Epimedii Herba

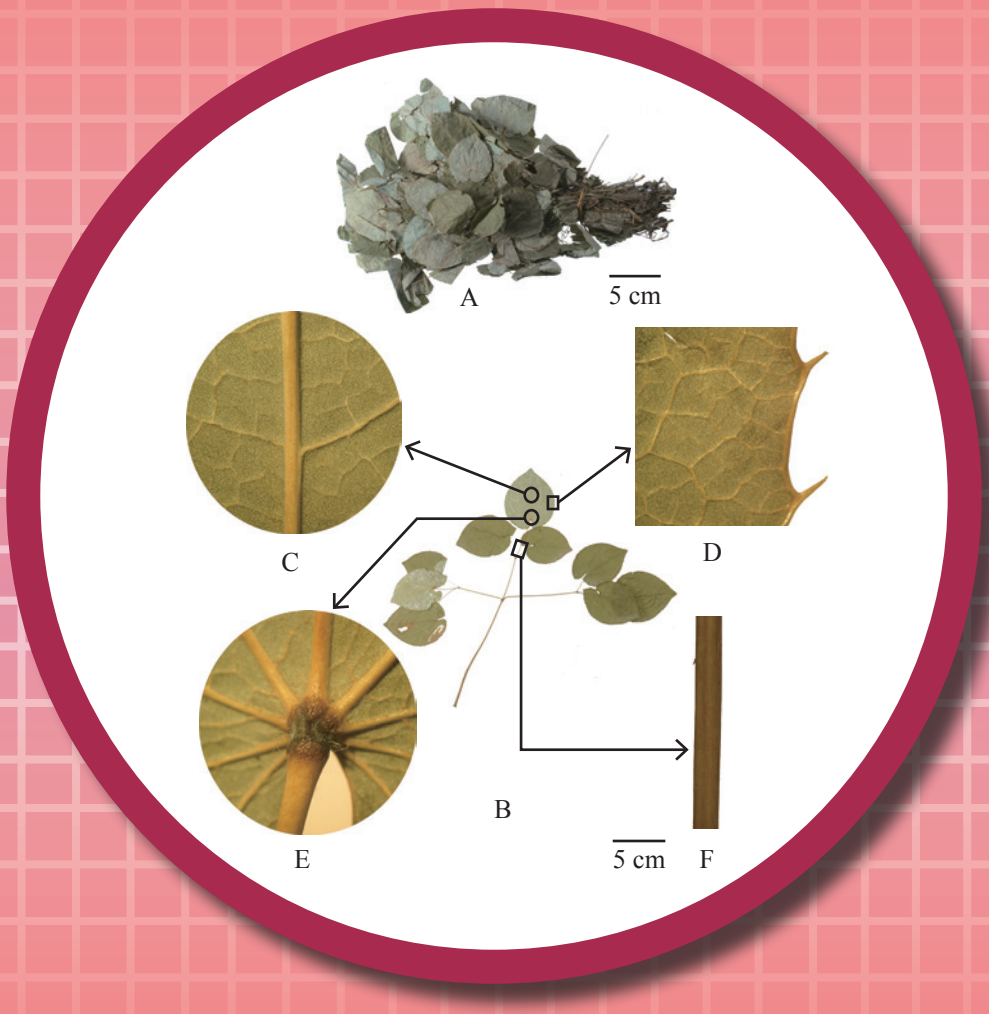

Figure 1 (i) A photograph of dried aerial part of *Epimedium brevicornu* Maxim.

A. Aerial part of herb

B. Intact flattened leaves showing biternately compound leaves after soaking into water

C. Magnified image of lower surface of leaf

D. Magnified image of spinose serrulations on margin of leaf

E. Magnified image of base of leaf

F. Magnified image of petiolule

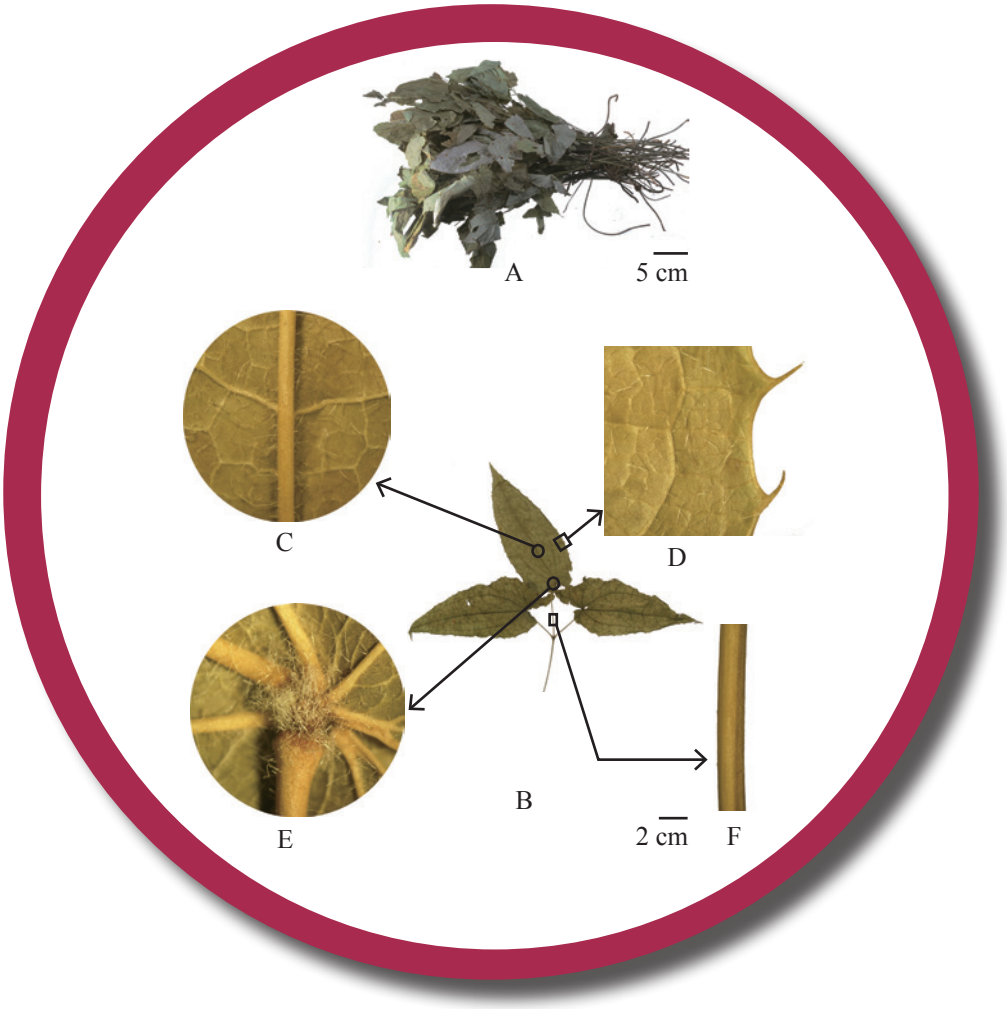

Figure 1 (ii) A photograph of dried aerial part of *Epimedium sagittatum* (Sieb. et Zucc.) Maxim.

A. Aerial part of herb

B. Intact flattened leaves showing ternately compound leaves after soaking into water

C. Magnified image of lower surface of leaf

D. Magnified image of spinose serrulations on margin of leaf

E. Magnified image of base of leaf

F. Magnified image of petiolule

Figure 1 (iii) A photograph of dried aerial part of *Epimedium pubescens* Maxim.

A. Aerial part of herb

B. Intact flattened leaves showing ternately compound leaves after soaking into water

C. Magnified image of lower surface of leaf

D. Magnified image of spinose serrulations on margin of leaf

E. Magnified image of base of leaf

F. Magnified image of petiolule

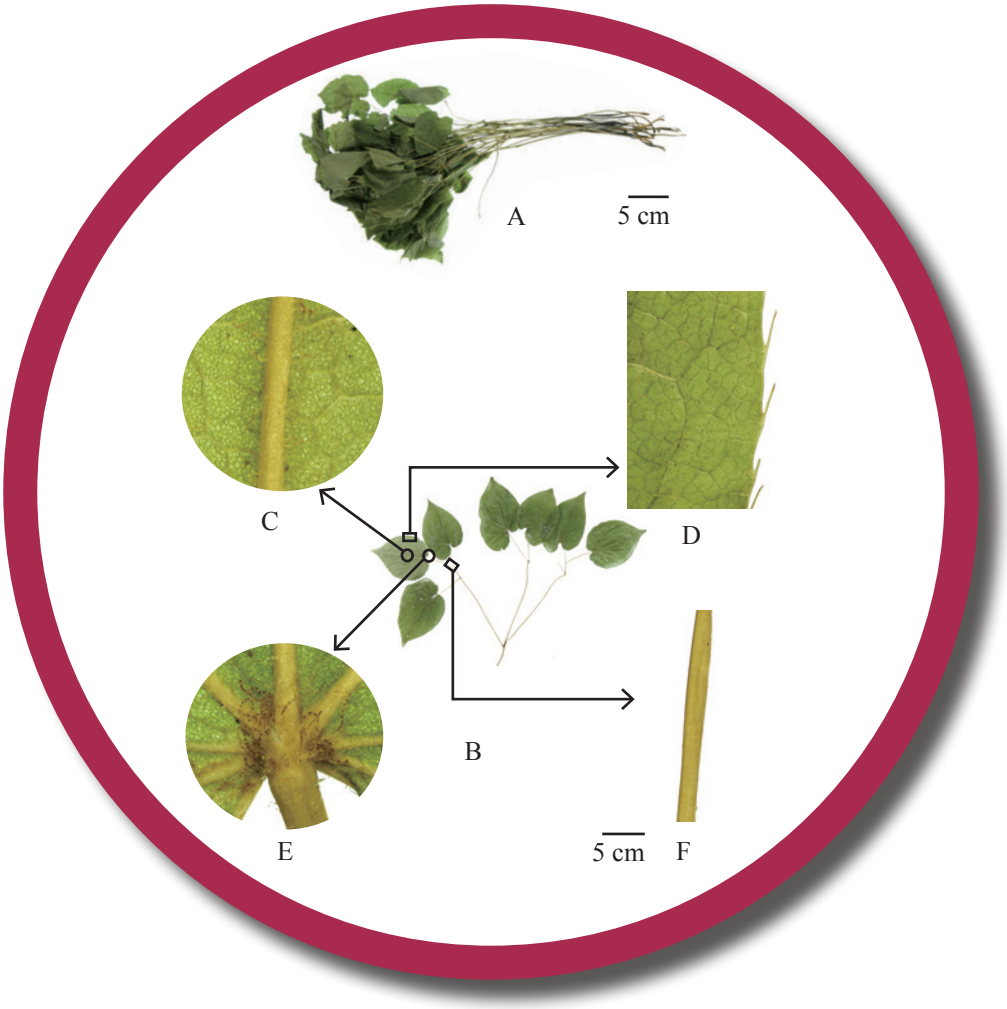

Figure 1 (iv) A photograph of dried aerial part of *Epimedium koreanum* Nakai

A. Aerial part of herb

B. Intact flattened leaves showing biternately compound leaves after soaking into water

C. Magnified image of lower surface of leaf

D. Magnified image of spinose serrulations on margin of leaf

E. Magnified image of base of leaf

F. Magnified image of petiolule

1. NAMES

Official Name: Epimedii Herba

Chinese Name: 淫羊藿

Chinese Phonetic Name: Yinyanghuo

2. SOURCE

Epimedii Herba is the dried aerial part of *Epimedium brevicornu* Maxim., *Epimedium sagittatum* (Sieb. et Zucc.) Maxim., *Epimedium pubescens* Maxim., or *Epimedium koreanum* Nakai (Berberidaceae). The aerial part is collected in summer and autumn when foliage branch growing luxuriantly, thick stalks and foreign matter removed, then dried under the sun or in a shaded area to obtain Epimedii Herba.

3. DESCRIPTION

***Epimedium brevicornu* Maxim.:** Stems slender-cylindrical, 5-28 cm long, mostly hollow. Externally yellowish-green or pale green, lustrous. Stem leaves mostly biternately compound. Leaflets ovate, 2-10 cm long, 2-8 cm wide; slightly acute at the apex, terminal leaflets cordate at the base, lateral leaflets oblique-cordate at the base, relatively large on one side, auriculate, margin with yellow spinose serrulations; the upper surface yellowish-green, the lower surface greyish-green, main vein 7-9, sparsely covered with slender hairs at the base, veinlets protuberant on both surfaces, reticulate veins distinct; petiolules 1-7 cm long. Lamina papyraceous or near leathery. Texture fragile, easily broken. Odour slight; taste slightly bitter [Fig. 1 (i)].

***Epimedium sagittatum* (Sieb. et Zucc.) Maxim.:** Stems 15-55 cm long. Stem leaves ternately compound. Leaflets long-ovate to ovate-lanceolate, 4-15 cm long, 2-8 cm wide; acuminate at the apex; bilateral leaflets distinctly oblique at the base, arrow-shaped on one side; the lower surface sparsely strigillose, or sparsely covered with slender hairs, sometimes nearly glabrous; petiolules 3-7 cm long. Lamina leathery [Fig. 1 (ii)].

***Epimedium pubescens* Maxim.:** Stems 7-60 cm long. Stem leaves ternately compounded. Leaflets long-ovate to ovate-lanceolate, 3-20 cm long, 2-8 cm wide; the lower surface densely covered with white long pubescence; petiolules 1-7 cm long, pilose. Lamina thin and leathery [Fig. 1 (iii)].

***Epimedium koreanum* Nakai:** Stems 10-25 cm long. Stem leaves biternately compound. Leaflets ovate, relatively large, 3-10 cm long, 2.5-8 cm wide; long-acuminate at the apex; the upper surface dark green, the lower surface pale green, the lower surface sparsely covered with slender hairs or nearly glabrous; petiolules 1-7 cm long. Lamina thin membranous or papyraceous [Fig. 1 (iv)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Stem: Epidermis consists of 1 layer of cells, cells mostly flattened, arranged tightly, covered with cuticle. Cortex relatively narrow, cells subrounded. Fibres consist of 3-10 layers of cells, arranged in a ring, beneath cortex. Vascular bundles 8-27, collateral, scattered in parenchymatous cells, arranged in an interrupted ring. Phloem cells arranged tightly. Xylem vessels subrounded to subelliptic. Pith broad, sometimes hollow in the centre, parenchymatous cells relatively large [Fig. 2 (i), (ii), (iii) and (iv)].

(No significant differences in the transverse section of the stem for 4 species)

Leaf

***Epimedium brevicornu* Maxim.:** Upper and lower epidermis each consists of 1 layer of cells, cells rectangular or subsquare, covered with cuticle. Multicellular non-glandular hairs and remnant occasionally present on lower epidermis. Palisade tissue consists of 1-2 layers of cells, arranged irregularly, containing brown content. Spongy tissue arranged loosely. Vascular bundles of midrib 3, collateral. Vessels and fibres present in xylem. Several layers of sclerenchymatous cells present in the inner side of epidermis of the midrib [Fig. 2 (i)].

***Epimedium sagittatum* (Sieb. et Zucc.) Maxim.:** Remnant of multicellular non-glandular hairs occasionally found on the lower surface of epidermis. Vascular bundles of midrib 5-7 [Fig. 2 (ii)].

***Epimedium pubescens* Maxim.:** Remnant of multicellular non-glandular hairs numerous, present on the lower epidermis. Vascular bundles of midrib 5 [Fig. 2 (iii)].

***Epimedium koreanum* Nakai:** Remnant of multicellular non-glandular hairs present on the lower epidermis. Vascular bundles of midrib 3-5 [Fig. 2 (iv)].

Powder

***Epimedium brevicornu* Maxim.:** Colour yellowish-green. Non-glandular hairs multicellular, usually broken, basal cells short, gradually longer towards the apex, apical cell with the longest length, straight, curved or slightly twisted, some cells contain yellowish-brown contents. Anticlinal walls of upper and lower epidermal cells sinuately curved in surface view, unevenly thickened, lower epidermis with numerous anomocytic stomata, guard cells nearly semicircular, subsidiary cells 3-5. Columnar crystals of calcium oxalate frequently visible, mostly scattered among the sclerenchymatous cells; polychromatic under the polarized microscope. Fibres with visible wall pits [Fig. 3 (i)].

***Epimedium sagittatum* (Sieb. et Zucc.) Maxim.:** Non-glandular hairs with short basal cells, apical cells long fusiform, forming obtuse angle with basal cells. Anticlinal walls of upper and lower epidermal cells sinuously curved in surface view, outer periclinal walls of lower epidermal cells mostly with papillae protuberance, double circle-shaped in surface view [Fig. 3 (ii)].

***Epimedium pubescens* Maxim.:** Non-glandular hairs numerous, basal cells usually shorter, apical cells very long, mostly twisted or straight. Anticlinal walls of upper and lower epidermal cells sinuately curved in surface view [Fig. 3 (iii)].

***Epimedium koreanum* Nakai:** Non-glandular hairs of 2 types, first type slender, apical cell with the longest length; second type the length of each cell nearly equal, usually with one cell collapsed; both types of non-glandular hairs contain yellowish-brown contents. Anticlinal walls of upper and lower epidermal cells sinuously curved in surface view [Fig. 3 (iv)].

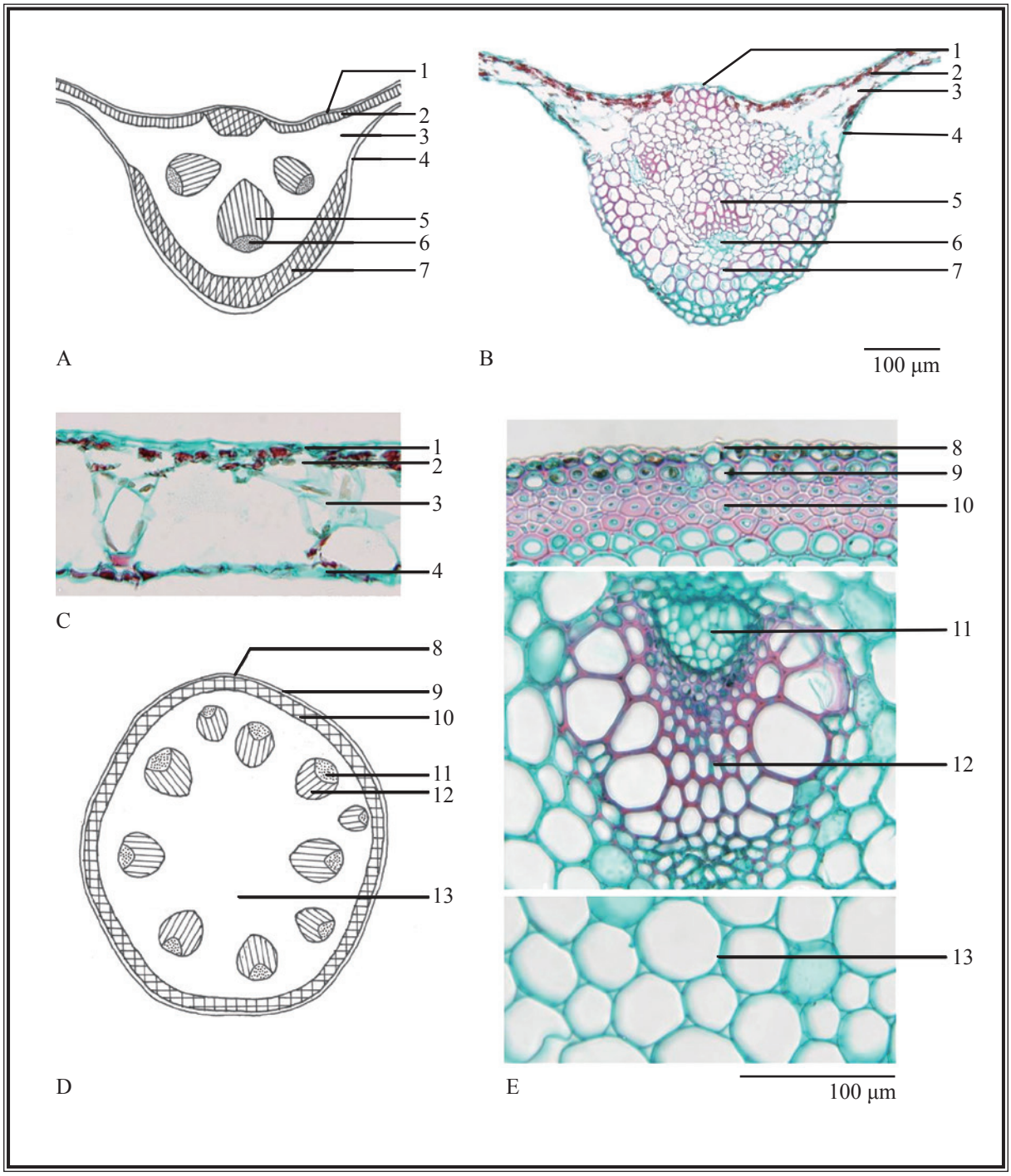

Figure 2 (i) Microscopic features of transverse section of dried aerial part of *Epimedium brevicornu* Maxim.

A. Sketch of leaf B. Section illustration of leaf C. Section magnified of mesophyll
D. Sketch of stem E. Section illustration of stem

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Lower epidermis
5. Xylem 6. Phloem 7. Sclerenchyma 8. Epidermis 9. Cortex 10. Fibres
11. Phloem 12. Xylem 13. Pith

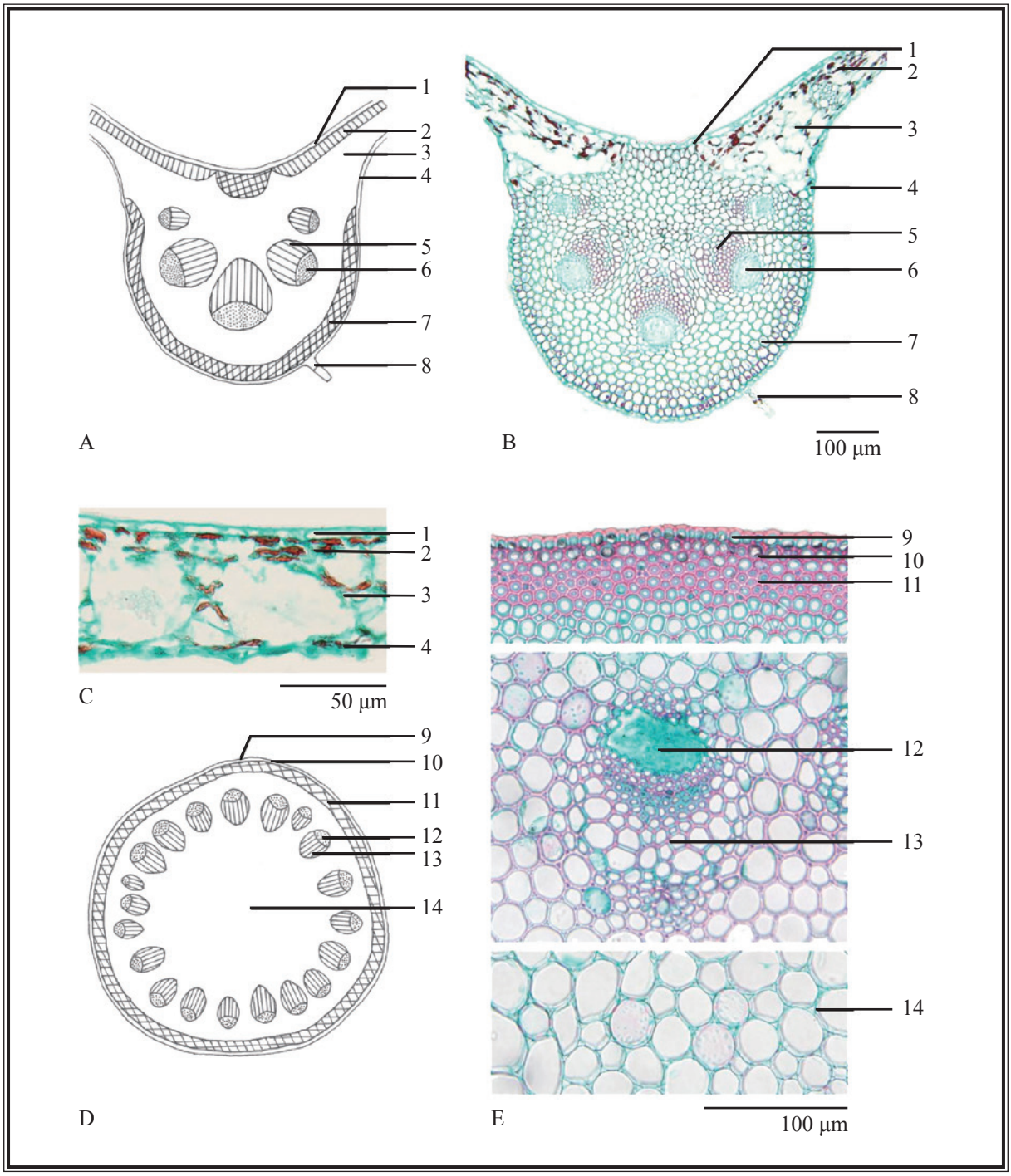

Figure 2 (ii) Microscopic features of transverse section of dried aerial part of *Epimedium sagittatum* (Sieb. et Zucc.) Maxim.

A. Sketch of leaf B. Section illustration of leaf C. Section magnified of mesophyll
D. Sketch of stem E. Section illustration of stem

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Lower epidermis 5. Xylem
6. Phloem 7. Sclerenchyma 8. Remnant of non-glandular hair 9. Epidermis
10. Cortex 11. Fibres 12. Phloem 13. Xylem 14. Pith

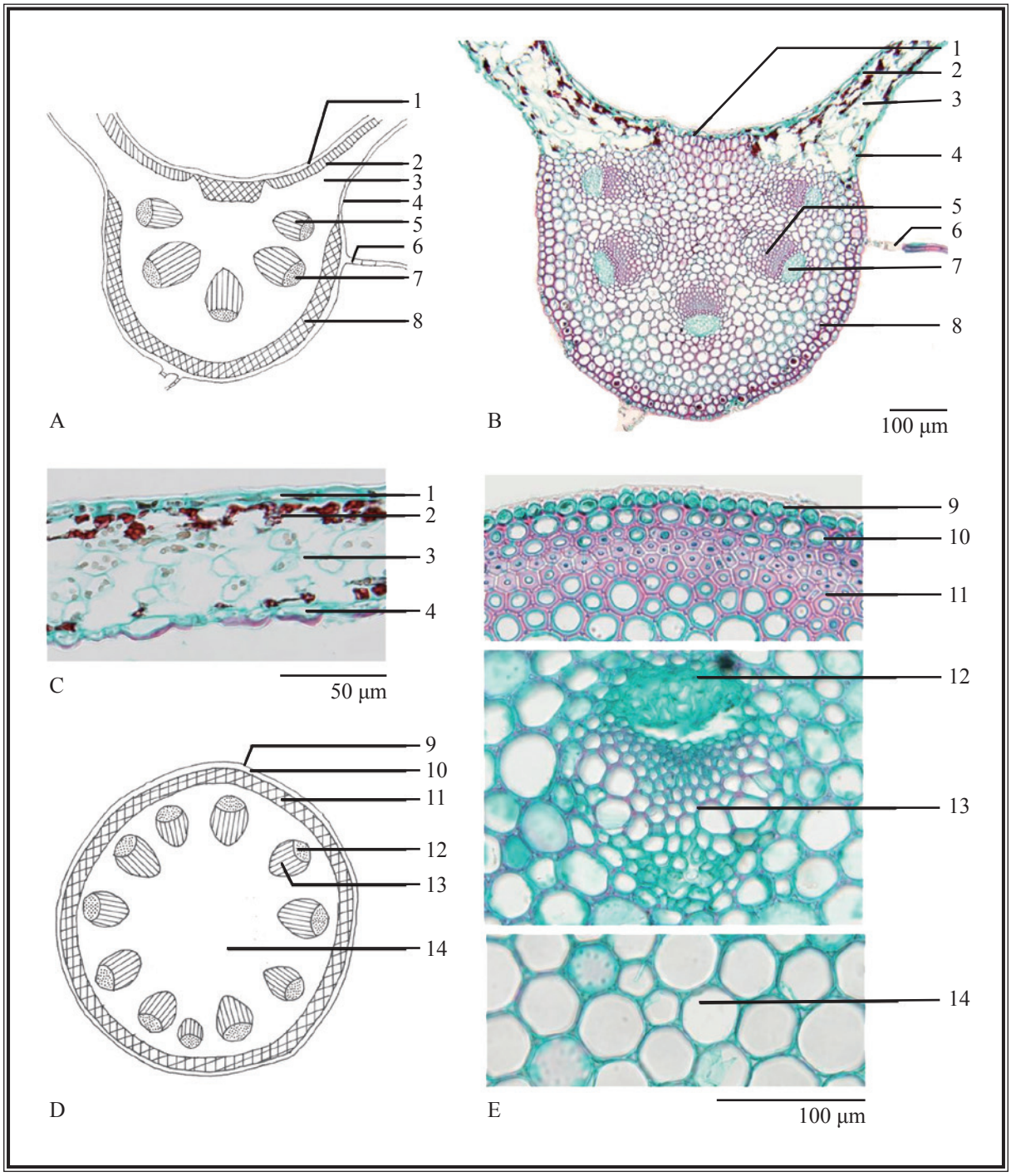

Figure 2 (iii) Microscopic features of transverse section of dried aerial part of *Epimedium pubescens* Maxim.

A. Sketch of leaf B. Section illustration of leaf C. Section magnified of mesophyll
D. Sketch of stem E. Section illustration of stem

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Lower epidermis 5. Xylem
6. Remnant of non-glandular hair 7. Phloem 8. Sclerenchyma 9. Epidermis
10. Cortex 11. Fibres 12. Phloem 13. Xylem 14. Pith

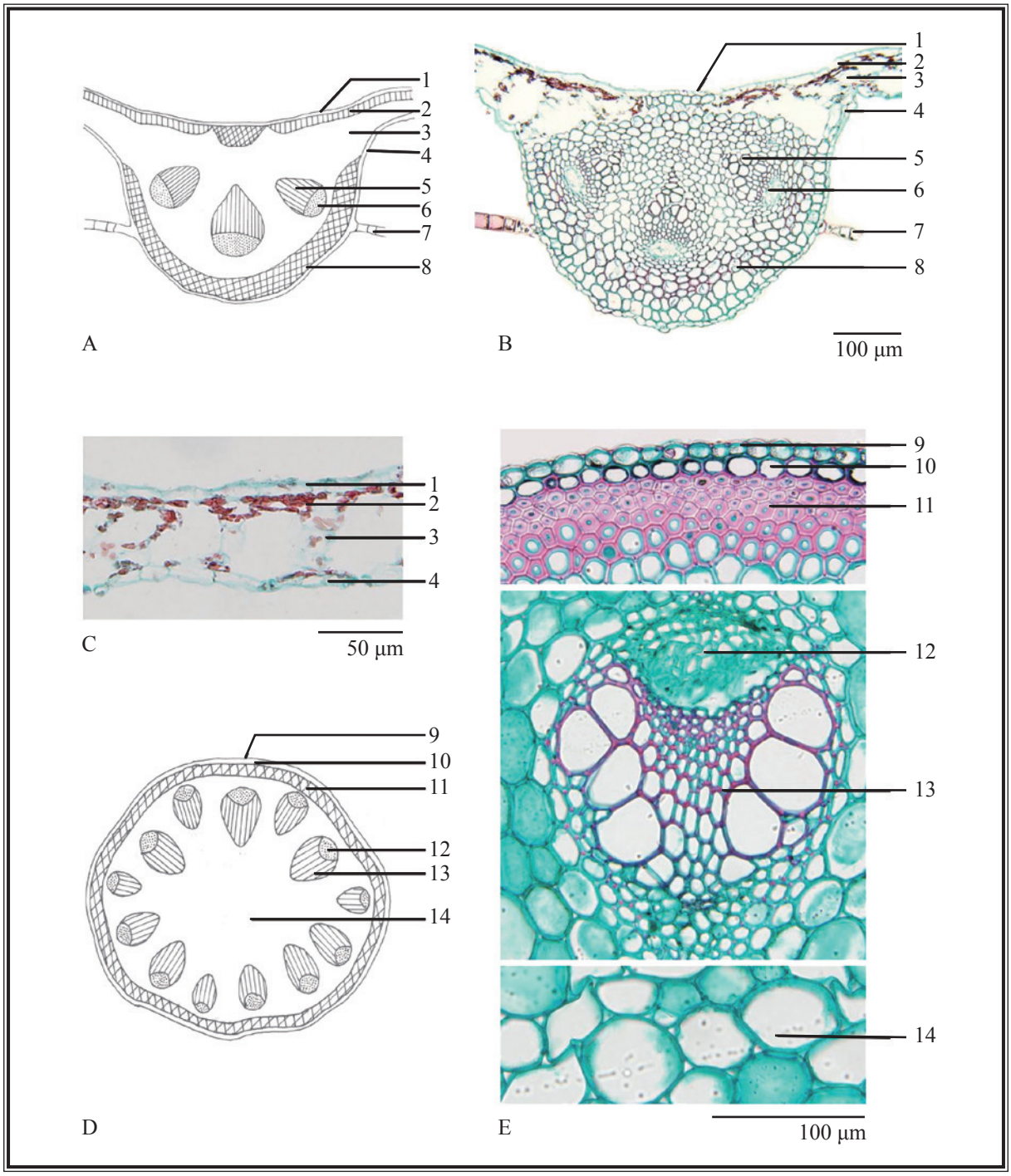

Figure 2 (iv) Microscopic features of transverse section of dried aerial part of *Epimedium koreanum* Nakai

A. Sketch of leaf B. Section illustration of leaf C. Section magnified of mesophyll
D. Sketch of stem E. Section illustration of stem

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Lower epidermis 5. Xylem
6. Phloem 7. Remnant of non-glandular hair 8. Sclerenchyma 9. Epidermis
10. Cortex 11. Fibres 12. Phloem 13. Xylem 14. Pith

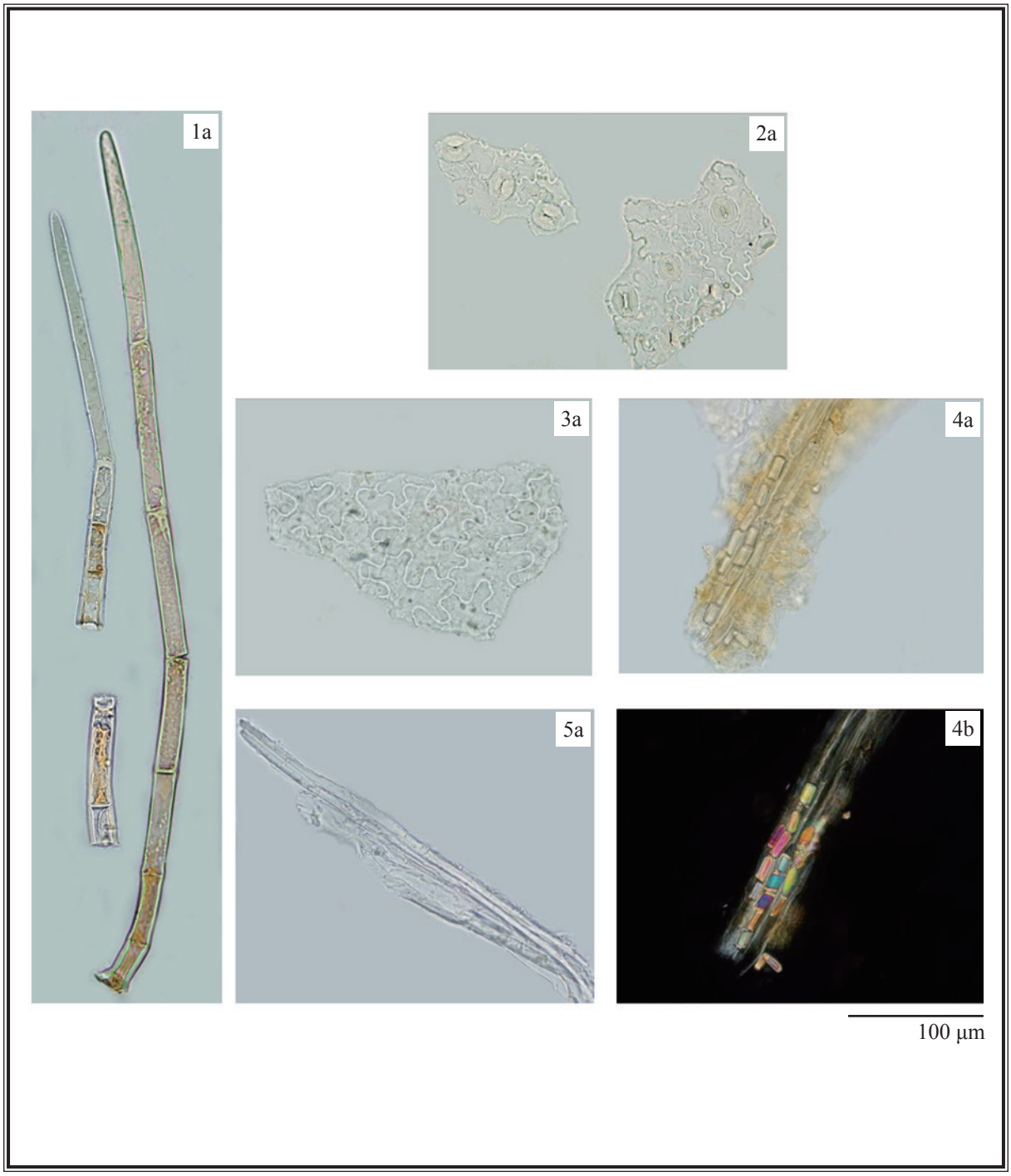

Figure 3 (i) Microscopic features of powder of dried aerial part of *Epimedium brevicornu* Maxim.

1. Non-glandular hairs 2. Lower epidermal cells with stomata 3. Upper epidermal cells
4. Columnar crystals of calcium oxalate (in sclerenchymatous cells) 5. Fibres

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

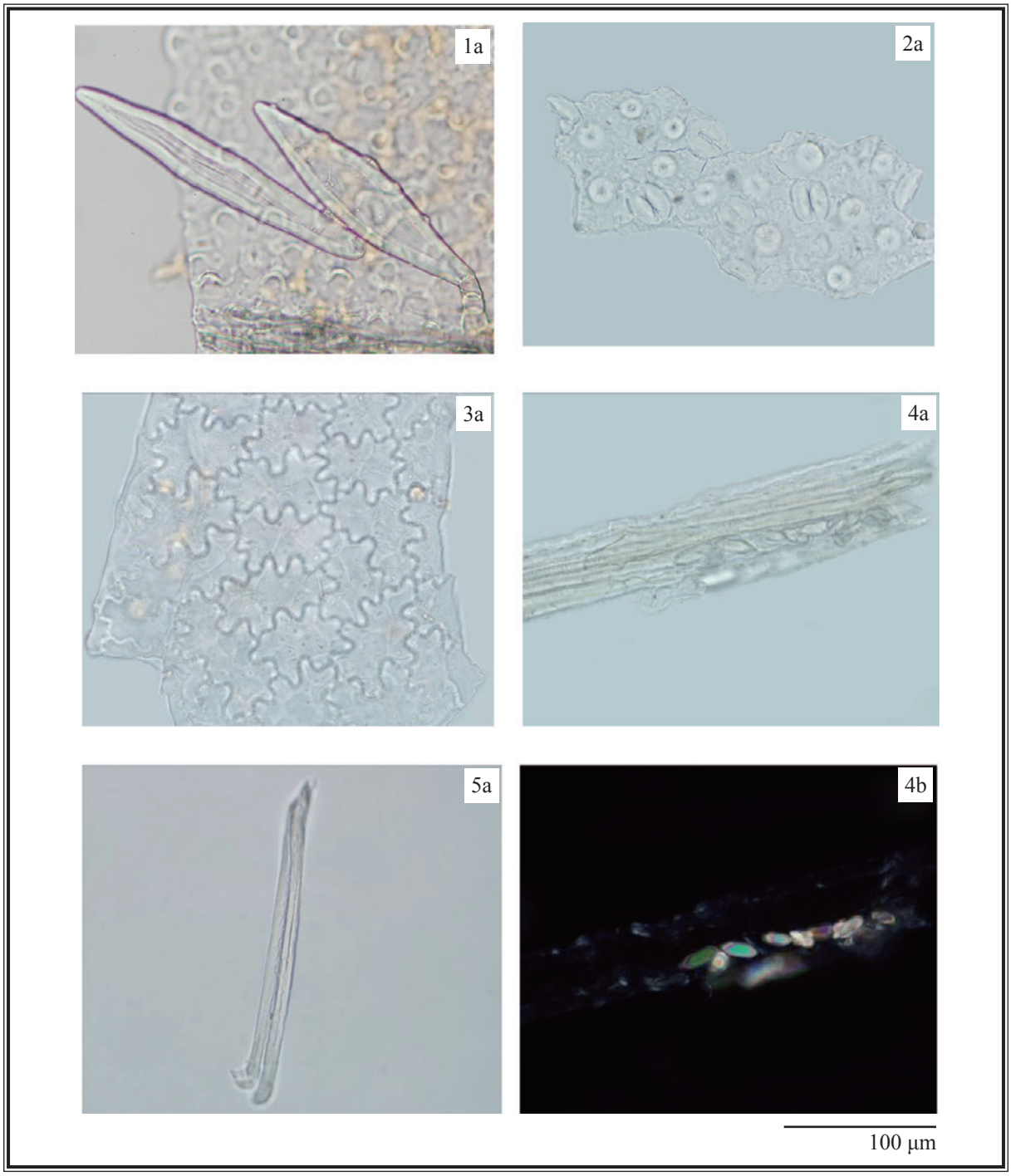

Figure 3 (ii) Microscopic features of powder of dried aerial part of *Epimedium sagittatum* (Sieb. et Zucc.) Maxim.

1. Non-glandular hairs 2. Lower epidermal cells with stomata 3. Upper epidermal cells
4. Columnar crystals of calcium oxalate (in sclerenchymatous cells) 5. Fibre

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

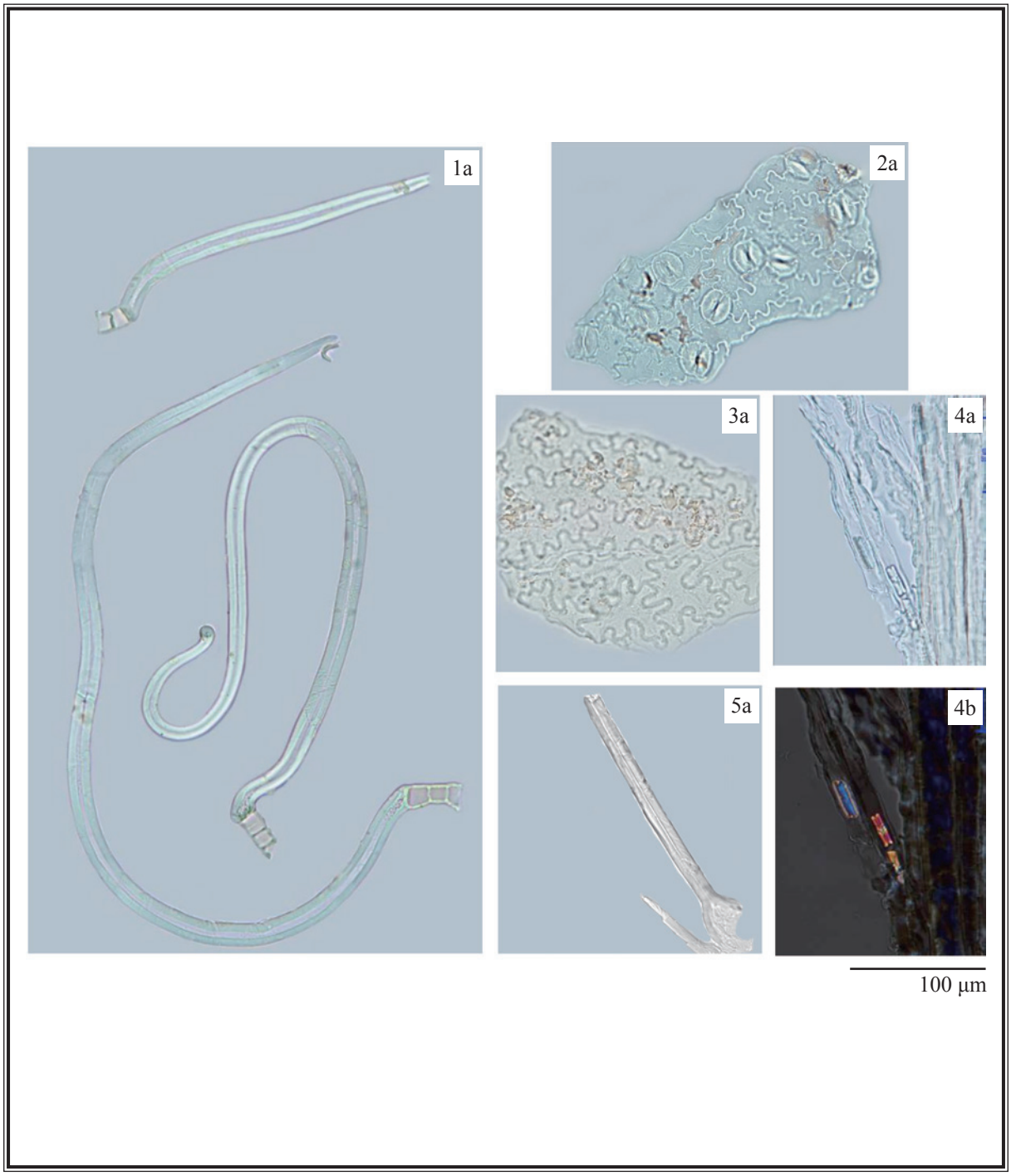

Figure 3 (iii) Microscopic features of powder of dried aerial part of *Epimedium pubescens* Maxim.

1. Non-glandular hairs 2. Lower epidermal cells with stomata 3. Upper epidermal cells
4. Columnar crystals of calcium oxalate (in sclerenchymatous cells) 5. Fibres

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

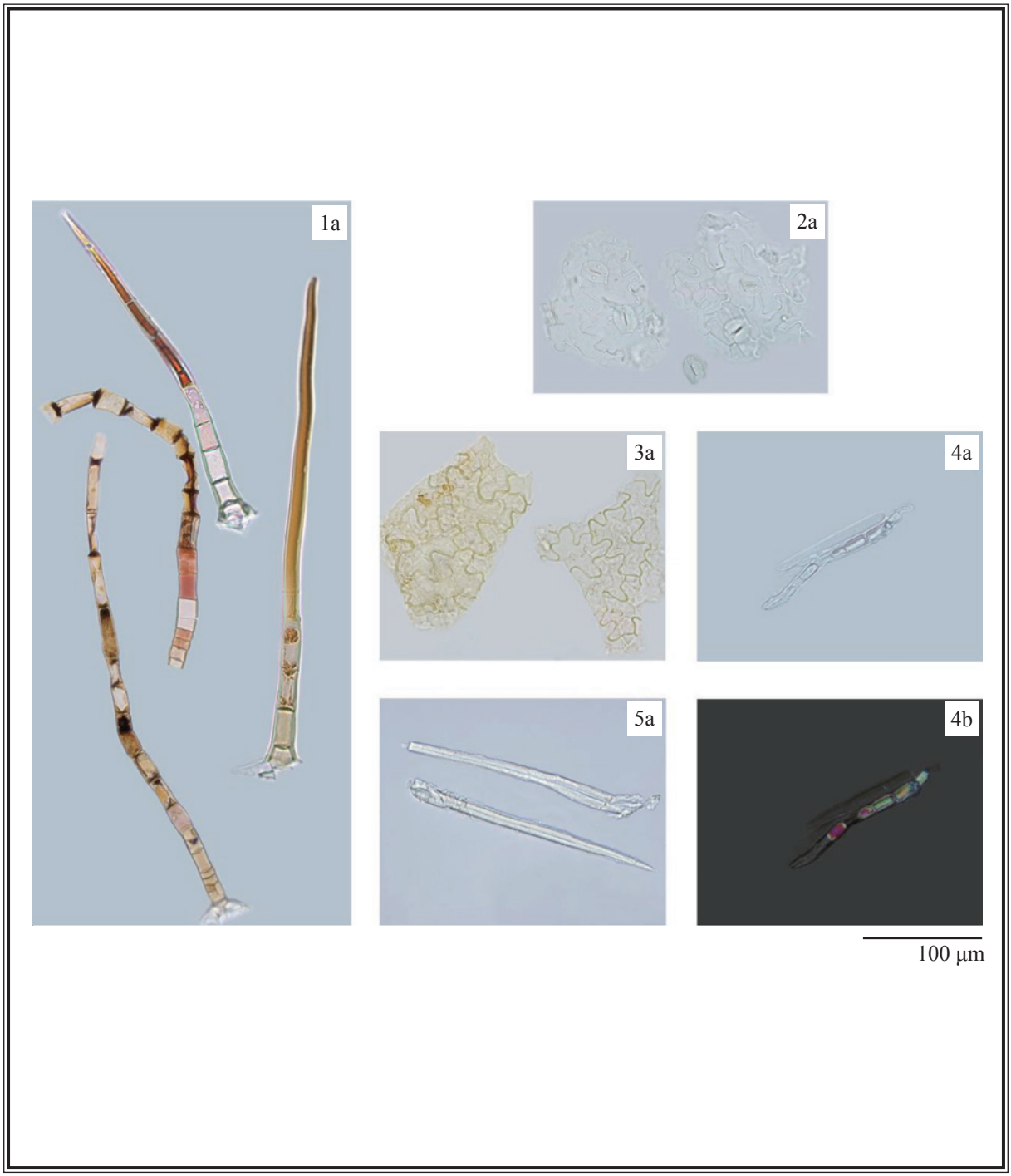

Figure 3 (iv) Microscopic features of powder of dried aerial part of *Epimedium koreanum* Nakai

1. Non-glandular hairs 2. Lower epidermal cells with stomata 3. Upper epidermal cells
4. Columnar crystals of calcium oxalate (in sclerenchymatous cells) 5. Fibres

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

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

Standard solution

Icariin standard solution

Weigh 2.0 mg of icariin CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (7:1:1, v/v).

Spray reagent

Weigh 1 g of aluminium 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 conical flask, then add 10 mL of ethanol. Sonicate (250 W) the mixture for 30 min. Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately icariin standard solution (1 μL) and the test solution (2 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 10 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 heat at about 105ºC (about 5 min). Spray the plate evenly with the spray reagent and dry in air (about 20 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

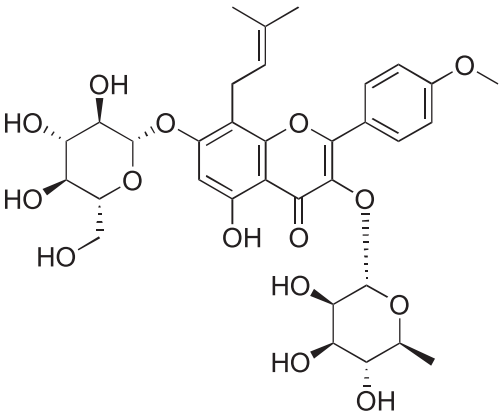

Figure 4 Chemical structure of icariin

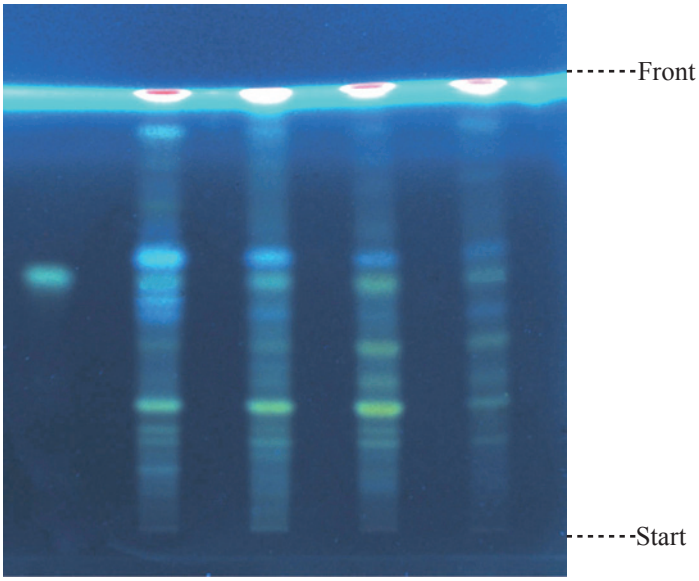

Figure 5 A reference HPTLC chromatogram of Epimedii Herba extract observed under UV light (366 nm) after staining

1. Icariin standard solution
2. Test solution of
(i) dried aerial part of *Epimedium brevicornu* Maxim.
(ii) dried aerial part of *Epimedium sagittatum* (Sieb. et Zucc.) Maxim.
(iii) dried aerial part of *Epimedium pubescens* Maxim.
(iv) dried aerial part of *Epimedium koreanum* Nakai

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 icariin (Fig. 5).

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

Standard solution

Icariin standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 5.0 mg of icariin CRS and dissolve in 100 mL of ethanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (300 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times each with 7 mL of ethanol (50%). Combine the supernatants and make up to the mark with ethanol (50%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	78 → 76	22 → 24	linear gradient
35 – 45	76 → 55	24 → 45	linear gradient
45 – 60	55 → 30	45 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of icariin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of icariin should not be more than 5.0%; the RSD of the retention time of icariin peak should not be more than 2.0%; the column efficiency determined from icariin peak should not be less than 15000 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 (i), (ii), (iii) or (iv)].

Procedure

Separately inject icariin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of icariin peak in the chromatogram of icariin Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i), (ii), (iii) or (iv)] in the chromatogram of the test solution. Identify icariin peak in the chromatogram of the test solution by

comparing its retention time with that in the chromatogram of icariin Std-FP. The retention times of icariin 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 Epimedii Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (epimedin A)	0.77	± 0.03
2 (epimedin B)	0.84	± 0.03
3 (epimedin C)	0.94	± 0.03
4 (marker, icariin)	1.00	-

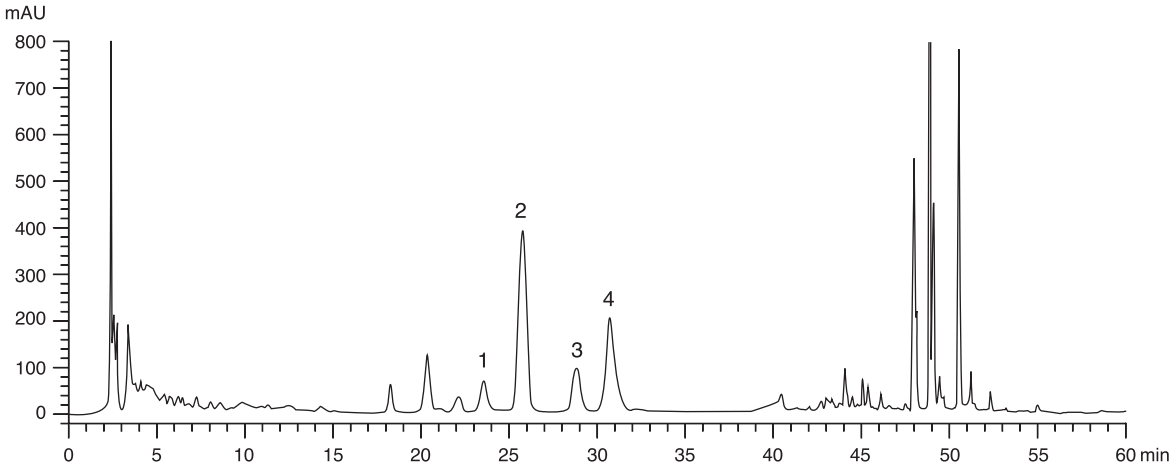

Figure 6 (i) A reference fingerprint chromatogram of dried aerial part of *Epimedium brevicornu* Maxim. extract

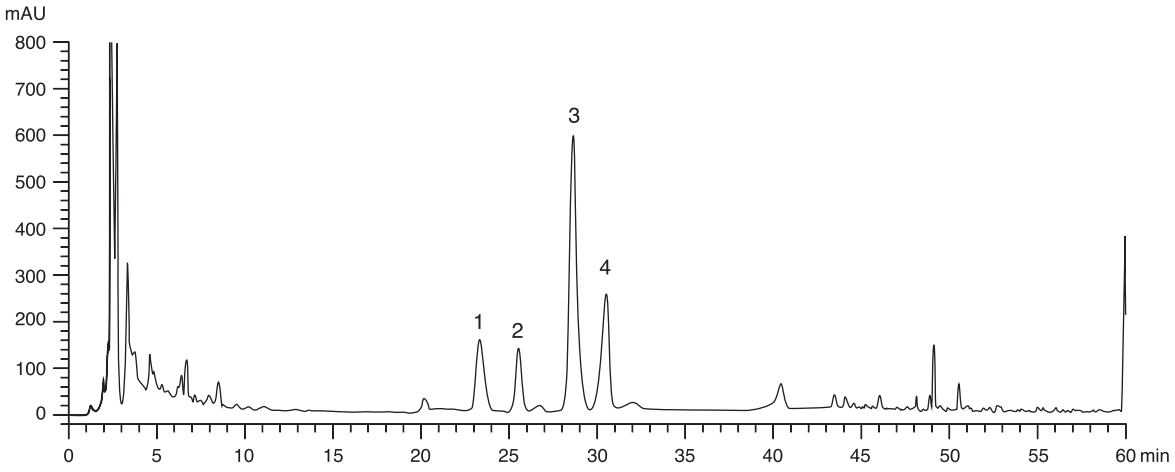

Figure 6 (ii) A reference fingerprint chromatogram of dried aerial part of *Epimedium sagittatum* (Sieb. et Zucc.) Maxim. extract

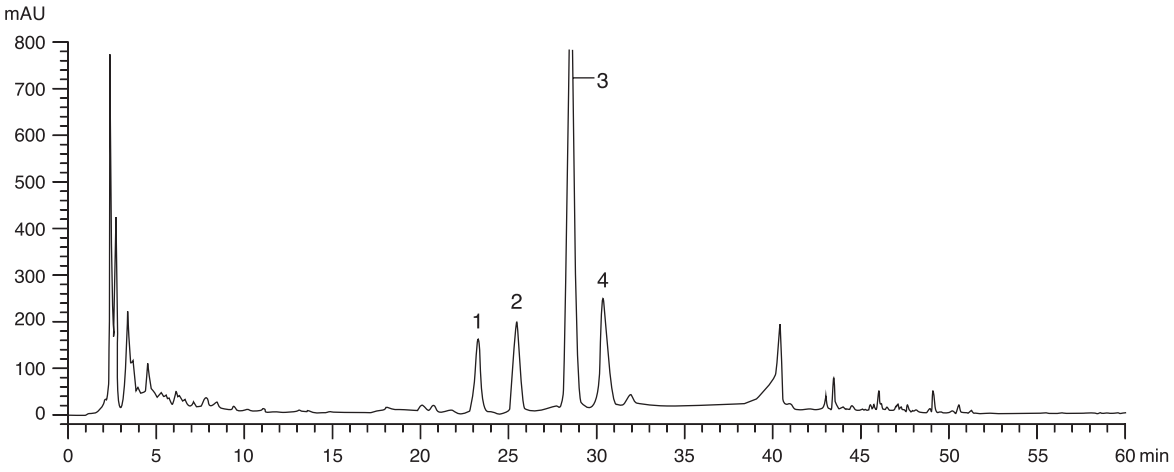

Figure 6 (iii) A reference fingerprint chromatogram of dried aerial part of *Epimedium pubescens* Maxim. extract

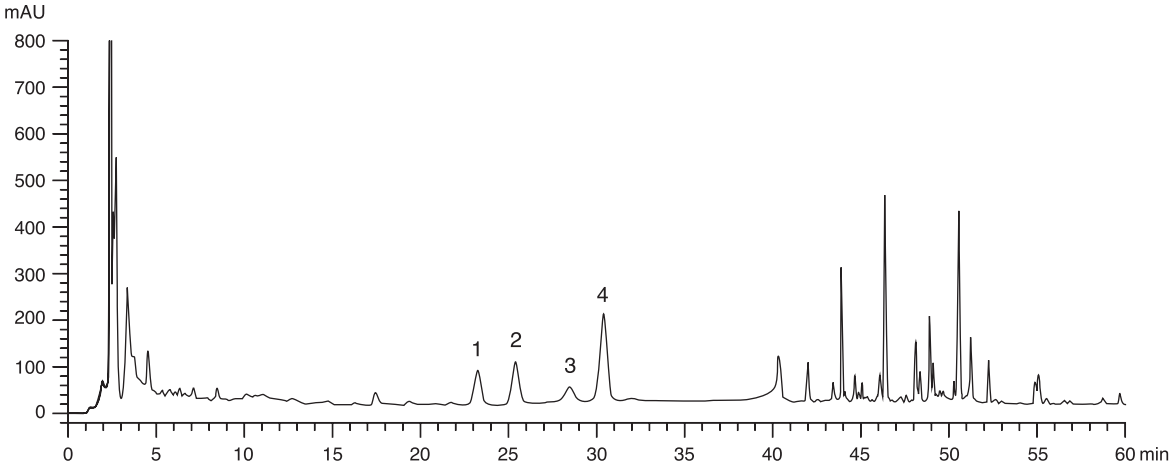

Figure 6 (iv) A reference fingerprint chromatogram of dried aerial part of *Epimedium koreanum* Nakai extract

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 respective reference fingerprint chromatograms [Fig. 6 (i), (ii), (iii) or (iv)].

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 10.0%.
Ethanol-soluble extractives (hot extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution
Icariin standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 10.0 mg of icariin CRS and dissolve in 10 mL of ethanol (50%).
Icariin standard solution for assay, Std-AS
Measure accurately the volume of the icariin Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 1, 10, 100, 200, 500 mg/L for icariin.

Test solution
Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (300 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times each with 7 mL of ethanol (50%). Combine the supernatants and make up to the mark with ethanol (50%). Filter through a 0.45-μm nylon filter.

Chromatographic system
The liquid chromatograph is equipped with a DAD (270 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	78 → 76	22 → 24	linear gradient
35 – 45	76 → 55	24 → 45	linear gradient
45 – 60	55 → 30	45 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of icariin Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of icariin should not be more than 5.0%; the RSD of the retention time of icariin peak should not be more than 2.0%; the column efficiency determined from icariin peak should not be less than 15000 theoretical plates.

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

Calibration curve

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

Limits

The sample contains not less than 0.055% of icariin ($C_{33}H_{40}O_{15}$), calculated with reference to the dried substance.