Artemisiae Scopariae Herba

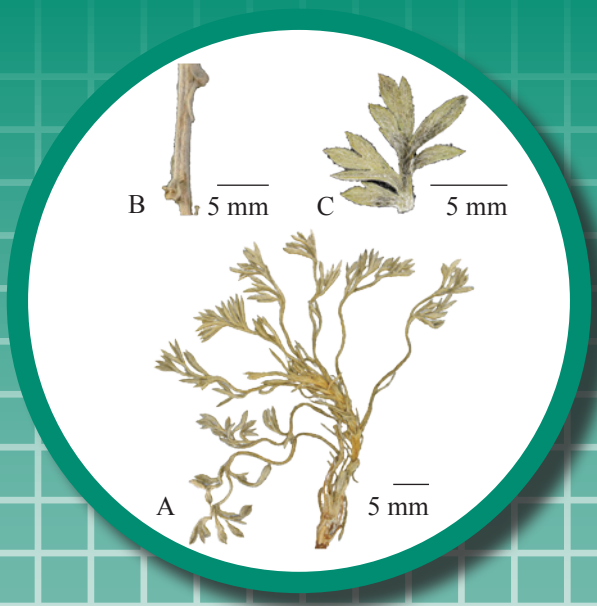


Figure 1 (i) A photograph of dried aerial part of *Artemisia scoparia* Waldst. et Kit.

A. Aerial part of Herba B. Magnified stem C. Magnified leaf

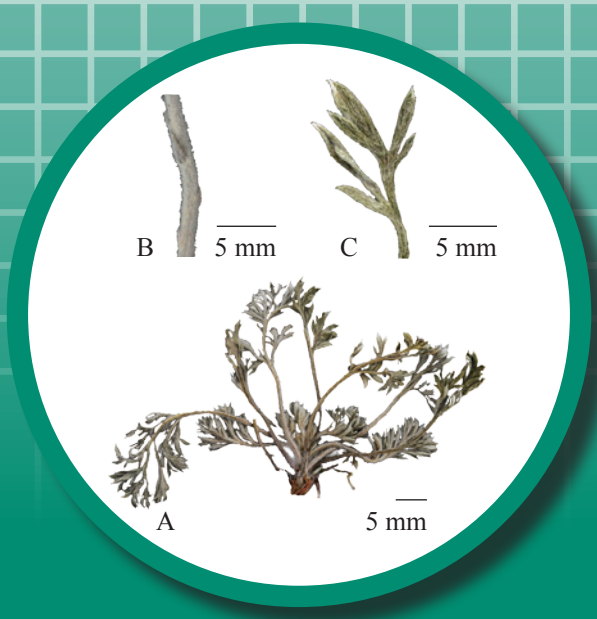


Figure 1 (ii) A photograph of dried aerial part of *Artemisia capillaris* Thunb.

A. Aerial part of Herba B. Magnified stem C. Magnified leaf

1. NAMES

Official Name: Artemisiae Scopariae Herba

Chinese Name: 茵陳

Chinese Phonetic Name: Yinchen

2. SOURCE

Artemisiae Scopariae Herba is the dried aerial part of *Artemisia scoparia* Waldst. et Kit. or *Artemisia capillaris* Thunb. (Asteraceae). The aerial part is collected in spring when the seedling is 6-10 cm high, foreign matter and older stem removed, then dried under the sun to obtain Artemisiae Scopariae Herba.

3. DESCRIPTION

***Artemisia scoparia* Waldst. et Kit.:** Mostly pieces are rolled into masses, greyish-white or greyish-green, densely covered with soft, white pubescence throughout. Stem thin, small, cylindrical, 1.5-2.5 cm long, 1-2 mm in diameter, with longitudinal striations underneath the white pubescence; texture fragile, easily broken. Leaves petioled, pinnately to tripinnately compound, when intact flattened out, lamina 1.5-3.5 cm long and 1-3 cm wide; segments ovate to oblanceolate, apex acute. Odour delicately aromatic; taste slightly bitter [Fig. 1 (i)].

***Artemisia capillaris* Thunb.:** Stem base woody, silky pubescent or glabrescent, 1.5-4.0 cm long and 1-3 mm in diameter. Lamina 1.6-8.2 cm long and 1.0-2.6 cm wide [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem:

***Artemisia scoparia* Waldst. et Kit.:** Non-glandular hair occurring abundantly on the epidermis. Epidermis consists of 1 layer of cells, cells elliptical to polygonal, with slightly thickened walls. Cortex consists of 8-9 layers of parenchymatous cells. Vascular bundles 12-15, collateral, arranged in an interrupted ring, corresponding to phloem fibres. Resin canals distinct in between

two vascular bundles. Pith consists of lots of large rounded parenchymatous cells, occupied nearly 2/3 of the stem [Fig. 2 (i)].

***Artemisia capillaris* Thunb.:** Vascular bundles 11-14, arranged in an interrupted ring [Fig. 2 (ii)].

Petiole:

Non-glandular hair occurring abundantly on the surface of the epidermis. Epidermis consists of 1 layer of cells, cells elliptical to polygon, arranged regularly and fragile. Palisade tissue consists of 1 layer of cells, beneath the epidermal cells or collenchymous cells. Collenchyma consists of 1-2 layers of collenchymatous cells, beneath the epidermis, mostly located at the edges and corners of the petiole. Vascular bundles 3-5, the middle one relative large, 2 collateral at the corner. Resin canal next to the vascular bundle, containing bright yellow resin in it [Fig. 3 (i) and (ii)].

Leaf:

***Artemisia scoparia* Waldst. et Kit.:** Non-glandular hairs occuring abundantly on the epidermis. Epidermis consists of 1 layer of cells, often broken. Palisade tissue consists of 1-2 layers of cells, beneath the epidermal cells. Spongy tissue presents in the middle, occurring half of the lamina. Resin canal visible right above the vascular bundle. Xylem locates in the upper of the middle vascular of the midvein, phloem in the lower correspondingly [Fig. 4 (i)].

***Artemisia capillaris* Thunb.:** Palisade tissue consists of 1-2 layers of cells in the upper surface, and 1 layer in the lower surface, arranged relatively loose [Fig. 4 (ii)].

Powder

***Artemisia scoparia* Waldst. et Kit.:** Colour greyish-green. Upper epidermal cells of leaf subrectangular, walls relatively smooth, stomata visible. Lower epidermal cells of leaf nearly colourless, walls sinuately curved, stomata visible, anomocytic, 22-36 μm long, 15-24 μm wide, residues of non-glandular hairs infrequently visible in epidermis. Spiral vessels in bundles or irregular crossed, colourless to pale yellow, 3-15 μm in diameter. Glandular hairs consist of 4-8 oppositely overlapped cells, without stalk, subrounded to oblong in surface view. Non-glandular hairs T-shaped, slightly V-shaped at the centre with unequal arms, apical cell walls 2-6 μm thick [Fig. 5 (i)].

***Artemisia capillaris* Thunb.:** Stomata anomocytic, 20-32 μm long and 12-21 μm wide. Vessels spial, 5-16 μm in diameter. Apical cell walls of non-glandular hairs 2-5 μm thick [Fig. 5 (ii)].

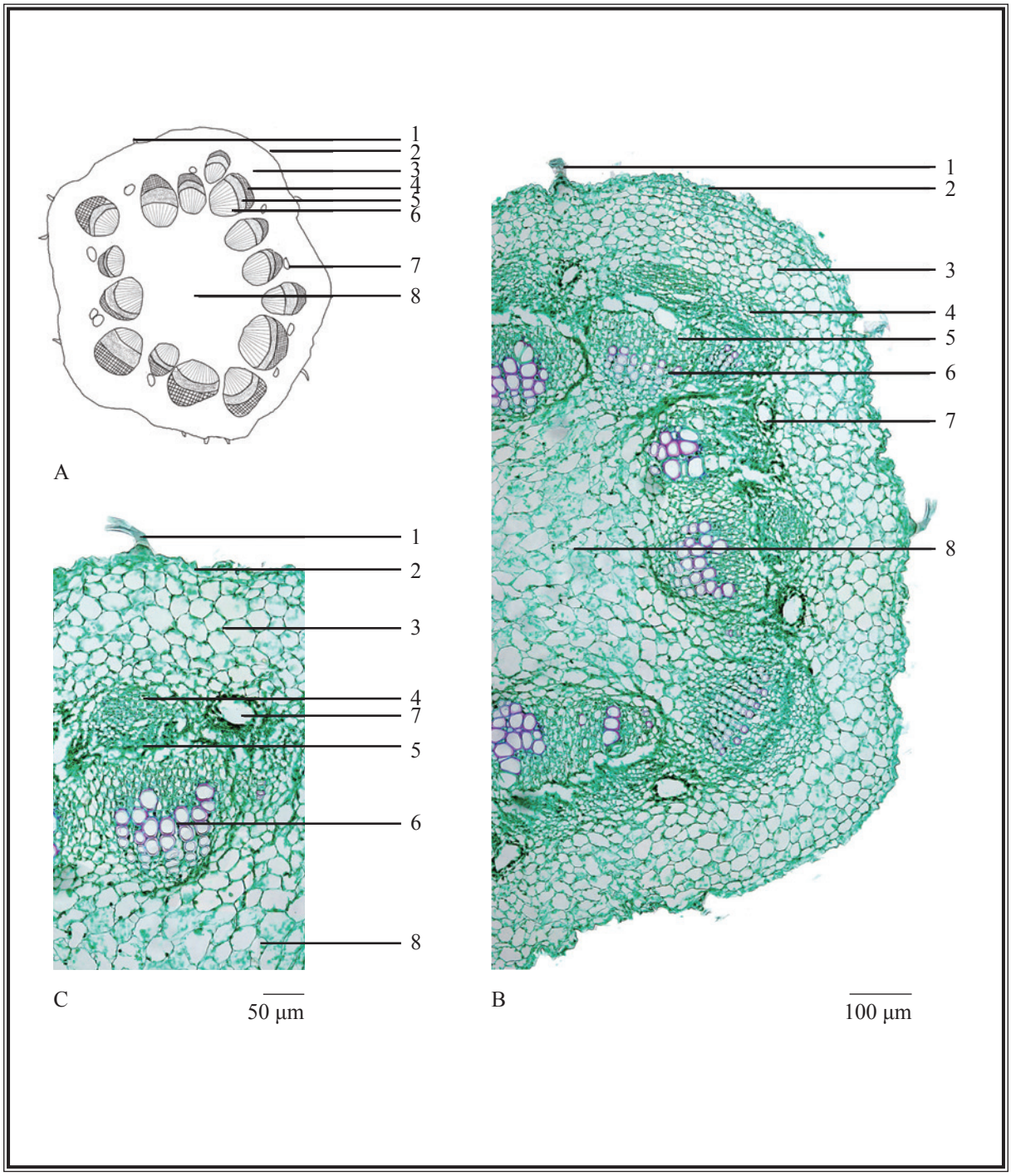


Figure 2 (i) Microscopic features of transverse section of dried stem of *Artemisia scoparia* Waldst. et Kit.

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Epidermis 3. Cortex 4. Phloem fibres 5. Phloem
6. Xylem 7. Resin canal 8. Pith

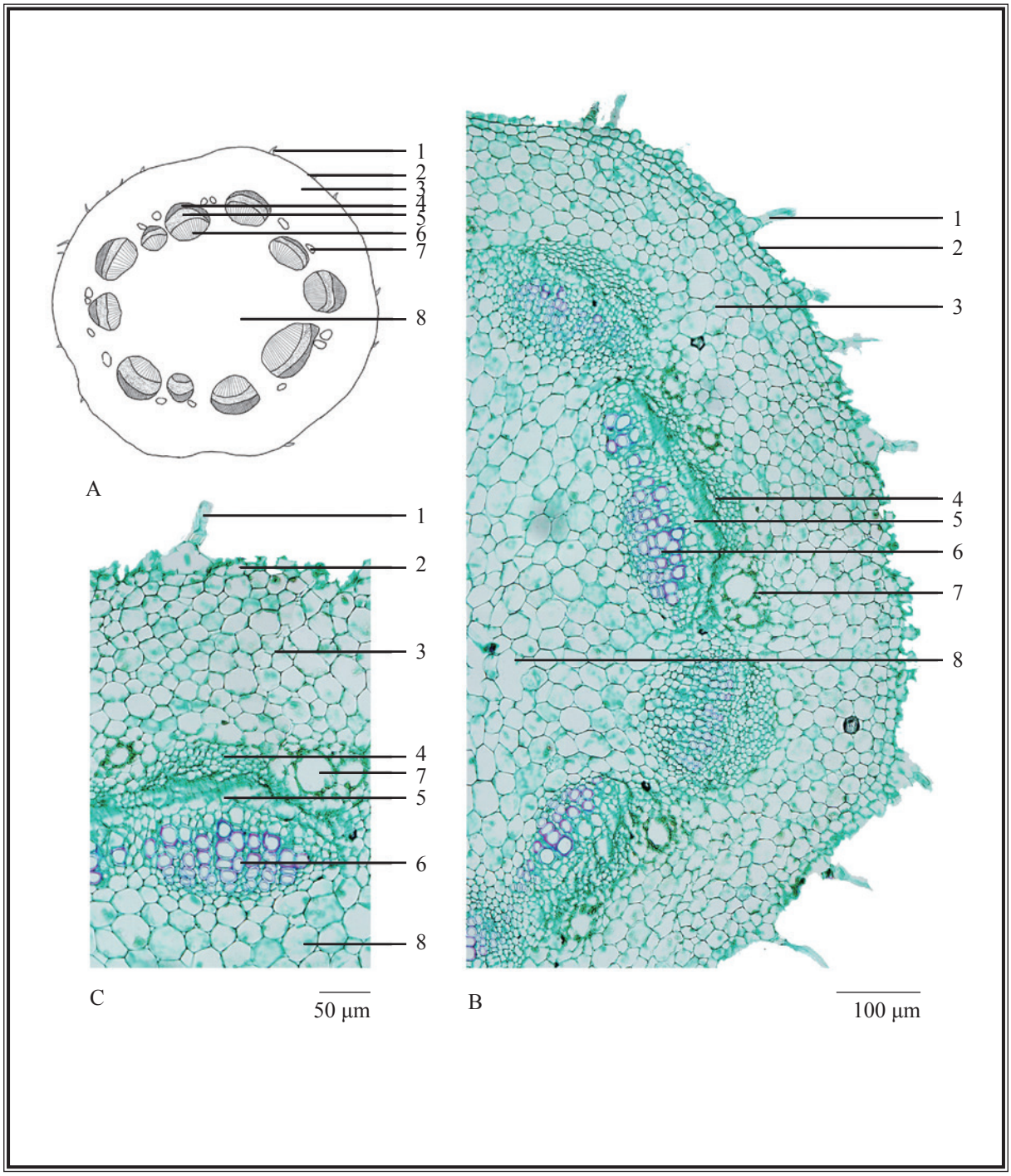


Figure 2 (ii) Microscopic features of transverse section of dried stem of *Artemisia capillaris* Thunb.

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Epidermis 3. Cortex 4. Phloem fibres 5. Phloem
6. Xylem 7. Resin canal 8. Pith

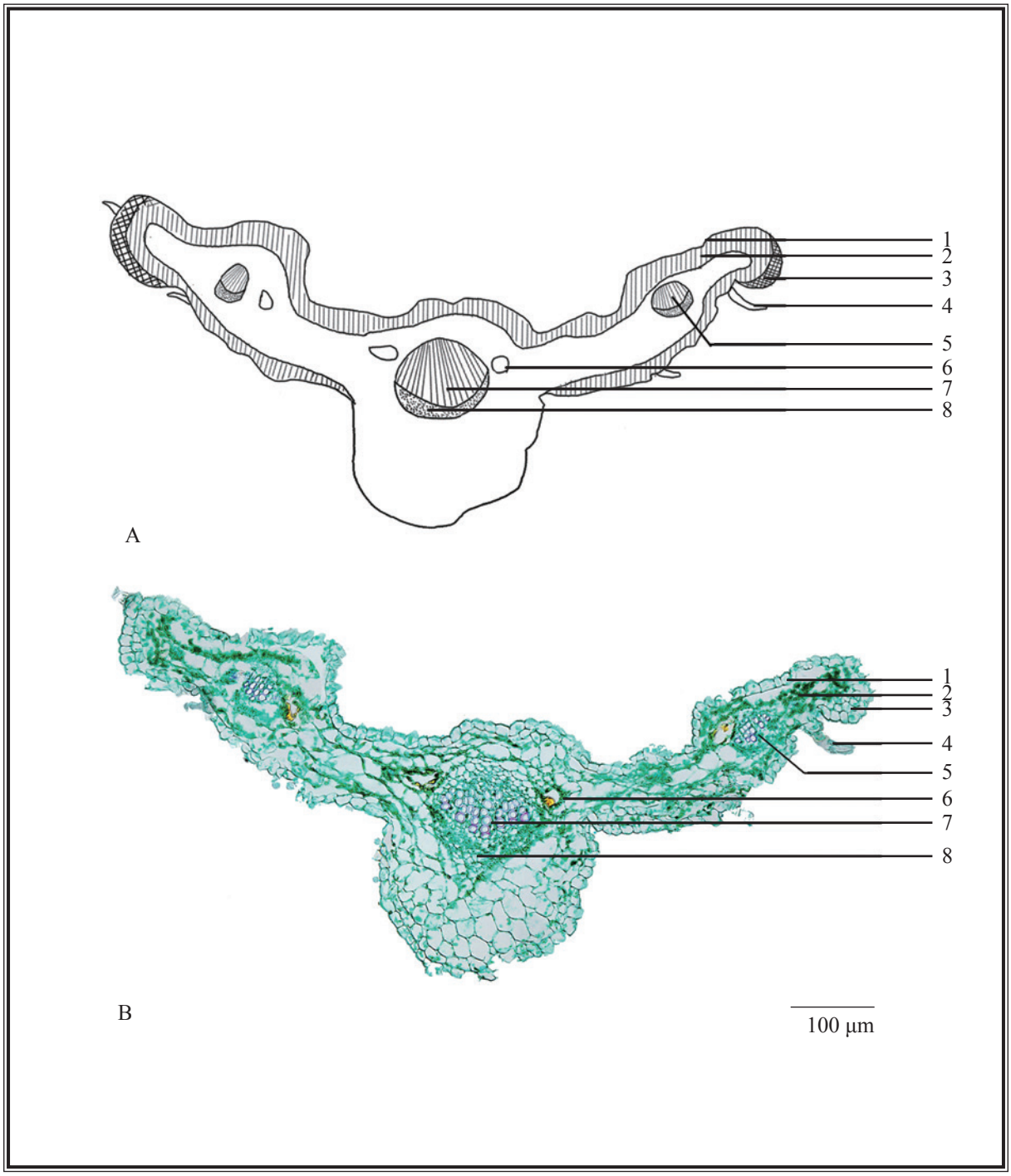


Figure 3 (i) Microscopic features of transverse section of dried petiole of *Artemisia scoparia* Waldst. et Kit.

A. Sketch B. Section illustration

1. Epidermis 2. Palisade tissue 3. Collenchyma 4. Non-glandular hair
5. Vascular bundle 6. Resin canal 7. Xylem 8. Phloem

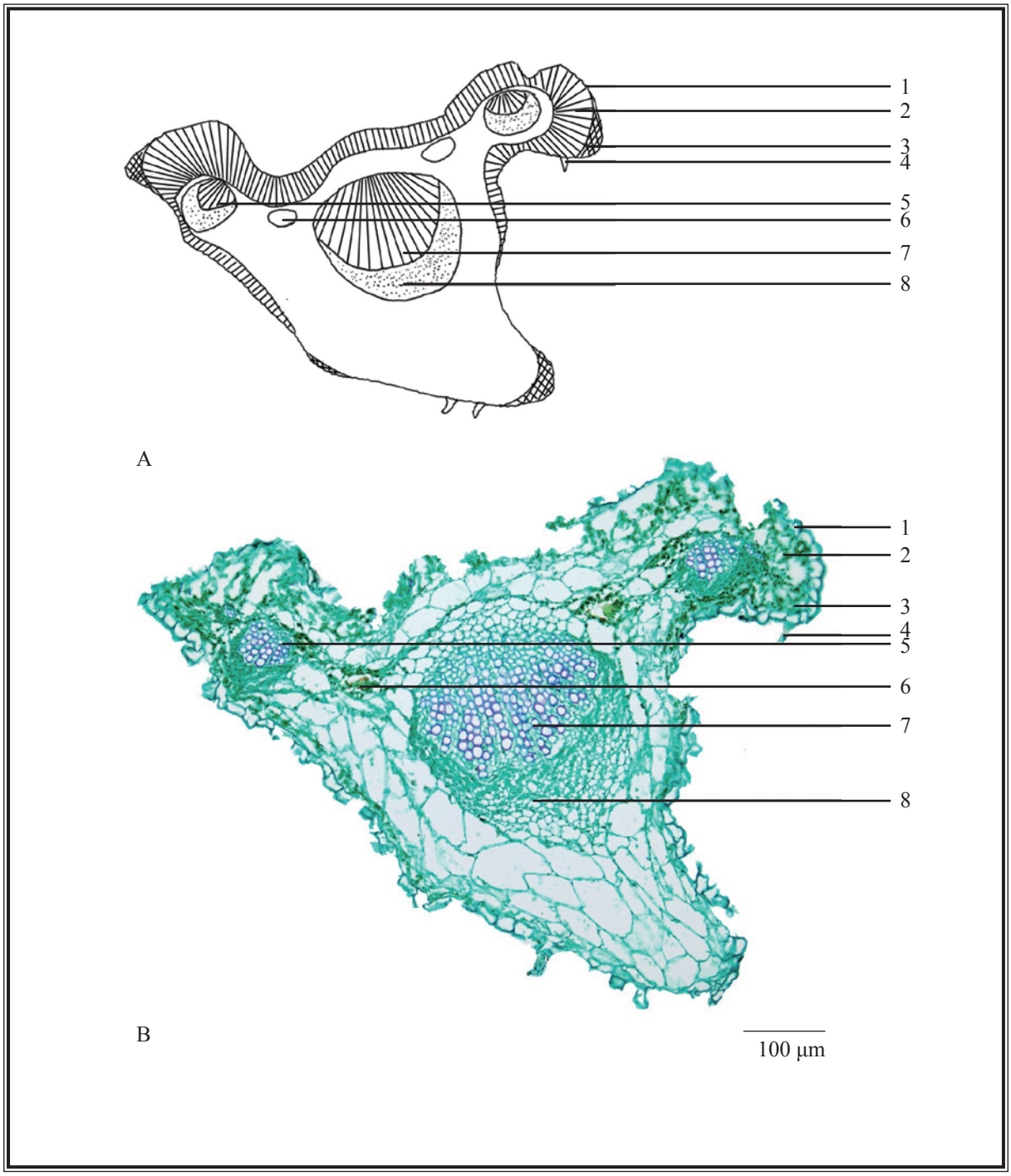


Figure 3 (ii) Microscopic features of transverse section of dried petiole of *Artemisia capillaris* Thunb.

A. Sketch B. Section illustration

1. Epidermis 2. Palisade tissue 3. Collenchyma 4. Non-glandular hair
5. Vascular bundle 6. Resin canal 7. Xylem 8. Phloem

Figure 4 (i) Microscopic features of transverse section of dried leaf of *Artemisia scoparia* Waldst. et Kit.

A. Sketch B. Section illustration

1. Non-glandular hair 2. Epidermis 3. Palisade tissue 4. Spongy tissue
5. Resin canal 6. Xylem 7. Phloem

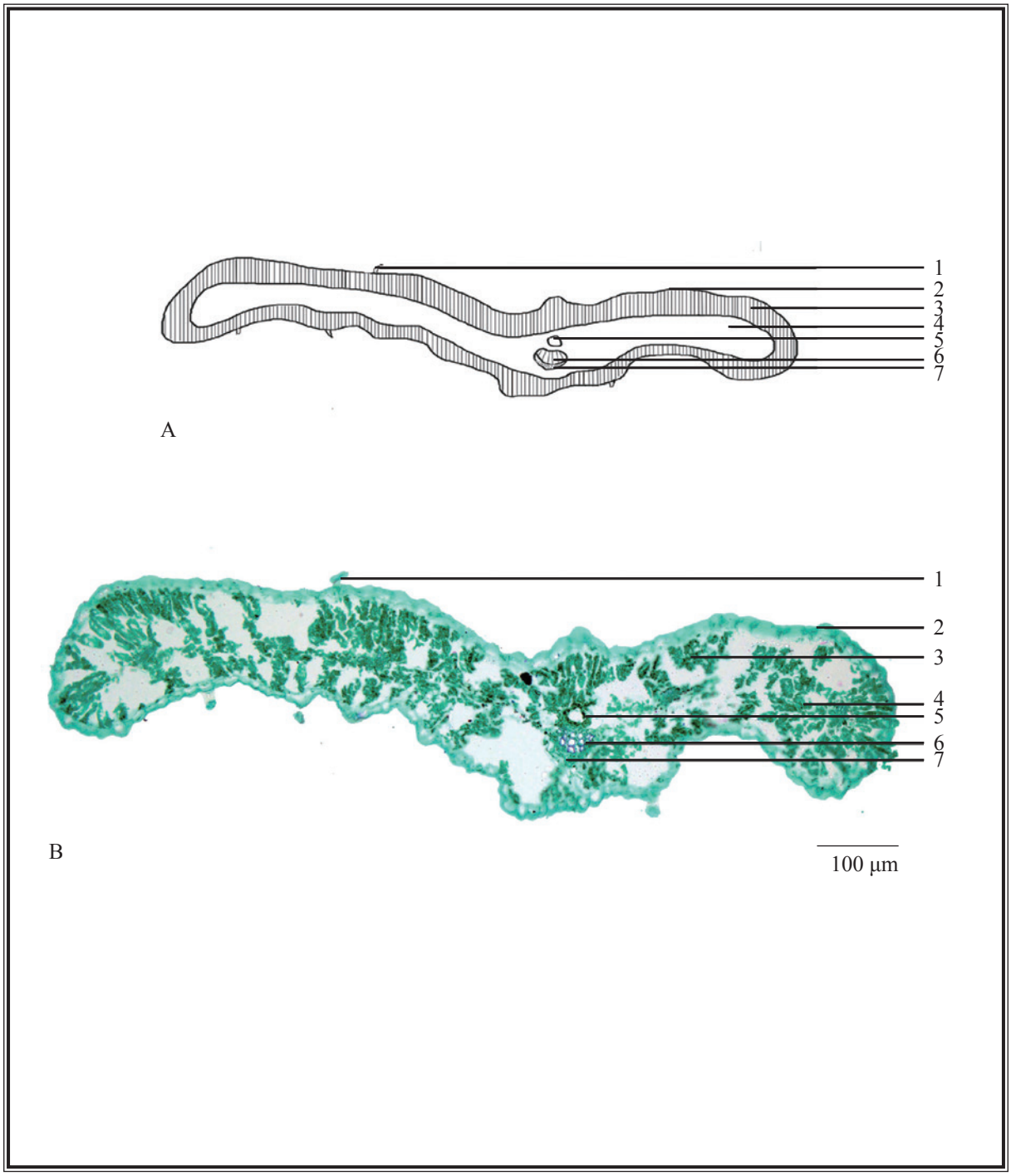


Figure 4 (ii) Microscopic features of transverse section of dried leaf of *Artemisia capillaris* Thunb.

A. Sketch B. Section illustration

1. Non-glandular hair 2. Epidermis 3. Palisade tissue 4. Spongy tissue
5. Resin canal 6. Xylem 7. Phloem

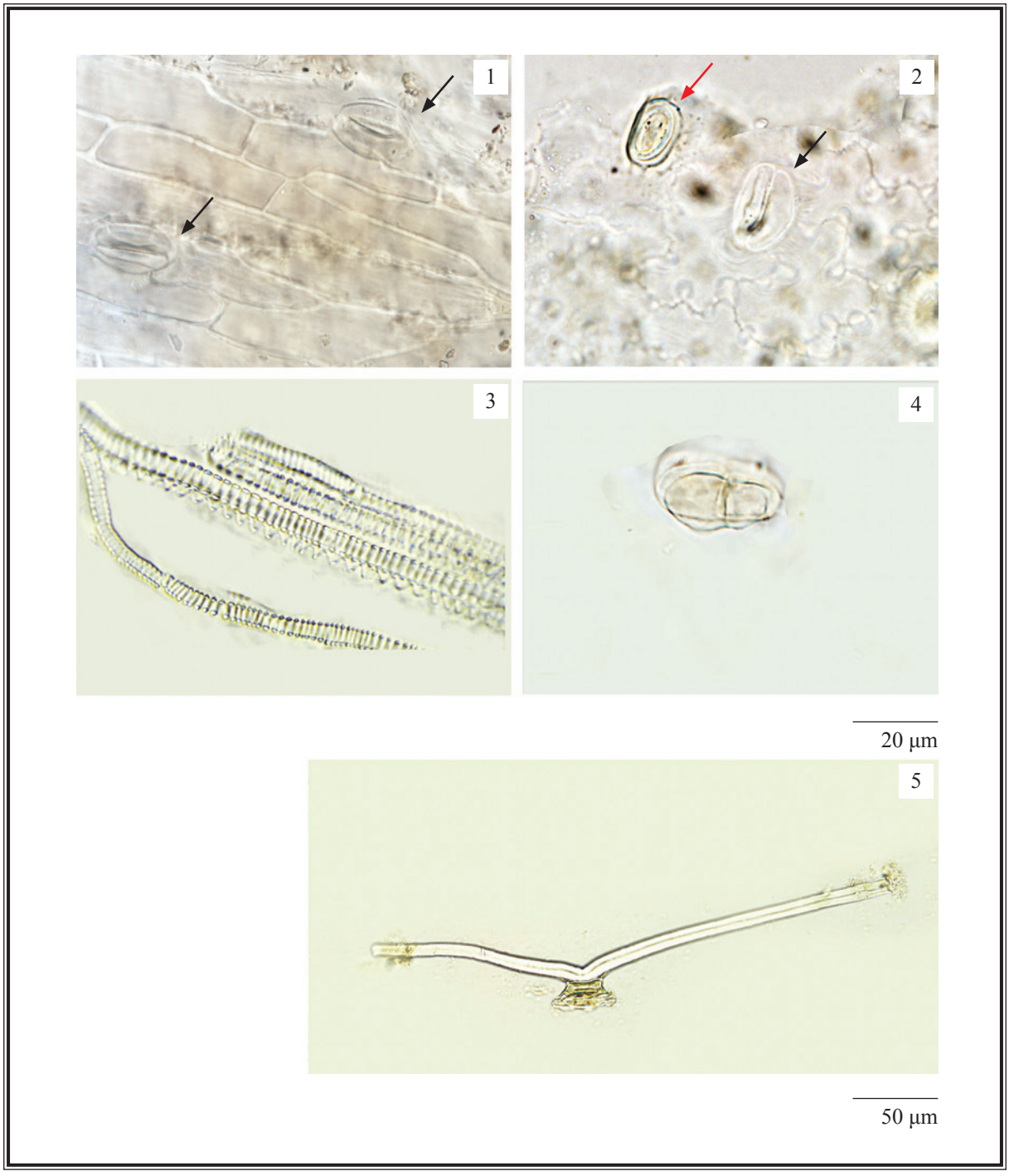


Figure 5 (i) Microscopic features of powder of dried aerial part of *Artemisia scoparia* Waldst. et Kit. (under the light microscope)

1. Upper epidermal cells with stomata (⟶)
2. Lower epidermal cells with stomata (⟶) and residue of non-glandular hair (⟶)
3. Spiral vessels 4. Glandular hair 5. Non-glandular hair

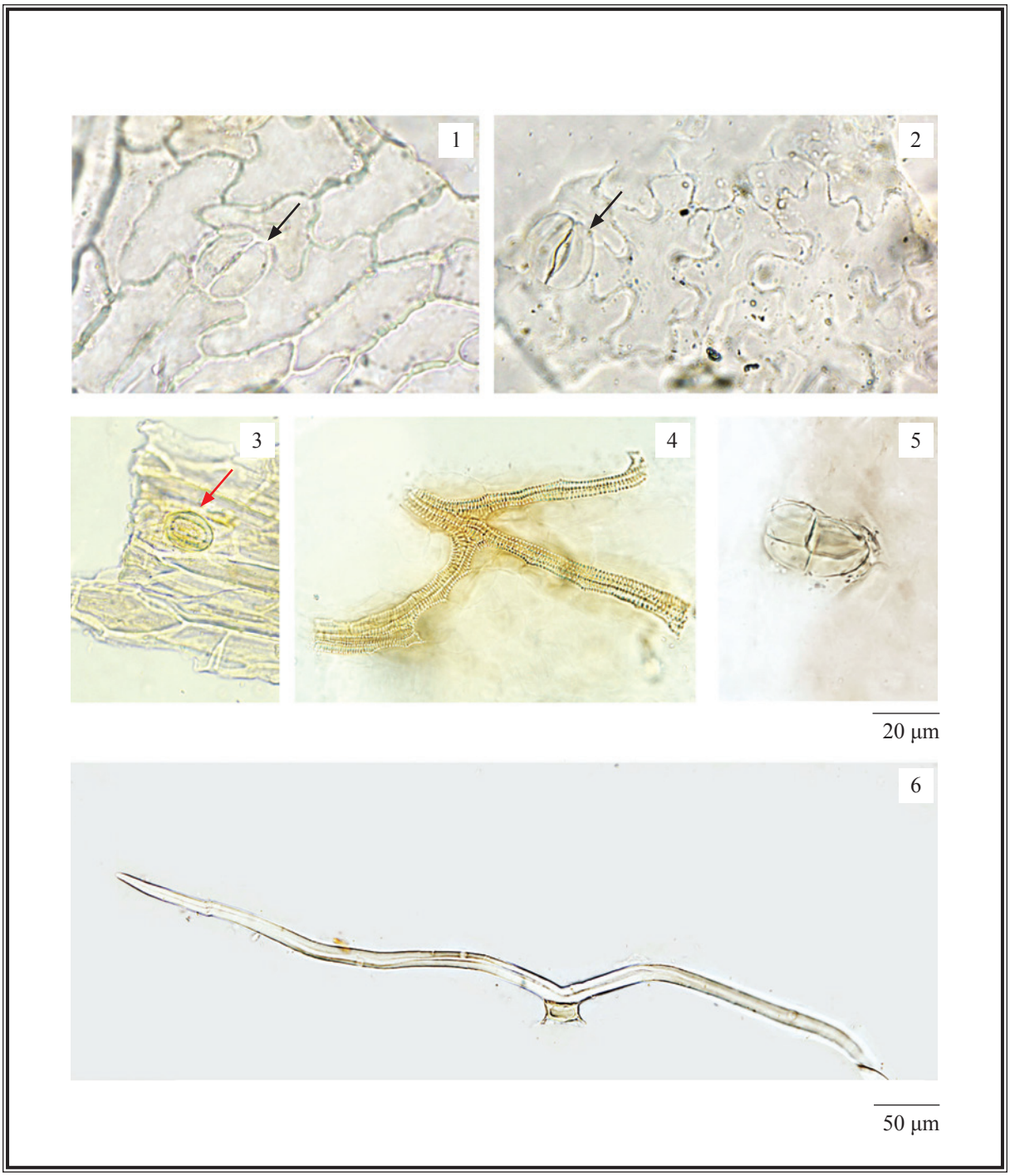


Figure 5 (ii) Microscopic features of powder of dried aerial part of *Artemisia capillaris* Thunb. (under the light microscope)

1. Upper epidermal cells with stomata (⟶) 2. Lower epidermal cells with stomata (⟶)
3. Lower epidermal cells with residue of non-glandular hair (⟶) 4. Spiral vessels
5. Glandular hair 6. Non-glandular hair

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

Standard solution

Chlorogenic acid standard solution

Weigh 1.0 mg of chlorogenic acid CRS (Fig. 6) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of *n*-butyl acetate, formic acid and water (28:13:10, v/v). Shake well and use the upper layer.

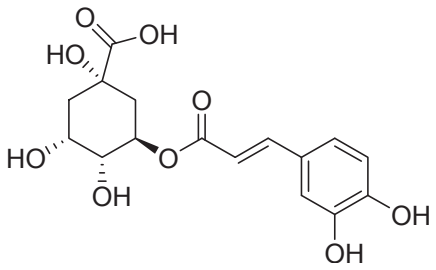
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (50%). Sonicate (220 W) the mixture for 30 min. Filter the mixture.

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 chlorogenic acid standard solution (1 μL) and the test solution (2 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. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

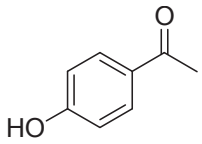


Figure 6 Chemical structures of (i) chlorogenic acid and (ii) 4-hydroxyacetophenone

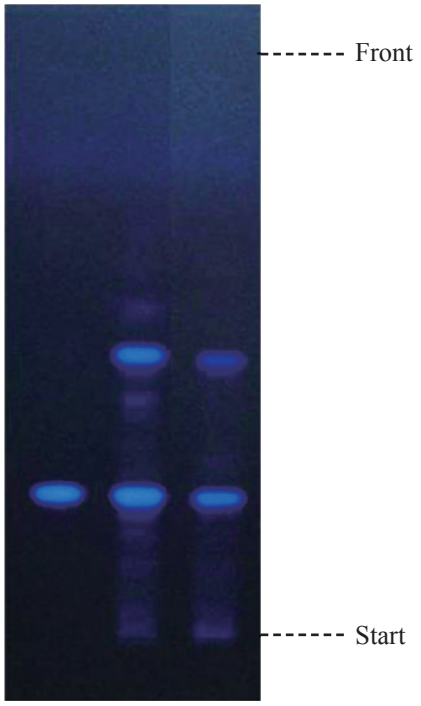


Figure 7 A reference HPTLC chromatogram of Artemisiae Scopariae Herba extract observed under UV light (366 nm)

1. Chlorogenic acid standard solution
2. Test solution of
(i) dried aerial part of *Artemisia scoparia* Waldst. et Kit.
(ii) dried aerial part of *Artemisia capillaris* Thunb.

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 chlorogenic acid (Fig. 7).

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

Standard solutions

Chlorogenic acid standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol.

4-Hydroxyacetophenone standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of 4-hydroxyacetophenone CRS (Fig. 6) and dissolve in 10 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (50%). Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. 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 (275 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)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	75	25	isocratic
10 – 30	75 → 55	25 → 45	linear gradient
30 – 50	55	45	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of chlorogenic acid Std-FP and 4-hydroxyacetophenone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and 4-hydroxyacetophenone should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and 4-hydroxyacetophenone peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and 4-hydroxyacetophenone peaks should not be less than 8500 and 20000 theoretical plates respectively.

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 1.0 [Fig. 8 (i) or (ii)].

Procedure

Separately inject chlorogenic acid Std-FP, 4-hydroxyacetophenone 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 4-hydroxyacetophenone peaks in the chromatograms of chlorogenic acid Std-FP, 4-hydroxyacetophenone Std-FP and the retention times of the three characteristic peaks [Fig. 8 (i) or (ii)] in the chromatogram of the test solution. Identify chlorogenic acid and 4-hydroxyacetophenone peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chlorogenic acid Std-FP and 4-hydroxyacetophenone Std-FP. The retention times of chlorogenic acid and 4-hydroxyacetophenone 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 three characteristic peaks of Artemisiae Scopariae Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Artemisiae Scopariae Herba extract

Peak No.	RRT	Acceptable Range
1 (chlorogenic acid)	0.79	± 0.06
2 (marker, 4-hydroxyacetophenone)	1.00	-
3	1.57	± 0.06

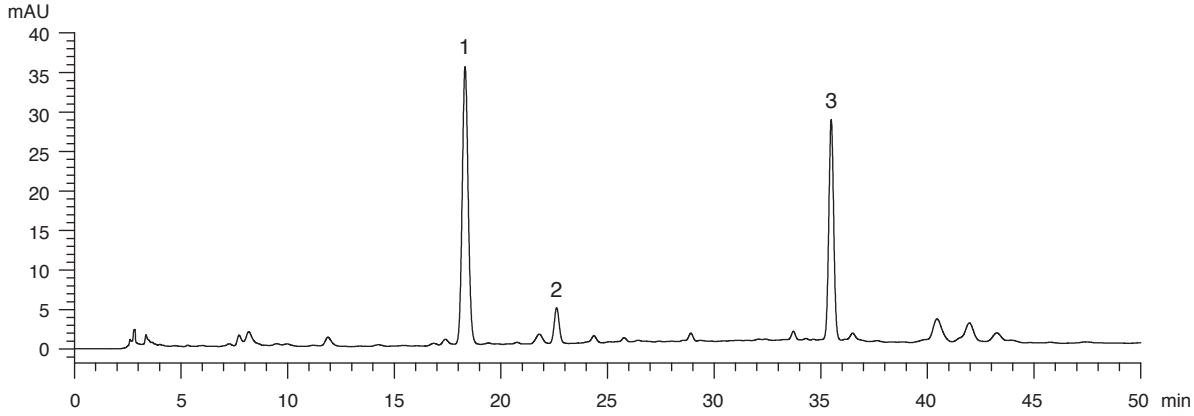


Figure 8 (i) A reference fingerprint chromatogram of dried aerial part of *Artemisia scoparia* Waldst. et Kit. extract

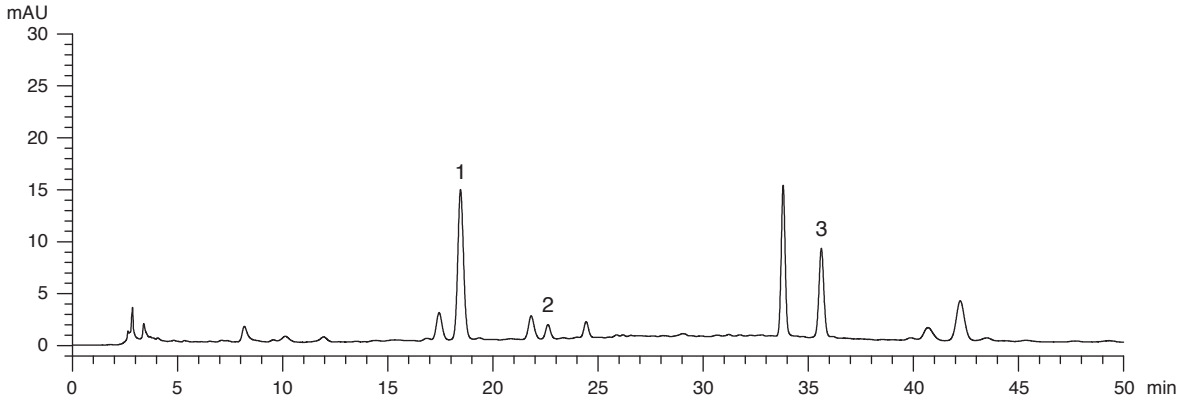


Figure 8 (ii) A reference fingerprint chromatogram of dried aerial part of *Artemisia capillaris* Thunb. extract

For positive identification, the sample must give the above three characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference fingerprint chromatograms [Fig. 8 (i) or (ii)].

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 5.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 32.0%.
Acid-insoluble ash: not more than 23.0%.

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 25.0%.
Ethanol-soluble extractives (hot extraction method): not less than 15.0%.

7. ASSAY

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

Standard solution

Chlorogenic acid standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 5.0 mg of chlorogenic acid CRS and dissolve in 50 mL of methanol.

Chlorogenic acid standard solution for assay, Std-AS

Measure accurately the volume of the chlorogenic acid Std-Stock, dilute with methanol to produce a series of solutions of 3, 5, 10, 30, 50 mg/L for chlorogenic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for two more times. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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. The mobile phase is a mixture of 0.1% phosphoric acid and methanol (75:25, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of chlorogenic acid Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of chlorogenic acid should not be more than 5.0%; the RSD of the retention time of chlorogenic acid peak should not be more than 2.0%; the column efficiency determined from chlorogenic acid peak should not be less than 4000 theoretical plates.

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

Limits

The sample contains not less than 0.50% of chlorogenic acid ($C_{16}H_{18}O_9$), calculated with reference to the dried substance.