Lysimachiae Herba

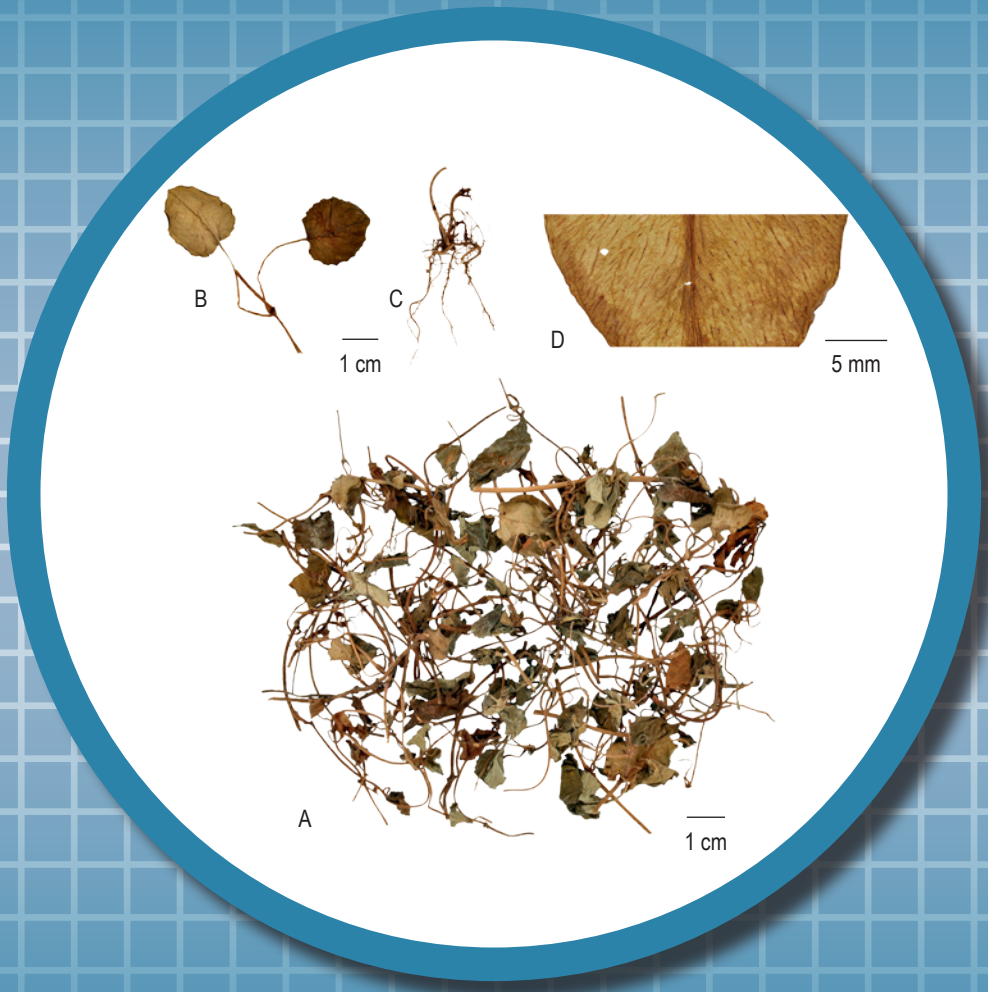

Figure 1 A photograph of Lysimachiae Herba

A. Lysimachiae Herba B. Leaves C. Rootlets
D. Leaf after soaking in water

1. NAMES

Official Name: Lysimachiae Herba

Chinese Name: 金錢草

Chinese Phonetic Name: Jinqiancao

2. SOURCE

Lysimachiae Herba is the dried herb of *Lysimachia christinae* Hance (Primulaceae). The whole plant is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Lysimachiae Herba.

3. DESCRIPTION

Herb usually twisted into masses, glabrous or sparsely pubescent. Stem twisted, externally brown to dark brownish-red, with longitudinal striations; stem nodes of the lower part sometimes with rootlets, fracture solid. Leaves opposite, mostly shriveled, when intact and flattened, broad ovate or cordate, 1-4 cm long, 1-4.5 cm wide, base slightly concave, margin entire; petioles 0.8-5 cm long; upper surface greyish-green to reddish-brown, lower surface relatively pale, midrib distinctly protuberant; after soaking in water, the black or brown stripes visible under the light. Odour slight; taste weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Epidermal cells subsquare to rectangular, slightly lignified. Cortex relatively broad, consisting of approximately 10 layers of parenchymatous cells. Stele occupies about 1/2 of the root. Endodermis consists of 1 layer of cells, arranged in a ring, located in the outermost part of stele. Phloem narrow. Xylem broad [Fig. 2 (i)].

Stem: Glandular hairs present occasionally, each with an unicellular head and a 1- to 2-celled stalk. Epidermis covered with cuticle. Cortex broad, scattered with reddish-brown secretion. Secretory canals scattered, containing reddish-brown lumpy secretion. Endodermis distinct. Pericyclic fibres arranged in an interrupted ring, with slightly lignified wall. Phloem narrow. Xylem arranged in a ring. Pith usually hollowed, parenchymatous cells contain starch granules [Fig. 2 (ii)].

Leaf: Upper epidermal cells subsquare, covered with cuticle, without stoma. Palisade tissue normally 1 layer of cells, seldom 2 layers. Spongy tissue consists of 4-6 layers of cells. Secretory canal scattered, containing reddish-brown lumpy secretion. Midvein 1 distinctly protruding, consisting of xylem, phloem and collenchymatous cells. Lateral veins small, undeveloped. Collenchymatous cells located next to the phloem and near the lower epidermis. Lower epidermis similar to the upper epidermis, but with stomata. Glandular hairs present occasionally, each with an unicellular head and a 1- to 2-celled stalk [Fig. 2 (iii)].

Powder

Colour pale brown to brown. Upper epidermal cells with slightly sinuous wall. Lower epidermal cells with slightly sinuous wall, stomata anisocytic or anomocytic, 26-65 μm long and 24-48 μm wide, subsidiary cells 3-5. Glandular hairs reddish-brown, consisting of unicellular head and 1- to 2-celled stalk, head 18-41 μm in diameter. Glandular hairs always fallen off from epidermis, leaving a cicatrix surrounded by fine striations. Content of secretory canal numerous, brown to reddish-brown, subrounded or irregular. Fibres rectangular, lignified, 7-38 μm in diameter. Vessels mainly spiral, bordered-pitted or reticulate, 4-40 μm in diameter. Simple starch granules, subrounded, 4-25 μm in diameter, hilum cleft-like or pointed; black and cruciate-shaped under the polarized microscope; compound starch granules mostly composed of 2-5 units (Fig. 3).

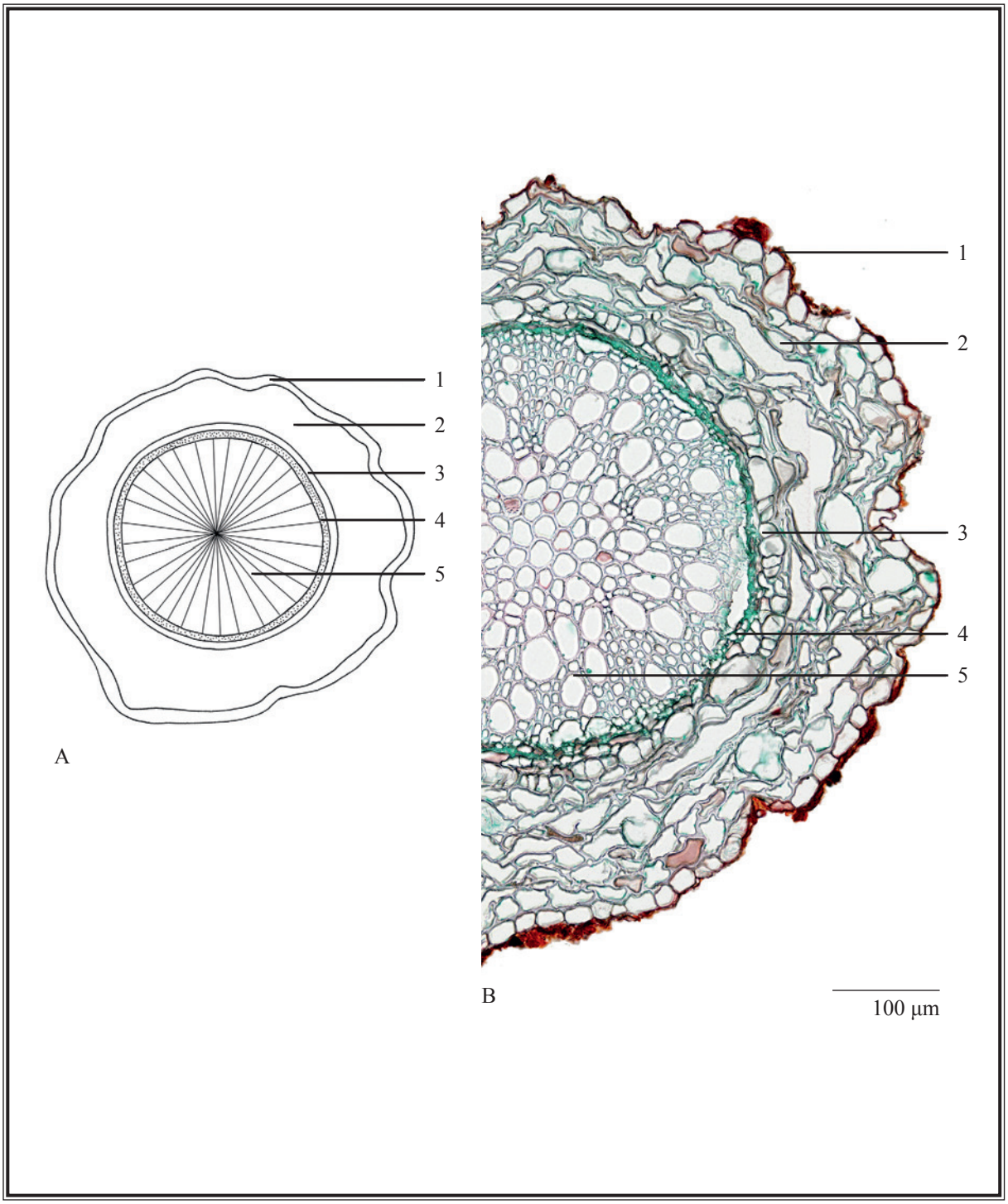

Figure 2 (i) Microscopic features of transverse section of root of Lysimachiae Herba

A. Sketch B. Section illustration

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

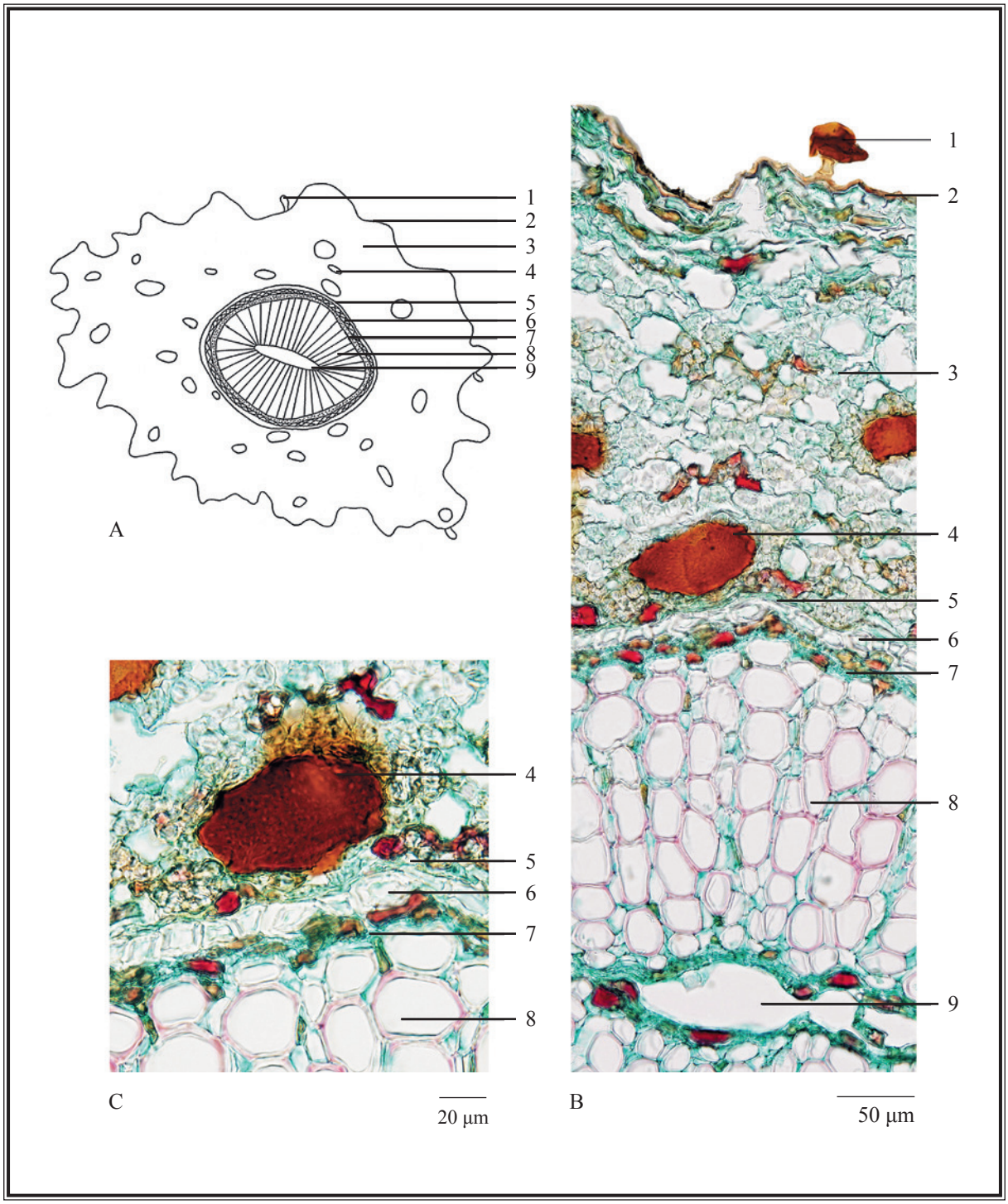

Figure 2 (ii) Microscopic features of transverse section of stem of Lysimachiae Herba

A. Sketch B. Section illustration C. Section magnified

1. Glandular hair 2. Epidermis 3. Cortex 4. Secretory canal 5. Endodermis
6. Pericyclic fibre 7. Phloem 8. Xylem 9. Pith

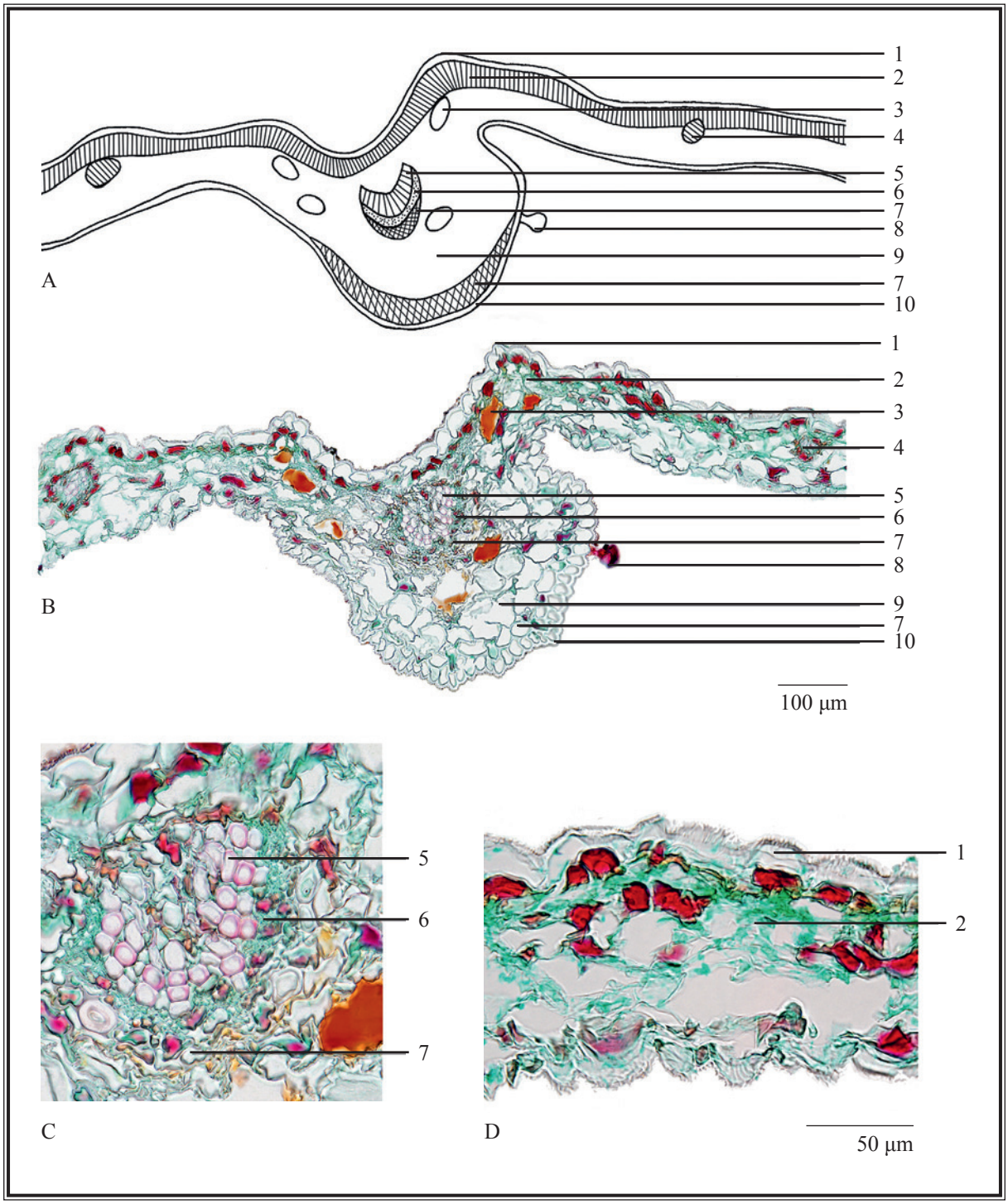

Figure 2 (iii) Microscopic features of transverse section of leaf of Lysimachiae Herba

A. Sketch B. Section illustration C. Vascular bundle D. Blade

1. Upper epidermis 2. Palisade tissue 3. Secretory canal 4. Lateral vein
5. Xylem 6. Phloem 7. Collenchyma 8. Glandular hair
9. Spongy tissue 10. Lower epidermis

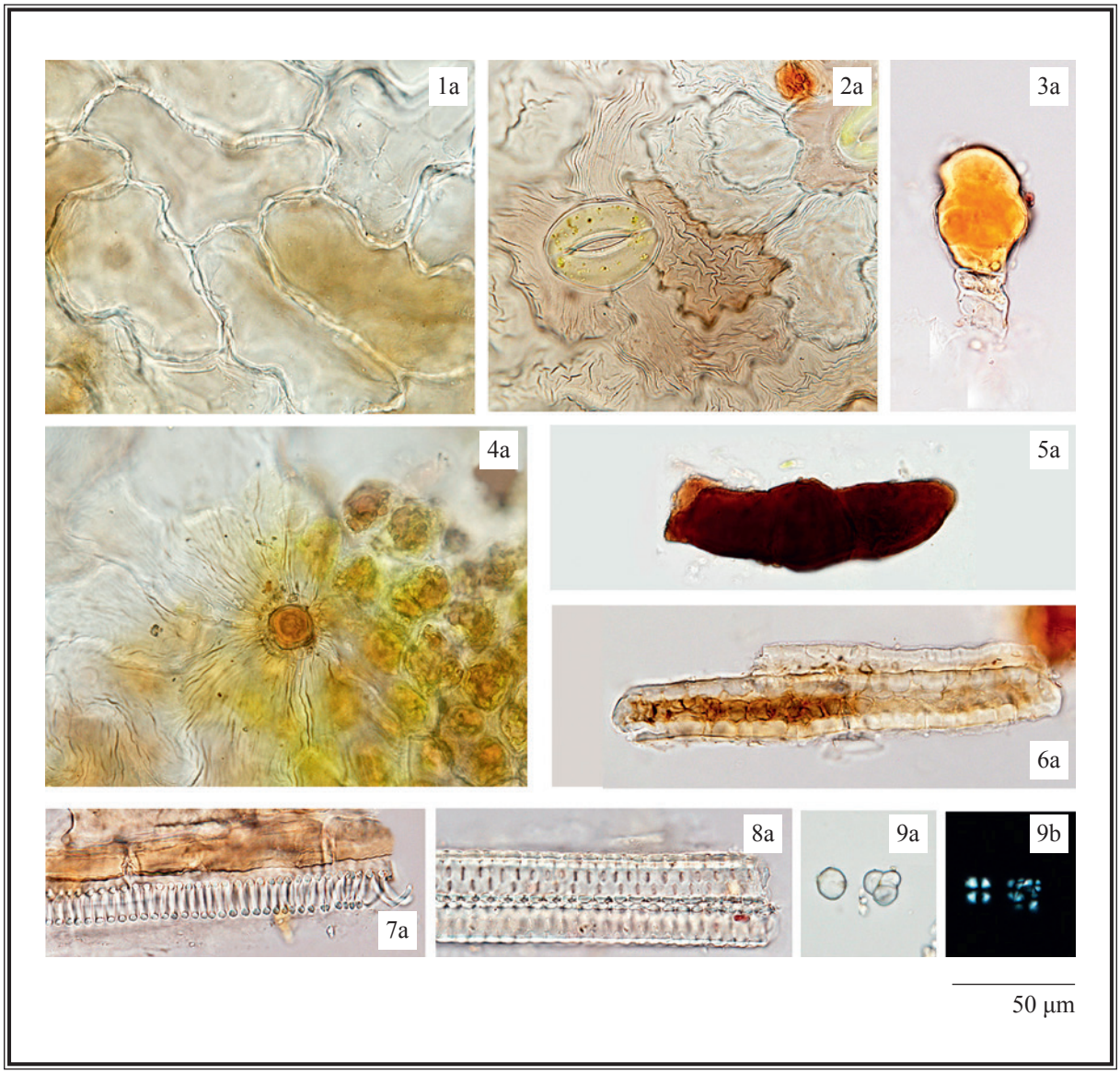

Figure 3 Microscopic features of powder of Lysimachiae Herba

1. Upper epidermal cells 2. Lower epidermal cells with stomata
3. Glandular hair 4. Cicatrix 5. Contents of secretory canal 6. Fibre
7. Spiral vessel 8. Bordered-pitted vessel 9. Starch granules

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

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

Standard solution

Kaempferol standard solution

Weigh 1.0 mg of kaempferol CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of n-hexane, ethyl acetate and formic acid (10:6:1, v/v).

Spray reagent

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

Test solution

Weigh 2.0 g of the powdered sample and place it in a 250-mL conical flask, then add 50 mL of ethanol (80%). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

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 kaempferol standard solution (1 µL) and the test solution (4 µL) to the plate. 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 it in air until the spots or bands become visible. Examine the plate under UV light (366 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 kaempferol.

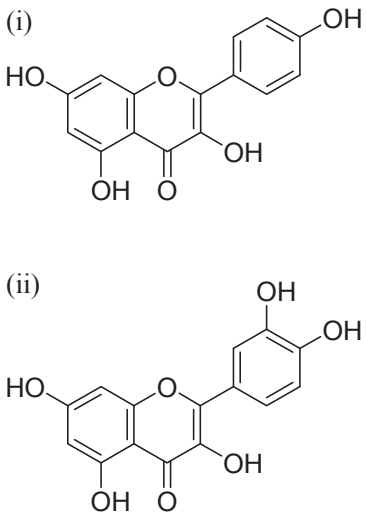

Figure 4 Chemical structures of (i) kaempferol and (ii) quercetin

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

Standard solutions

Kaempferol standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of kaempferol CRS and dissolve in 10 mL of methanol.

Quercetin standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of quercetin CRS (Fig. 4) and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (80%). Sonicate (220 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter and transfer the filtrate to a 10-mL volumetric flask. 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 (364 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 15	70 → 55	30 → 45	linear gradient
15 – 35	55	45	isocratic
35 – 60	55 → 30	45 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of kaempferol Std-FP and quercetin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of kaempferol and quercetin should not be more than 5.0%; the RSD of the retention times of kaempferol and quercetin peaks should not be more than 2.0%; the column efficiencies determined from kaempferol and quercetin peaks should not be less than 100000 and 50000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject kaempferol Std-FP, quercetin Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention times of kaempferol and quercetin peaks in the chromatograms of kaempferol Std-FP, quercetin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify kaempferol and quercetin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of kaempferol Std-FP and quercetin Std-FP. The retention times of kaempferol and quercetin 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 Lysimachiae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.37	± 0.03
2	0.39	± 0.03
3 (quercetin)	0.85	± 0.03
4 (marker, kaempferol)	1.00	-

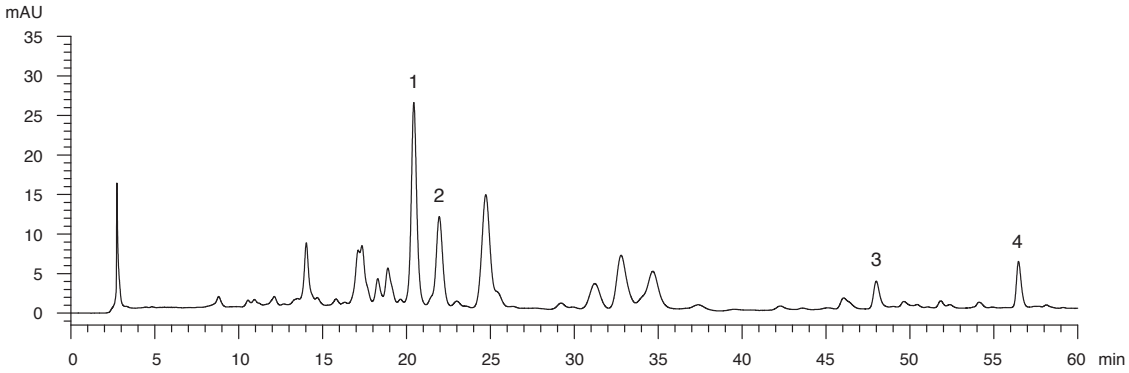

Figure 5 A reference fingerprint chromatogram of Lysimachiae Herba extract

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. 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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 13.0%.
Acid-insoluble ash: not more than 5.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (hot extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

Mixed kaempferol and quercetin standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of kaempferol CRS and 2.0 mg of quercetin CRS, and dissolve in 10 mL of methanol.

Mixed kaempferol and quercetin standard solution for assay, Std-AS

Measure accurately the volume of the mixed kaempferol and quercetin Std-Stock, dilute with methanol to produce a series of solutions of 0.5, 1, 2, 5, 10 mg/L for both kaempferol and quercetin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of methanol (80%). Shake the mixture overnight (16 h). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for two more times each with 30 mL of methanol (80%) without shaking overnight. Combine the filtrates. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 25 mL of methanol (80%) and 5 mL of hydrochloric acid. Reflux the mixture for 1.5 h. Cool down in an ice water bath immediately. Filter and transfer the filtrate to a 100-mL volumetric flask. 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 (364 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)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 25	50 → 25	50 → 75	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed kaempferol and quercetin Std-AS (2 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of kaempferol and quercetin should not be more than 5.0%; the RSD of the retention times of kaempferol and quercetin peaks should not be more than 2.0%; the column efficiencies determined from kaempferol and quercetin peaks should not be less than 30000 and 20000 theoretical plates respectively.

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

Limits

The sample contains not less than 0.10% of the total content of kaempferol ($C_{15}H_{10}O_6$) and quercetin ($C_{15}H_{10}O_7$), calculated with reference to the dried substance.