Centellae Herba

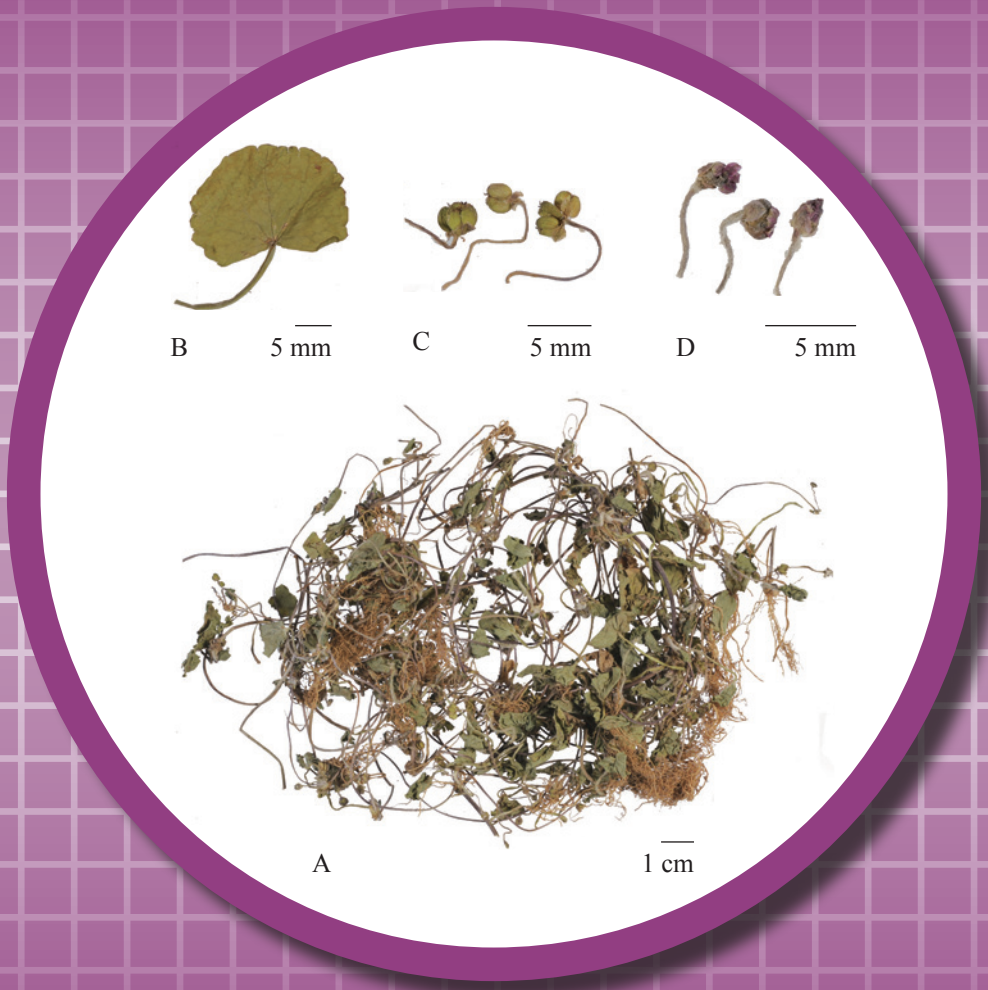

Figure 1 A photograph of Centellae Herba

A. Centellae Herba B. Magnified image of leaf
C. Magnified image of fruits D. Magnified image of flowers

1. NAMES

Official Name: Centellae Herba

Chinese Name: 積雪草

Chinese Phonetic Name: Jixuecao

2. SOURCE

Centellae Herba is the dried whole plant of *Centella asiatica* (L.) Urb. (Apiaceae). The whole plant is collected in summer and autumn, soil removed, then dried under the sun to obtain Centellae Herba.

3. DESCRIPTION

Usually crumpled into masses. Roots cylindrical, 2-4 cm long, 1-1.5 mm in diameter, externally pale yellow or greyish-brown. Stems slender and curved, yellowish-brown, with fine longitudinal wrinkles, and usually with fibrous roots on the nodes. Leaves mostly crumpled and broken, when intact flattened out, subrounded or reniform, 10-50 mm in diameter, greyish-green, margins roughly crenate; petioles 3-6 cm long, twisted. Umbels axillary, small. Cremocarps oblate, with distinctly protuberant longitudinal ridges and fine reticulate striations; fruit stalk extremely short. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of several layers of cells. Cortex narrow, consists of 3-4 layers of parenchymatous cells. Phloem broad, cells mostly crumpled. Cambium consists of 1 layer of cells, arranged in a ring. Xylem fibres visible; xylem ray consists 1-3 rows of cells [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of cells, cells subrounded or subsquare, with 2-4 layers of collenchymatous cells beneath. Cortex consists of 7-9 layers of parenchymatous cells, several outer layer cells with unevenly thickened walls. Collateral vascular bundles 6-8; slightly lignified fibre groups occurring at the outside of phloem; fascicular cambium distinct, consists of 2-3 layers of small cells; xylem vessels arranged radically. Pith large, consists of

large parenchymatous cells. Secretory canal located in cortex and rays, 23-34 μm in diameter, composed of 5-7 secretory cells [Fig. 2 (ii)].

Leaf: Upper epidermis consists of 1 layer of cells, cells polygonal, relatively large. Palisade tissue consists of 1 layer of cells. Spongy tissue arranged loosely. Collenchyma located at the upper side of midrib. Vascular bundles of midrib collateral. Lower epidermis consists of 1 layer of cells, cells polygonal, relatively large [Fig. 2 (iii)].

Powder

Colour brownish-yellow. Stomata anomocytic or anisocytic. Prisms of calcium oxalate abundant, 3-21 μm in diameter; polychromatic under the polarized microscope. Pollen grains spherical, 11-43 μm in diameter, sculpture indistinct, with 3 germinal pores. Clusters of calcium oxalate occasionally found. Vessels mainly spiral, 4-71 μm in diameter. Secretory canals contain numerous yellow substances. Non-glandular hairs multicellular, mostly broken, intact cells fusiform, relatively straight (Fig. 3).

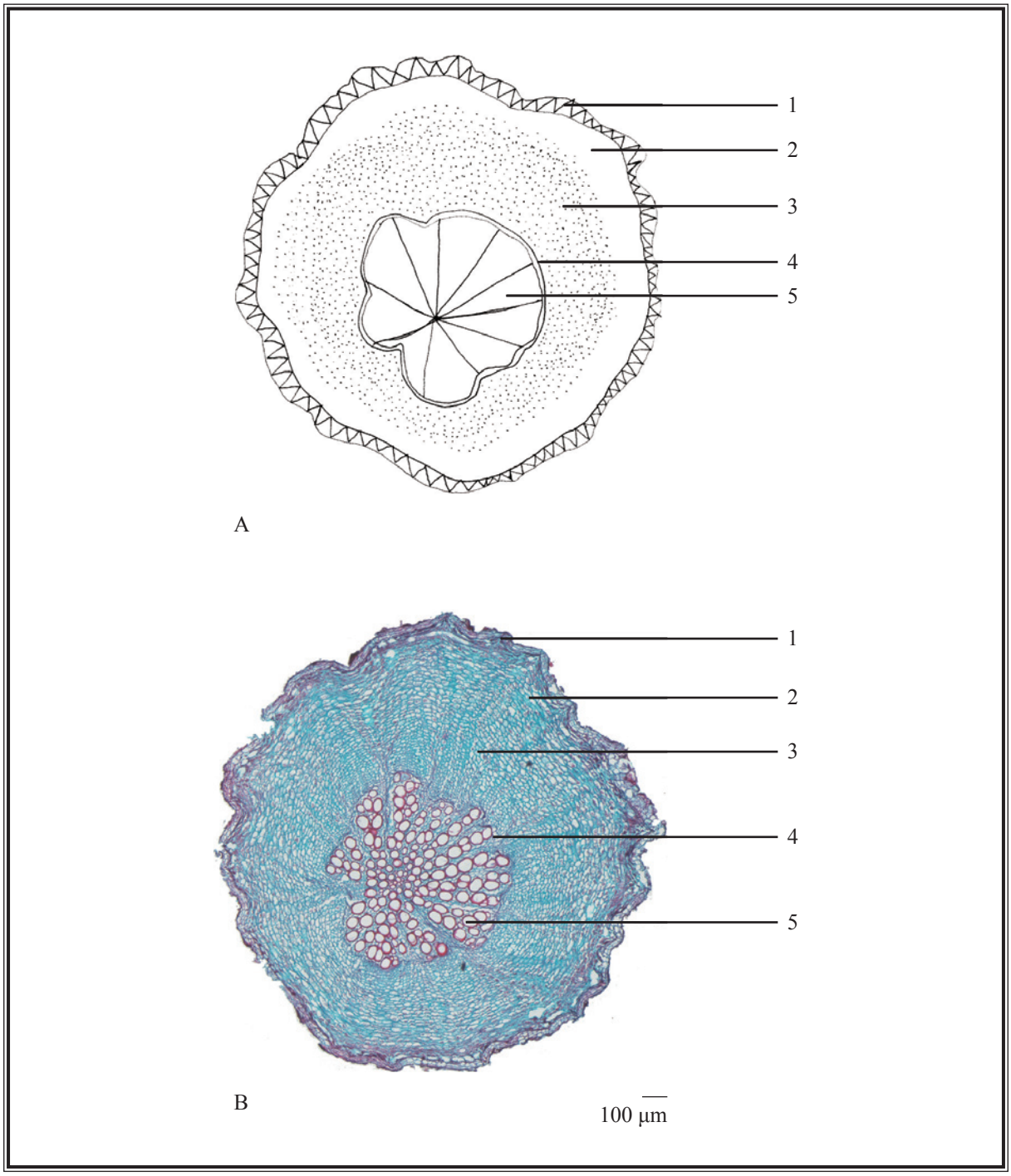

Figure 2 (i) Microscopic features of transverse section of root of Centellae Herba

A. Sketch B. Section illustration

1. Cork 2. Cortex 3. Phloem 4. Cambium 5. Xylem

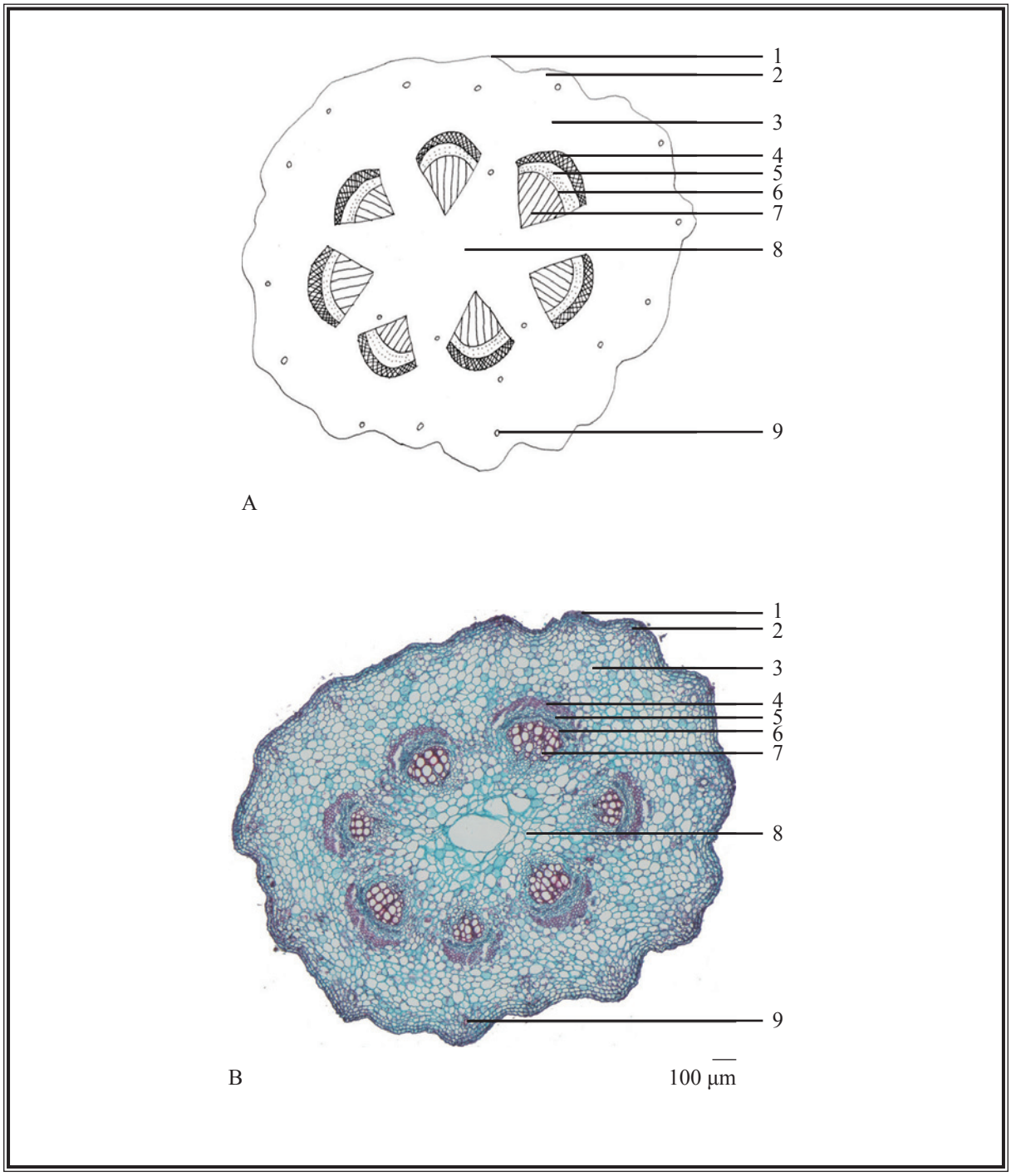

Figure 2 (ii) Microscopic features of transverse section of stem of Centellae Herba

A. Sketch B. Section illustration

1. Epidermis 2. Collenchymatous cell 3. Cortex 4. Fibres 5. Phloem 6. Fascicular cambium 7. Xylem 8. Pith 9. Secretory canal

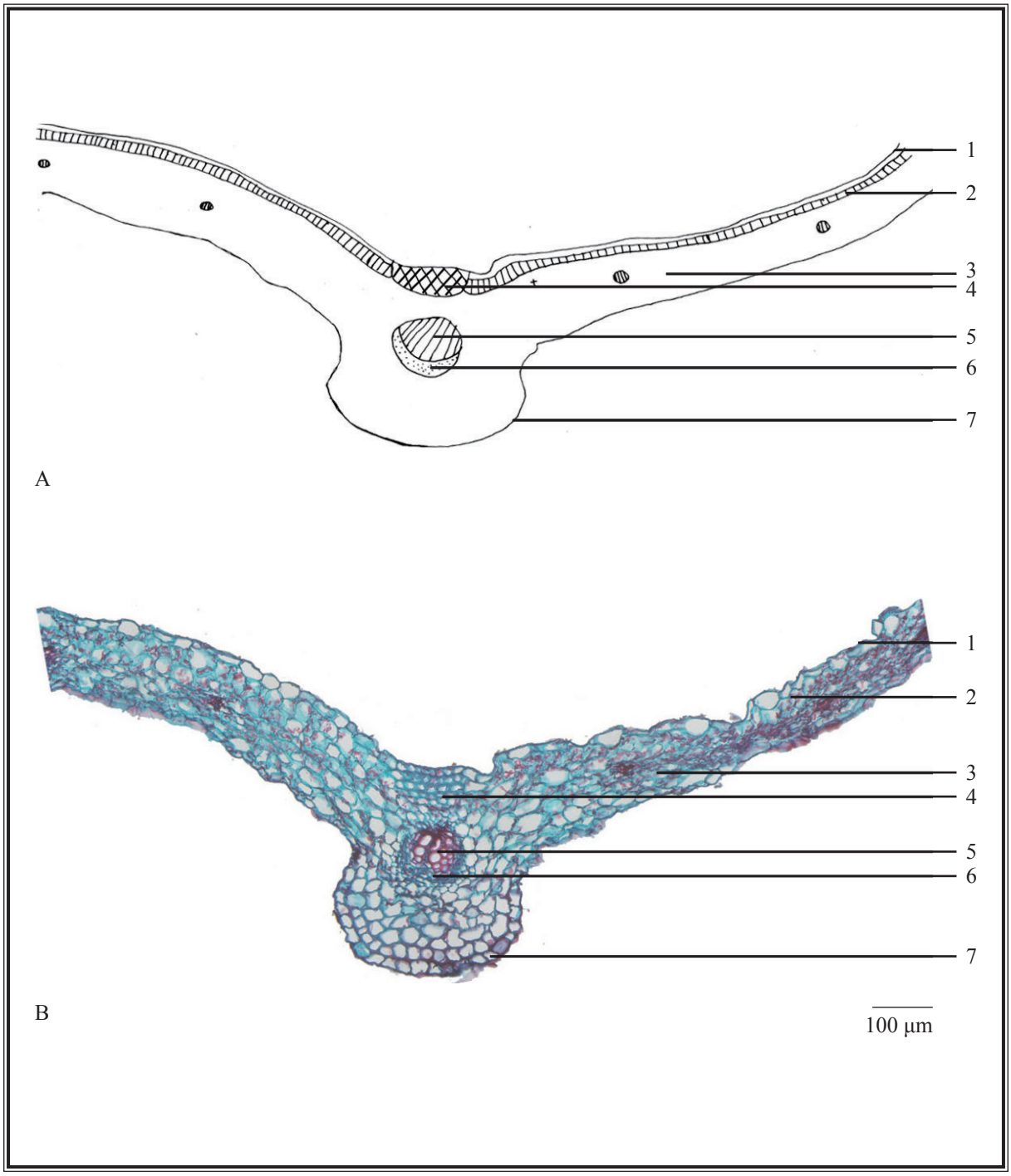

Figure 2 (iii) Microscopic features of transverse section of leaf of Centellae Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Collenchyma 5. Xylem 6. Phloem 7. Lower epidermis

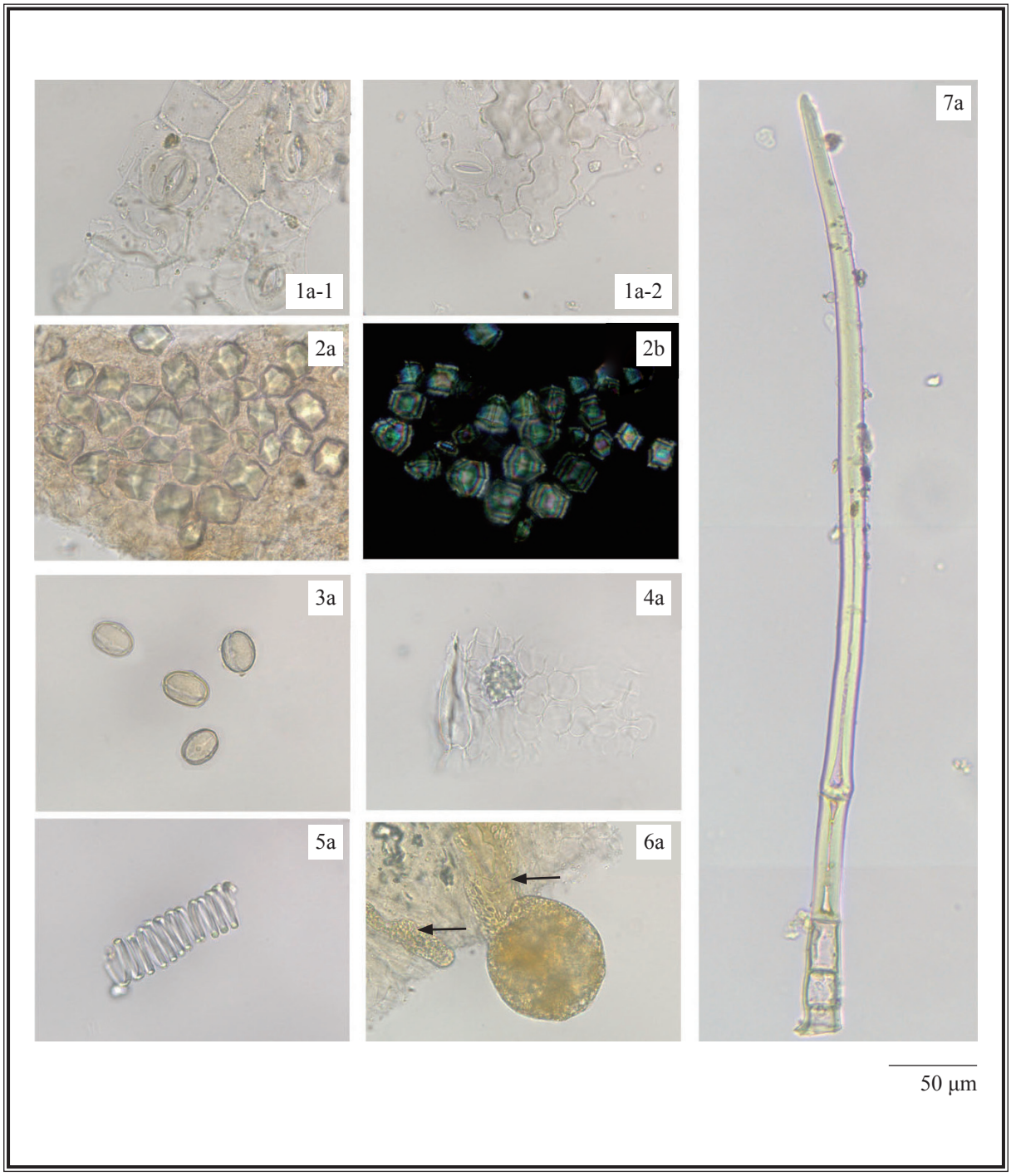

Figure 3 Microscopic features of powder of Centellae Herba

1. Stomata (1-1 anomocytic, 1-2 anisocytic) 2. Prisms of calcium oxalate 3. Pollen grains
4. Cluster of calcium oxalate 5. Spiral vessel 6. Secretory canals 7. Non-glandular hair

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

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

Standard solutions

Asiaticoside standard solution

Weigh 2.0 mg of asiaticoside CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Madecassoside standard solution

Weigh 2.0 mg of madecassoside CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, glacial acetic acid and water (8:2.6:2.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Filter the mixture.

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 asiaticoside standard solution (3 µL), madecassoside standard solution (4 µL) and the test solution (4 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 dry in air. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

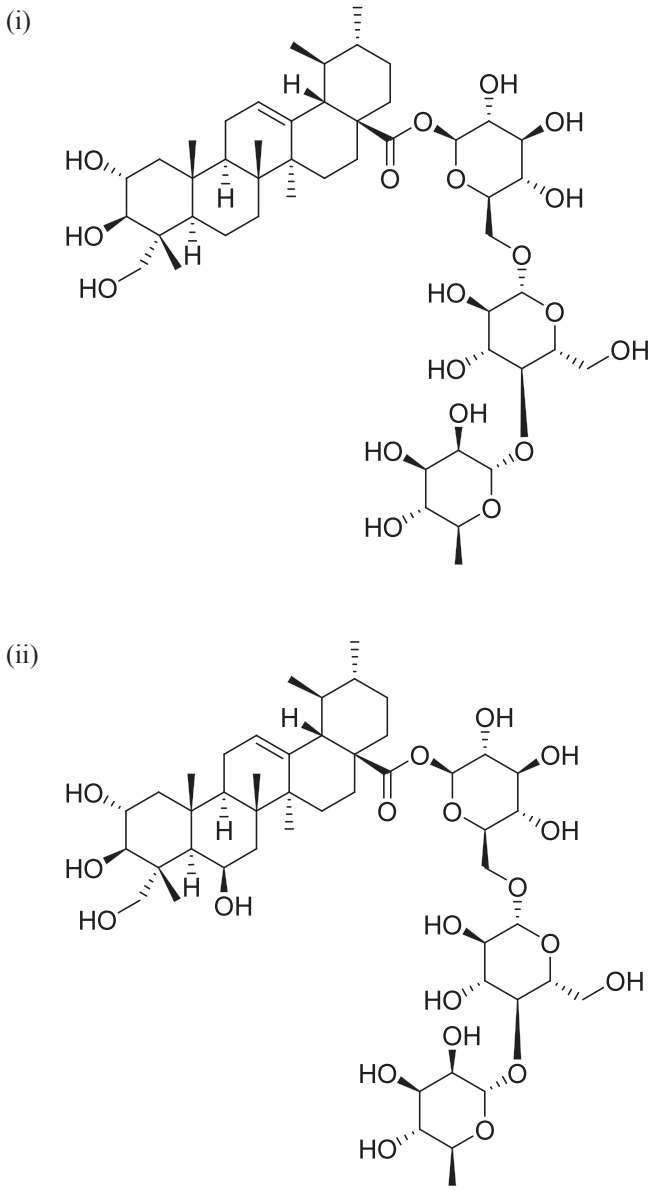

Figure 4 Chemical structures of (i) asiaticoside and (ii) madecassoside

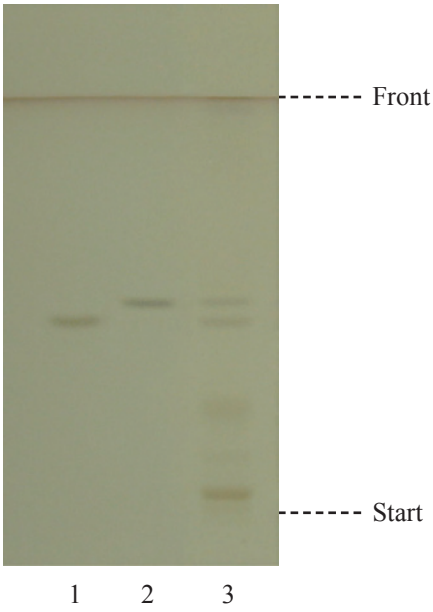

Figure 5 A reference HPTLC chromatogram of Centellae Herba extract observed under visible light after staining

1. Madecassoside standard solution 2. Asiaticoside 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 asiaticoside and madecassoside (Fig. 5).

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

Standard solutions

Asiaticoside standard solution for fingerprinting, Std-FP (250 mg/L)

Weigh 2.5 mg of asiaticoside CRS and dissolve in 10 mL of methanol (80%).

Madecassoside standard solution for fingerprinting, Std-FP (250 mg/L)

Weigh 2.5 mg of madecassoside CRS and dissolve in 10 mL of methanol (80%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of methanol (80%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (205 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 20ºC 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)	Acetonitrile (%, v/v)	0.15% Phosphoric acid (%, v/v)	Elution
0 – 15	21	79	isocratic
15 – 32	21 → 36	79 → 64	linear gradient
32 – 50	36 → 40	64 → 60	linear gradient
50 – 60	40 → 80	60 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of asiaticoside Std-FP and madecassoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of asiaticoside and madecassoside should not be more than 5.0%; the RSD of the retention times of asiaticoside and madecassoside peaks should not be more than 2.0%; the column efficiencies determined from asiaticoside and madecassoside peaks should not be less than 100000 and 200000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and 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. 6).

Procedure

Separately inject asiaticoside Std-FP, madecassoside Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention times of asiaticoside and madecassoside peaks in the chromatograms of asiaticoside Std-FP, madecassoside Std-FP and the retention times of the seven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify asiaticoside and madecassoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of asiaticoside Std-FP and madecassoside Std-FP. The retention times of asiaticoside and madecassoside 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 seven characteristic peaks of Centellae Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the seven characteristic peaks of Centellae Herba extract

Peak No.	RRT	Acceptable Range
1	0.98	± 0.03
2 (marker, madecassoside)	1.00	-
3 (asiaticoside)	1.16	± 0.03
4	1.22	± 0.03
5	1.50	± 0.03
6 (madecassic acid)	1.94	± 0.03
7 (asiatic acid)	2.37	± 0.04

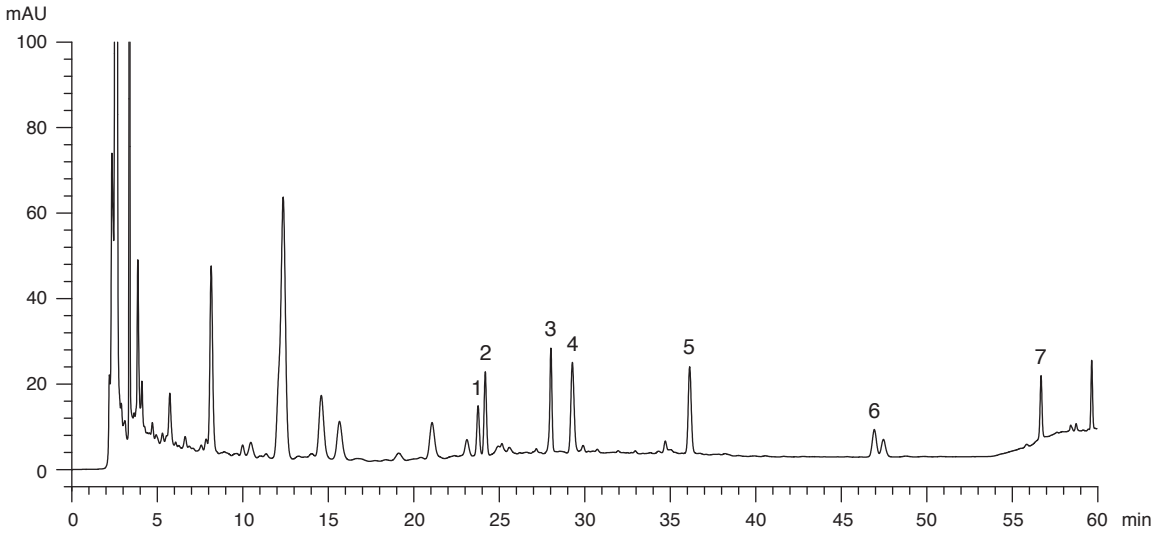

Figure 6 A reference fingerprint chromatogram of Centellae Herba extract

For positive identification, the sample must give the above seven 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 (except cadmium should not be more than 5.5 mg/kg).

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.0%.
Acid-insoluble ash: not more than 3.5%.

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 26.0%.
Ethanol-soluble extractives (cold extraction method): not less than 24.0%.

7. ASSAY

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

Standard solution

Mixed asiaticoside and madecassoside standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 5.0 mg of asiaticoside CRS and 5.0 mg of madecassoside CRS, and dissolve in 10 mL of methanol (80%).

Mixed asiaticoside and madecassoside standard solution for assay, Std-AS

Measure accurately the volume of the mixed asiaticoside and madecassoside Std-Stock, dilute with methanol (80%) to produce a series of solutions of 5, 10, 20, 40, 100 mg/L for asiaticoside and 10, 30, 50, 75, 100 mg/L for madecassoside.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of methanol (80%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (80%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (205 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 (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.15% Phosphoric acid (%, v/v)	Elution
0 – 10	21	79	isocratic
10 – 25	21 → 23	79 → 77	linear gradient
25 – 50	23	77	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed asiaticoside and madecassoside Std-AS (20 mg/L for asiaticoside and 50 mg/L for madecassoside). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of asiaticoside and madecassoside should not be more than 5.0%; the RSD of the retention times of asiaticoside and madecassoside peaks should not be more than 2.0%; the column efficiencies determined from asiaticoside and madecassoside peaks should not be less than 18000 theoretical plates.

The *R* value between asiaticoside peak and the closest peak; and the *R* value between madecassoside 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 asiaticoside and madecassoside Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of asiaticoside and madecassoside against the corresponding concentrations of the mixed asiaticoside and madecassoside 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 asiaticoside and madecassoside peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed asiaticoside and madecassoside Std-AS. The retention times of asiaticoside and madecassoside 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 asiaticoside and madecassoside in the test solution, and calculate the percentage contents of asiaticoside and madecassoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.97% of the total content of asiaticoside ($C_{48}H_{78}O_{19}$) and madecassoside ($C_{48}H_{78}O_{20}$), calculated with reference to the dried substance.

8. CAUTION

This CMM should be used after proper processing (such as decoction).