogenic acid standard solution 3. Test solution

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 chlorogenic acid and rutin (Fig. 5).

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

Standard solutions

Chlorogenic acid standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol (50%).

Rutin standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 3.0 mg of rutin CRS and dissolve in 10 mL of methanol. Pipette 1 mL of the solution to a 10-mL volumetric flask and make up to the mark with methanol (50%).

Test solution

Weigh 0.25 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more

time. Wash the residue with methanol (50%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (320 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.4% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 6	85	15	isocratic
6 – 25	85 → 70	15 → 30	linear gradient
25 – 35	70 → 60	30 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of chlorogenic acid Std-FP and rutin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and rutin should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and rutin peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and rutin peaks should not be less than 10000 theoretical plates.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1 (chlorogenic acid)	0.40	± 0.03
2 (marker, rutin)	1.00	-
3	1.22	± 0.03
4	1.34	± 0.03

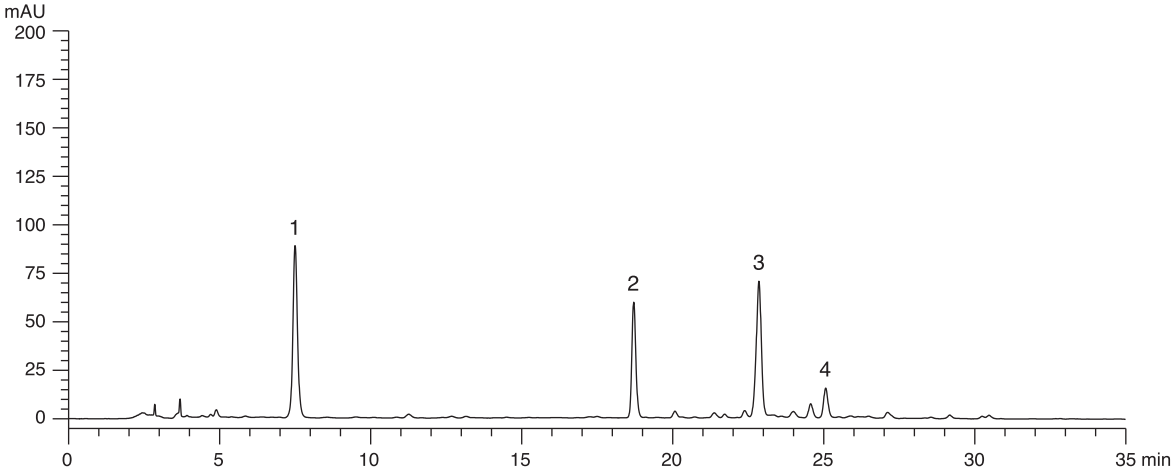

Figure 6 A reference fingerprint chromatogram of Saussureae Involucratae Herba extract

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 – Aflatoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVI)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.0%.

Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 22.0%.

Ethanol-soluble extractives (cold extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

Mixed chlorogenic acid and rutin standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 5.0 mg of chlorogenic acid CRS and 5.0 mg of rutin CRS, and dissolve in 10 mL of methanol.

Mixed chlorogenic acid and rutin standard solution for assay, Std-AS

Measure accurately the volume of the mixed chlorogenic acid and rutin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 1, 10, 40, 80, 100 mg/L for both chlorogenic acid and rutin.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.4% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 6	85	15	isocratic
6 – 25	85 → 70	15 → 30	linear gradient
25 – 35	70 → 60	30 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed chlorogenic acid and rutin Std-AS (40 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and rutin should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and rutin peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and rutin peaks should not be less than 10000 theoretical plates.

The *R* value between chlorogenic acid peak and the closest peak; and the *R* value between rutin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

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

Limits

The sample contains not less than 0.35% of chlorogenic acid ($C_{16}H_{18}O_9$) and not less than 0.49% of rutin ($C_{27}H_{30}O_{16}$), calculated with reference to the dried substance.

Saussureae Involucratae Herba（天山雪蓮）

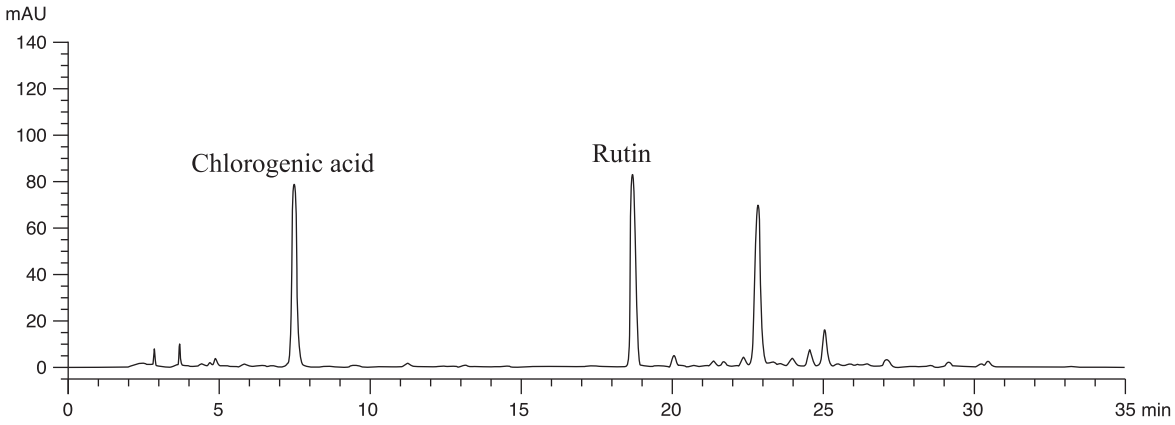

Figure 1 A reference assay chromatogram of Saussureae Involucratae Herba extract