Siegesbeckiae Herba

Figure 1 (i) A photograph of dried aerial part of *Siegesbeckia orientalis* L.

A. Aerial part of herb B. A small branch

C. Magnified image of upper surface of leaf

D. Magnified image of lower surface of leaf

E. Magnified image of capitulum F. Magnified image of achenes

Figure 1 (ii) A photograph of dried aerial part of *Siegesbeckia pubescens* Makino

A. Aerial part of herb B. A small branch

C. Magnified image of upper surface of leaf

D. Magnified image of lower surface of leaf

E. Magnified image of capitula

F. Magnified image of achenes

Figure 1 (iii) A photograph of dried aerial part of *Siegesbeckia glabrescens* Makino

A. Aerial part of herb B. A small branch

C. Magnified image of upper surface of leaf

D. Magnified image of lower surface of leaf

E. Magnified image of capitula

F. Magnified image of achenes

1. NAMES

Official Name: Siegesbeckiae Herba

Chinese Name: 豨薟草

Chinese Phonetic Name: Xixiancao

2. SOURCE

Siegesbeckiae Herba is the dried aerial part of *Siegesbeckia orientalis* L., *Siegesbeckia pubescens* Makino or *Siegesbeckia glabrescens* Makino (Asteraceae). The aerial part is collected in summer and autumn before or at flowering period, foreign matter removed, then dried under the sun to obtain Siegesbeckiae Herba.

3. DESCRIPTION

***Siegesbeckia orientalis* L.:** Stem slightly subsquare, frequently branched, 30-110 cm long, 3-10 mm in diameter; externally greyish-green, yellowish-brown or purplish-brown, with longitudinal furrows and fine longitudinal striations, covered with grey pubescences; nodes distinct, slightly swollen; texture fragile, easily broken, fracture yellowish-white to pale green; pith broad, whitish, hollowed. Leaves simple, opposite, lamina frequently crumpled and rolled, ovate-lanceolate to triangular-ovate when intact, greyish-green, margins slightly undulate or entire; both surfaces covered with white pubescences, venation trinerved at base. Some stems with yellow capitula, involucre spatulate. Stipule 2, some with fruits, achenes, obovate tetragonal, slightly curved, greyish-black, about 3 mm long. Odour slight; taste slightly bitter [Fig. 1 (i)].

***Siegesbeckia pubescens* Makino:** Upper part of stem with more branches. Leaf ovate when intact, surface dark greyish-green, margins obtusely serrate. Stipule 1. Peduncle covered with dark brown pubescence [Fig. 1 (ii)].

***Siegesbeckia glabrescens* Makino:** Stem mostly purplish-brown, relatively thin and delicate, generally no more than 80 cm long, surface of the upper part of stem sparsely covered with greyish-white pubescences. Leaf ovate when intact, margins regularly serrate [Fig. 1 (iii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Stem

***Siegesbeckia orientalis* L.:** Epidermis consists of 1 layer of rectangular cells, covered with thin cuticle. Cortex relatively narrow, collenchyma consists of 3-6 layers of irregular polygonal or subpolygonal cells, thickened at the corners, located on the outer part of cortex. 2-5 layers of parenchymatous cells located on the inner part of cortex, cells shrunken, irregularly subpolygonal, wall sinuous. Several big and small vascular bundles arranged alternately, elongated ovate to triangular-ovate. Phloem relatively narrow, elliptic to crescent, arranged in an interrupted ring; fibre bundles located on the outer side of phloem, mostly lignified. Cambium distinct. Xylem vessels mostly arranged in a row. Pith large, central part hollow, relatively large [Fig. 2 (i)].

***Siegesbeckia pubescens* Makino:** Cortex extremely narrow, 5-8 layers of collenchymatous cells located on the outer part of cortex, 3-4 layers of parenchymatous cells located on the inner side of cortex. Phloem extremely narrow, phloem fibre bundle relatively small [Fig. 2 (ii)].

***Siegesbeckia glabrescens* Makino:** Cortex extremely narrow, 2-3 layers of collenchymatous cells located on the outer part of the cortex, 2-5 layers of parenchymatous cells located on the inner side of cortex. Pith solid in young stem and hollow in central part of old stem [Fig. 2 (iii)].

Leaf

***Siegesbeckia orientalis* L.:** Upper epidermal cells rectangular, relatively large, lower epidermal cells similar in features, but relatively small, both upper and lower epidermal cells covered with thin cuticle. Non-glandular hair raised from the upper and lower epidermis. Palisade tissue located beneath the upper epidermis, consisting of 1 layer of cells. Spongy tissue consists of several layers of cells. Collenchymatous cells thickened at the corners, located on the inner side of upper and lower epidermis of midvein. Midvein vascular bundles 3-5, the middle bundle the largest [Fig. 2 (i)].

***Siegesbeckia pubescens* Makino:** Vascular bundles of midvein arranged closely. Lower epidermis densely covered with non-glandular hairs [Fig. 2 (ii)].

***Siegesbeckia glabrescens* Makino:** Vascular bundle of midvein 1-3 [Fig. 2 (iii)].

Powder

Colour greyish-green. Non-glandular hairs consist of 1- to 8-celled, apical cell relatively slender and long, some middle cell relatively narrow, base 25-79 µm in diameter, 114-833 µm long, walls slightly thickened and vary in thickness. Glandular hairs two typed, one with multiseriate stalk and a rounded head, consisting of 10 to hundreds of cells in lateral view, head 49-302 µm in diameter, stalk consists of between 10- to 70-celled in lateral view, arranged in 2-10 rows, stalk 143-672 µm long, 44-510 µm in diameter near the base; another type glandular hairs rounded to oblong in surface view, 4- to 6-celled, cells arranged into 2 or 3 layers in pairs, 42-55 µm in diameter. Pollen grains subrounded, 27-29 µm in diameter, with 3 germinal pores, mostly indistinct, surface covered with spines of 3-5 µm long. Anticlinal walls of epidermal cells of leaf irregular sinuous in surface view, stomata anomocytic, subrounded or elliptic, 18-27 µm in diameter, 27-38 µm long, subsidiary cells 3-6. Spiral vessels 11-87 µm in diameter. Epidermal cells of achene irregular in shape in surface view, elongated, walls thickened and densely covered with straight or sinuous striations. Fibres scattered singly or in bundles, relatively long, with relatively thickened walls, 9-24 µm in diameter; polychromatic under the polarized microscope [Fig. 3 (i), (ii) and (iii)].

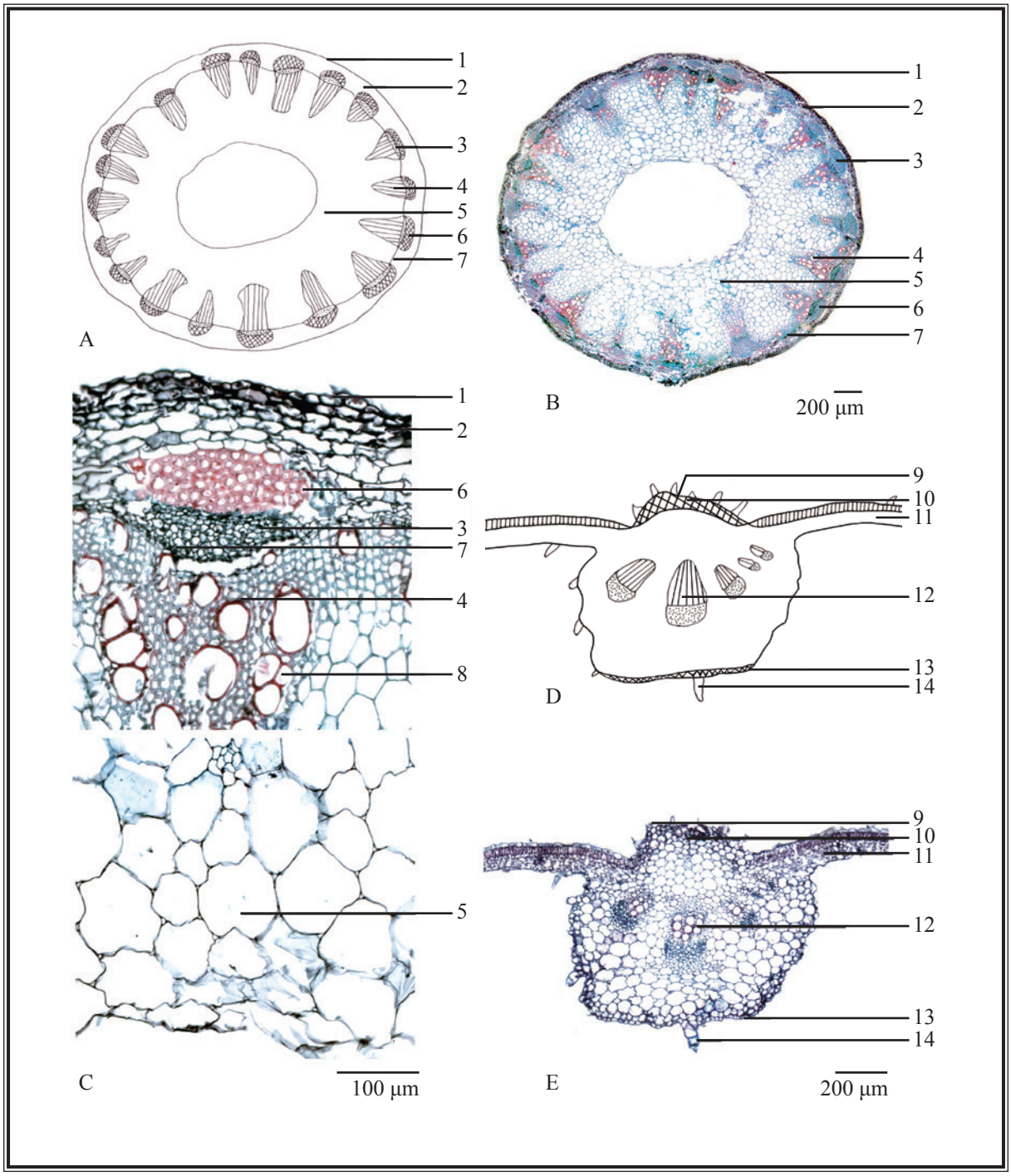

Figure 2 (i) Microscopic features of transverse section of dried stem and leaf of *Siegesbeckia orientalis* L.

A. Sketch of transverse section of stem B. Section illustration of stem
C. Magnified section illustration of stem D. Sketch of transverse section of leaf E. Section illustration of leaf

1. Epidermis 2. Cortex 3. Phloem 4. Xylem 5. Pith 6. Phloem fibre
7. Cambium 8. Vessel 9. Upper epidermis 10. Collenchyma
11. Mesophyll tissue 12. Vascular bundle 13. Lower epidermis 14. Non-glandular hair

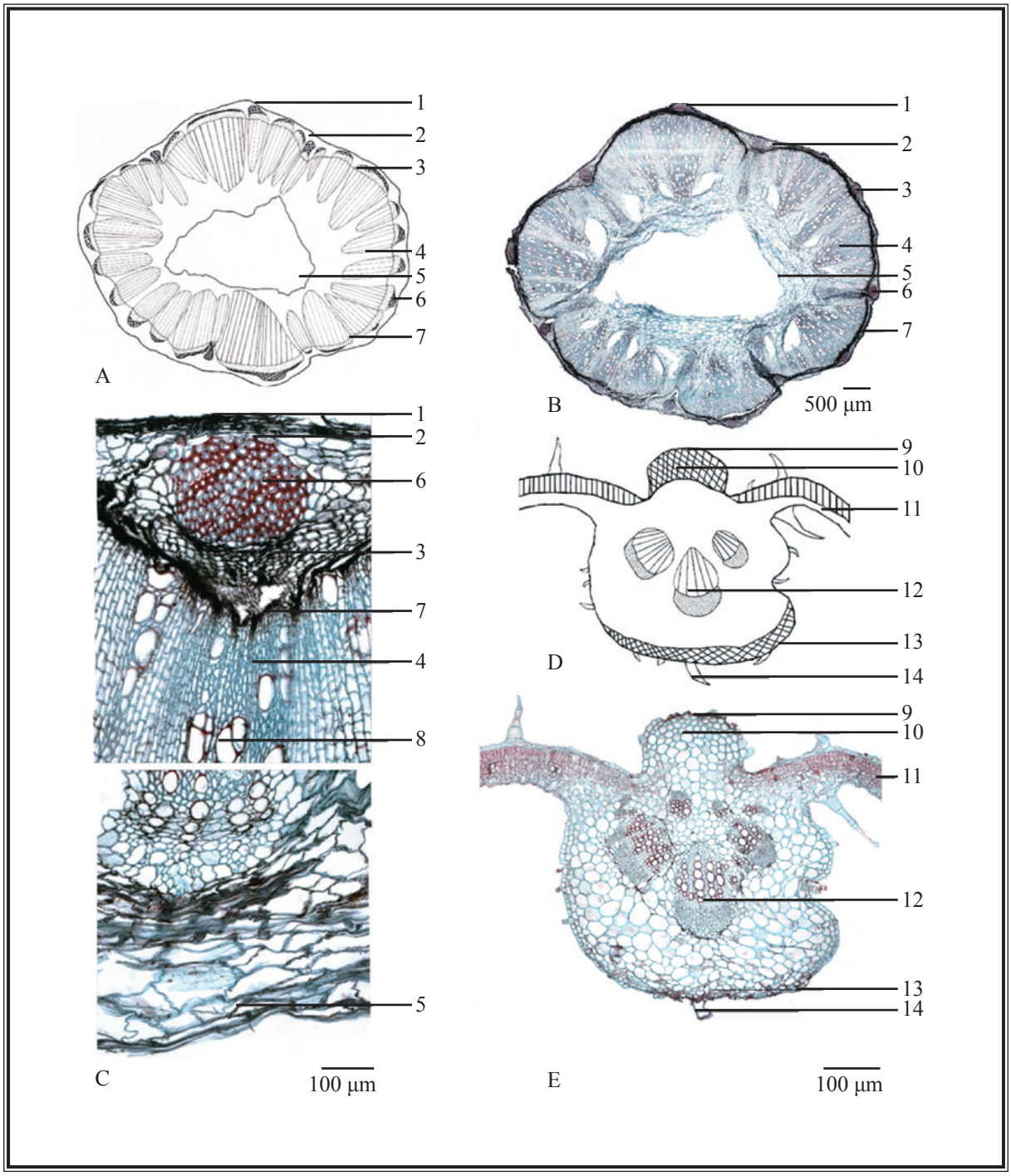

Figure 2 (ii) Microscopic features of transverse section of dried stem and leaf of *Siegesbeckia pubescens* Makino

A. Sketch of transverse section of stem B. Section illustration of stem
C. Magnified section illustration of stem D. Sketch of transverse section of leaf E. Section illustration of leaf

1. Epidermis 2. Cortex 3. Phloem 4. Xylem 5. Pith 6. Phloem fibre
7. Cambium 8. Vessel 9. Upper epidermis 10. Collenchyma 11. Mesophyll tissue
12. Vascular bundle 13. Lower epidermis 14. Non-glandular hair

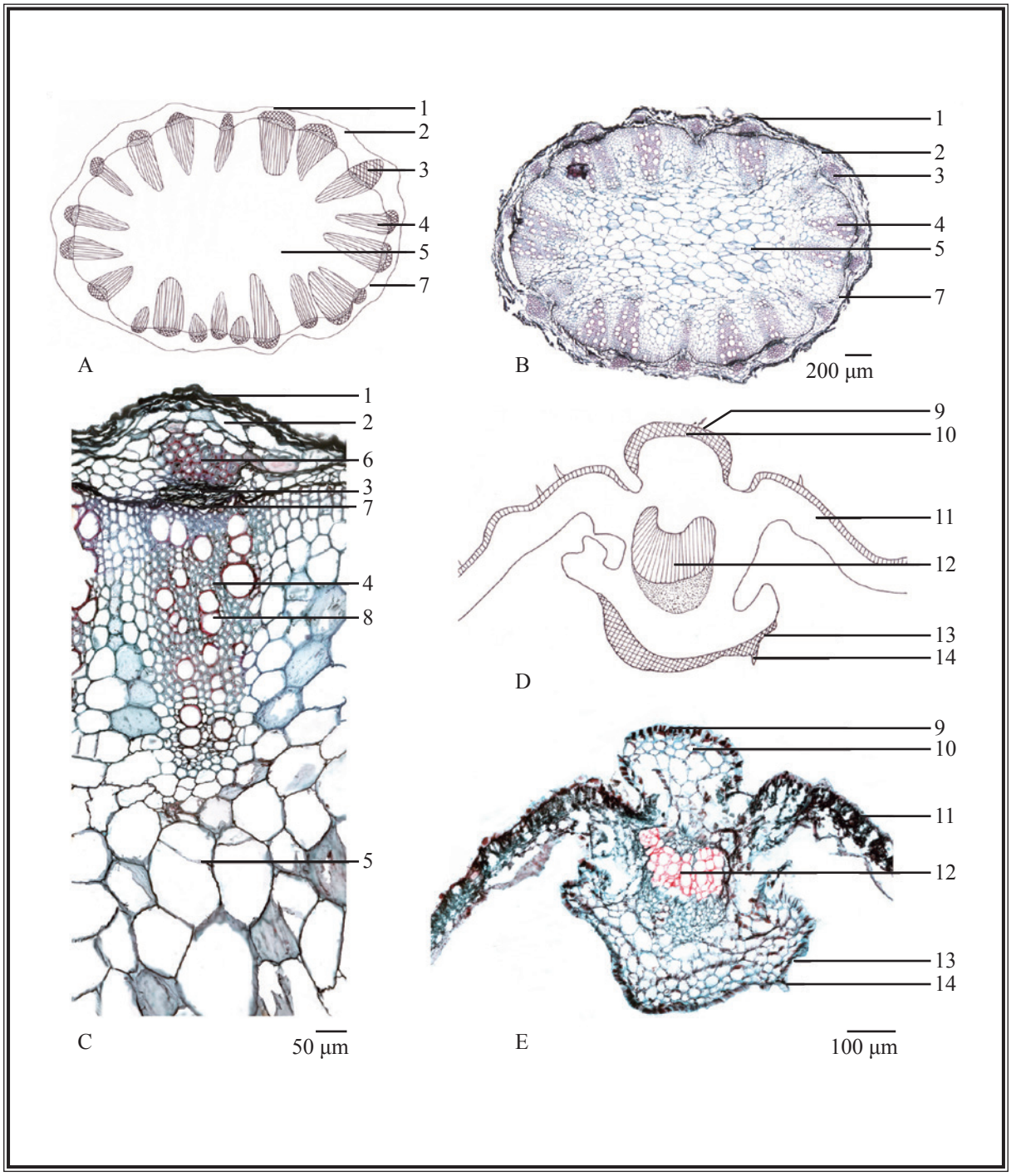

Figure 2 (iii) Microscopic features of transverse section of dried stem and leaf of *Siegesbeckia glabrescens* Makino

A. Sketch of transverse section of stem B. Section illustration of stem
C. Magnified section illustration of stem D. Sketch of transverse section of leaf E. Section illustration of leaf

1. Epidermis 2. Cortex 3. Phloem 4. Xylem 5. Pith 6. Phloem fibre
7. Cambium 8. Vessel 9. Upper epidermis 10. Collenchyma 11. Mesophyll tissue
12. Vascular bundle 13. Lower epidermis 14. Non-glandular hair

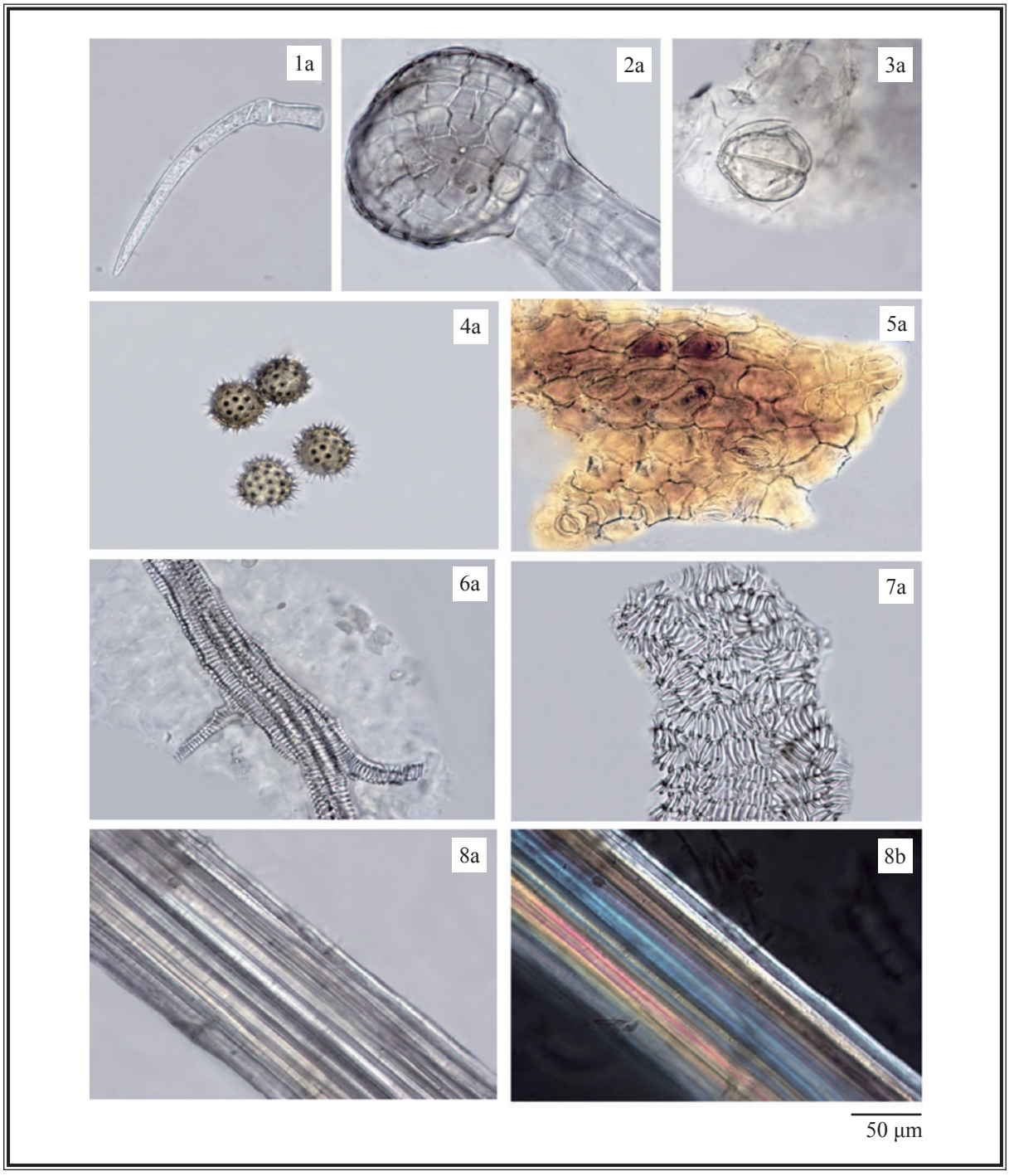

Figure 3 (i) Microscopic features of powder of dried ariel part of *Siegesbeckia orientalis* L.

1. Non-glandular hair 2. Glandular hair with multiseriate stalk 3. Glandular hair
4. Pollen grains 5. Epidermal cells of leaf 6. Spiral vessels in mesophyll
7. Epidermal cells of testa 8. Fibres

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

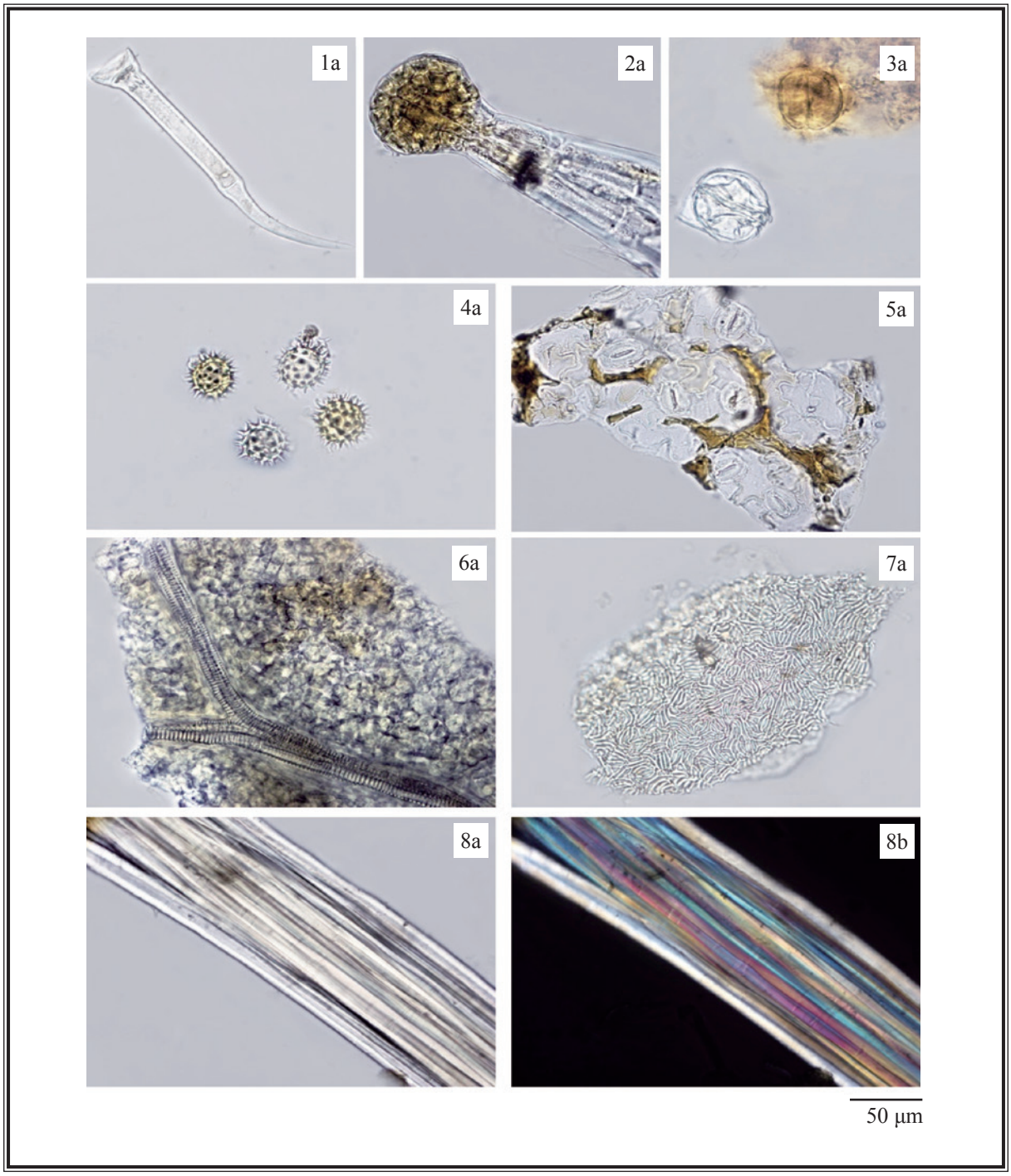

Figure 3 (ii) Microscopic features of powder of dried ariel part of *Siegesbeckia pubescens* Makino

1. Non-glandular hair 2. Glandular hair with multiseriate stalk 3. Glandular hairs
4. Pollen grains 5. Epidermal cells of leaf 6. Spiral vessels in mesophyll
7. Epidermal cells of testa 8. Fibres

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

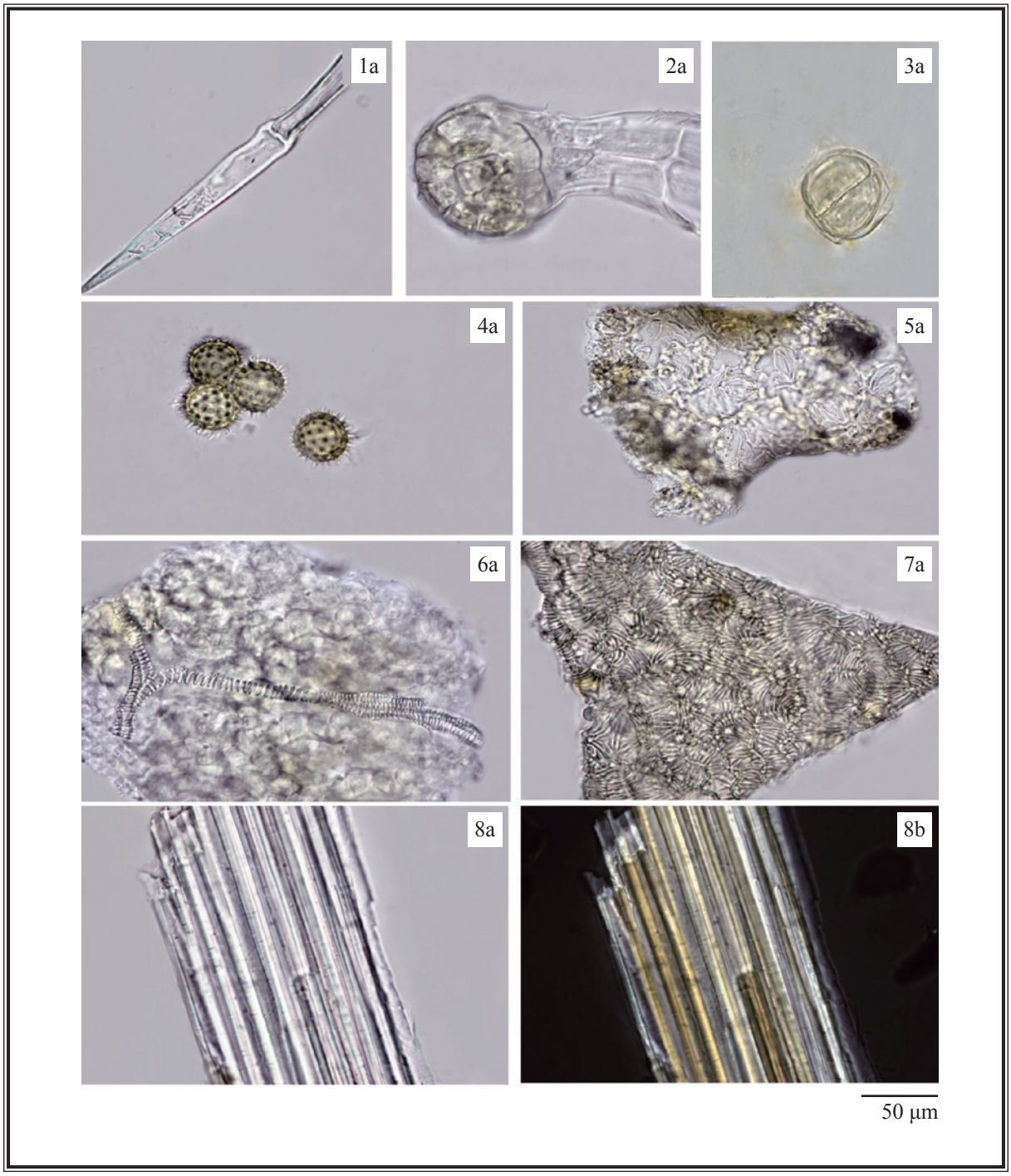

Figure 3 (iii) Microscopic features of powder of dried ariel part of *Siegesbeckiae glabrescens* Makino

1. Non-glandular hair 2. Glandular hair with multiseriate stalk 3. Glandular hair
4. Pollen grains 5. Epidermal cells of leaf 6. Spiral vessels in mesophyll
7. Epidermal cells of testa 8. Fibres

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

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

Standard solution

Kirenol standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane, acetone, methanol, water and formic acid (10:6:5:5:1, v/v). Use the lower layer.

Spray reagent

Weigh 1 g of vanillin and dissolve in 50 mL of sulphuric acid.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (240 W) the mixture for 25 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 kirenol standard solution (5 μL) and the test solution (12 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 7 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 5-10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

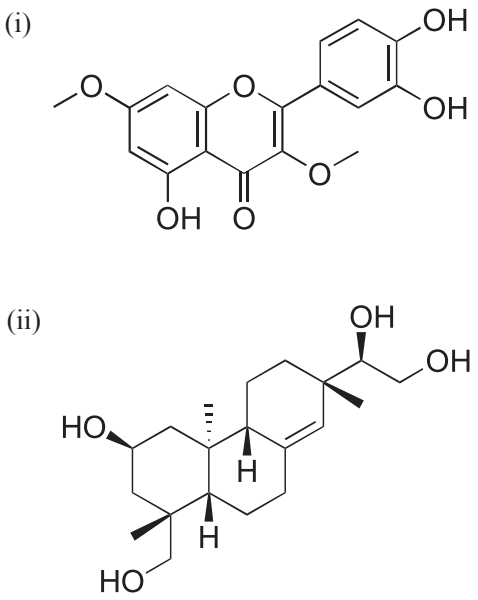

Figure 4 Chemical structures of (i) 3,7-di-*O*-methylquercetin and (ii) kirenol

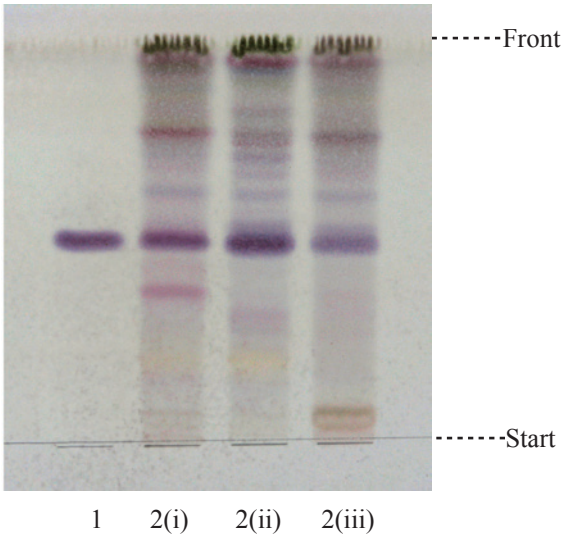

Figure 5 A reference HPTLC chromatogram of Siegesbeckiae Herba extract observed under visible light after staining

1. Kirenol standard solution
2. Test solution of
(i) dried aerial part of *Siegesbeckia orientalis* L.
(ii) dried aerial part of *Siegesbeckia pubescens* Makino
(iii) dried aerial part of *Siegesbeckia glabrescens* Makino

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 kirenol (Fig. 5).

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

Standard solutions

3,7-Di-O-methylquercetin standard solution for fingerprinting, Std-FP (15 mg/L)

Weigh 0.3 mg of 3,7-di-*O*-methylquercetin CRS (Fig. 4) and dissolve in 20 mL of methanol.

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

Weigh 0.8 mg of kirenol CRS and dissolve in 20 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 3 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. 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 (215 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 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	73	27	isocratic
20 – 60	73 → 60	27 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of 3,7-di-*O*-methylquercetin Std-FP and kirenol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 3,7-di-*O*-methylquercetin and kirenol should not be more than 5.0%; the RSD of the retention times of 3,7-di-*O*-methylquercetin and kirenol peaks should not be more than 2.0%; the column efficiencies determined from 3,7-di-*O*-methylquercetin and kirenol peaks should not be less than 75000 and 7500 theoretical plates respectively.

The *R* value between peak 1 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 (i), (ii) or (iii)].

Procedure

Separately inject 3,7-di-*O*-methylquercetin Std-FP, kirenol Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of 3,7-di-*O*-methylquercetin and kirenol peaks in the chromatograms of 3,7-di-*O*-methylquercetin Std-FP, kirenol Std-FP and the retention times of the three characteristic peaks [Fig. 6 (i), (ii) or (iii)] in the chromatogram of the test solution. Identify 3,7-di-*O*-methylquercetin and kirenol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of 3,7-di-*O*-methylquercetin Std-FP and kirenol Std-FP. The retention times of 3,7-di-*O*-methylquercetin and kirenol 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 Siegesbeckiae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (kirenol)	0.21	± 0.03
2	0.37	± 0.03
3 (marker, 3,7-di-*O*-methylquercetin)	1.00	-

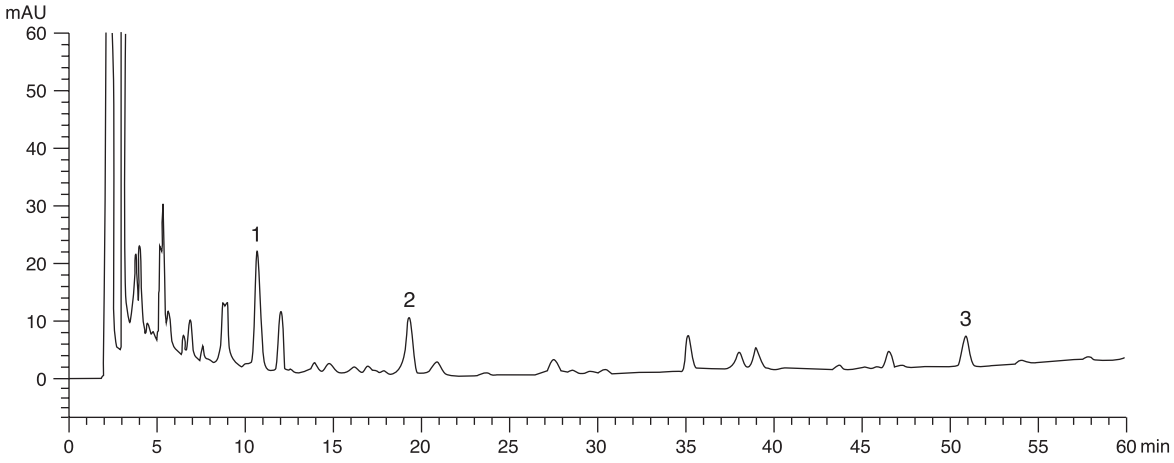

Figure 6 (i) A reference fingerprint chromatogram of dried aerial part of *Siegesbeckia orientalis* L. extract

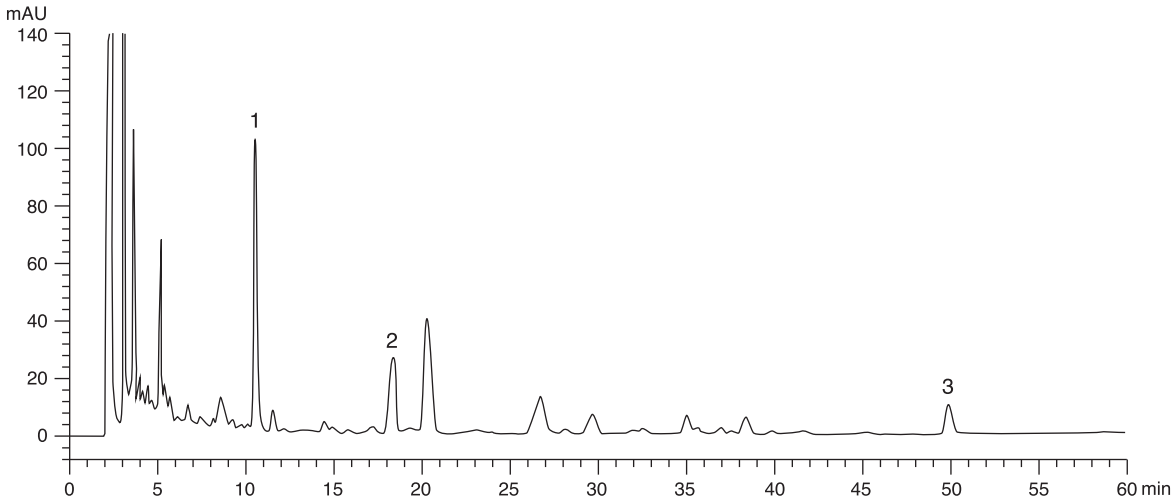

Figure 6 (ii) A reference fingerprint chromatogram of dried aerial part of *Siegesbeckia pubescens* Makino extract

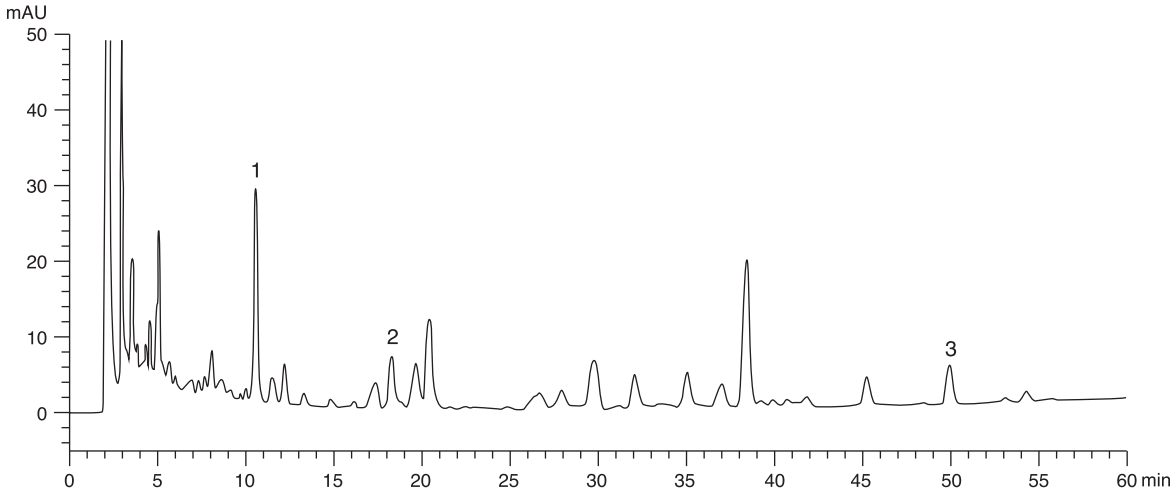

Figure 6 (iii) A reference fingerprint chromatogram of dried aerial part of *Siegesbeckia glabrescens* Makino extract

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. 6 (i), (ii) or (iii)].

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 4.0%.

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 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 11.0%.
Ethanol-soluble extractives (hot extraction method): not less than 10.0%.

7. ASSAY

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

Standard solution
Kirenol standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 1.0 mg of kirenol CRS and dissolve in 1 mL of methanol.
Kirenol standard solution for assay, Std-AS
Measure accurately the volume of the kirenol Std-Stock, dilute with methanol to produce a series of solutions of 2.5, 10, 20, 40, 60 mg/L for kirenol.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 3 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. 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 (215 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. The mobile phase is a mixture of water and acetonitrile (73:27, v/v). The elution time is about 35 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of kirenol Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of kirenol should not be more than 5.0%; the RSD of the retention time of kirenol peak should not be more than 2.0%; the column efficiency determined from kirenol peak should not be less than 7500 theoretical plates.

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

Limits

The sample contains not less than 0.079% of kirenol ($C_{20}H_{34}O_4$), calculated with reference to the dried substance.