Lonicerae Flos

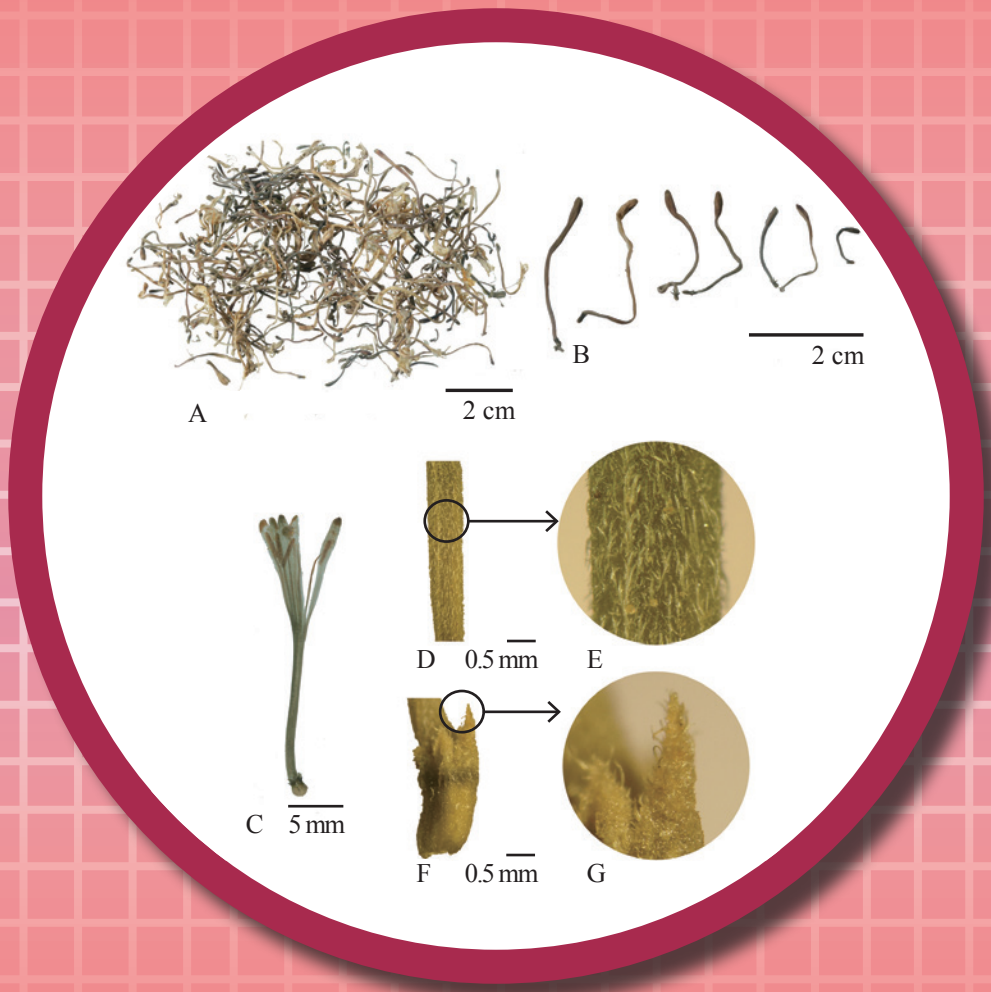

Figure 1 (i) A photograph of dried flower bud and opening flower of *Lonicera macranthoides* Hand.-Mazz.

A. Lonicerae Flos B. Flower buds C. Magnified image of dissected flower (spread after soaking)
D. Magnified image of corolla tube (surface view) E. Magnified image of corolla tube (partial view)
F. Magnified image of calyx (surface view) G. Magnified image of calyx tooth (partial view)

Figure 1 (ii) A photograph of dried flower bud and opening flower of *Lonicera hypoglauca* Miq.

A. Lonicerae Flos B. Flower buds C. Magnified image of dissected flower (spread after soaking)
D. Magnified image of corolla tube (surface view) E. Magnified image of corolla tube (partial view)
F. Magnified image of calyx (surface view) G. Magnified image of calyx tooth (partial view)

1. NAMES

Official Name: Lonicerae Flos

Chinese Name: 山銀花

Chinese Phonetic Name: Shanyinhua

2. SOURCE

Lonicerae Flos is the dried flower bud or with opening flower of *Lonicera macranthoides* Hand.-Mazz. or *Lonicera hypoglauca* Miq. (Caprifoliaceae). The flower bud is collected in early summer before anthesis, then dried to obtain Lonicerae Flos.

3. DESCRIPTION

***Lonicera macranthoides* Hand.-Mazz.:** Flower bud clavate, slightly curved, 1.5-5.4 cm long, upper part 1.5-3 mm in diameter, lower part 0.5-1.5 mm in diameter. Externally greenish-brown to yellowish-white. Corolla surface densely covered with yellowish-white bristles, occasionally scattered with pale yellow to orange-yellow glandular dots; calyx tube surface almost glabrous, apex 5-lobed, lobes long-triangular, sparsely pubescent, ciliate at the edges. Corolla of opening flower slightly tubular, bilabiate at apex, upper lip 4-lobed, lower lip entire, the length of lips shorter than half length of the corolla. Stamens 5, pistil 1, style glabrous. Texture slightly hard and springy. Odour delicately aromatic; taste slightly bitter and sweet [Fig. 1 (i)].

***Lonicera hypoglauca* Miq.:** Flower bud 1.5-4.5 cm long, upper part 1.3-3 mm in diameter, lower part 0.5-1.5 mm in diameter. Externally yellowish-white to dark brown. Corolla surface glabrous or sparsely pubescent, with scattered orange-yellow glandular spots; calyx tube and tooth surfaces almost glabrous, ciliate at the edges. Lips in opening flowers have the same length as or slightly longer than the corolla tube [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Corolla surface

***Lonicera macranthoides* Hand.-Mazz.:** Epidermis of corolla extremely thin, the cells subrectangular. Glandular hairs few, thick-walled non-glandular hairs relatively numerous on the epidermis. Head of glandular hairs mostly disk-shaped or inverted conical, top flat or slightly retuse, 5- to 16-celled in the lateral view, arranged in 1-3 layers, 37-125 μm in diameter, cells contain pale brown to yellowish-brown contents; stalk of glandular hairs 3- to 5-celled, 2(3-) cells always parallel arranged at the junction with the head, 30-168 μm long, 12-25 μm in diameter. Thick-walled non-glandular hairs unicellular, horn-shaped, 15-347 μm long, with few warty protuberances on the surface, spiral striations occasionally visible, lumen of the cells of short horn-shaped hairs relatively narrower compared with those of the long hairs; base slightly enlarged, 8-35 μm in diameter. Pollen grains usually adhered to the surface, yellow, subrounded to rounded-triangular. Clusters of calcium oxalate occasionally found, mainly scattered in lower layer of epidermal cells of corolla [Fig. 2 (i)].

***Lonicera hypoglauca* Miq.:** Epidermis of corolla with few glandular hairs, sparse thick-walled non-glandular hairs sparse, or nearly glabrous. Head of glandular hairs shield-like and large, mainly 13- to 20-celled in top view, 8- to 18-celled in lateral view, arranged in 1-2 layers, 71-150 μm in diameter, containing reddish-brown, brown or pale brown contents; stalk of glandular hairs 1- to 3-celled, extremely short, 15-60 μm long, 10-40 μm in diameter. Thick-walled non-glandular hair spouse of nearly glabrous, unicellular, 35-575 μm long, with small and dense warty protuberance on the surface, base 15-40 μm in diameter [Fig. 2 (ii)].

Powder

***Lonicera macranthoides* Hand.-Mazz.:** Colour yellowish-green to pale yellow. Pollen grains relatively numerous, yellow, rounded-triangular or subrounded, 50-87 μm in diameter, with fine granular sculptures visible on the surface of exine, with 3 furrows. Non-glandular hairs 2 types, first type thick walled non-glandular hairs on outer surface of corolla, unicellular, short horn-shaped, 8-35 μm in diameter, usually broken, with few warty protuberances on the surface; second type thin-walled non-glandular hairs on inner surface of corolla, long and curved, 15-25 μm in diameter, usually fragmented, with few fine warty protuberances on the surface. Clusters of calcium oxalate relatively abundant, scattered in parenchymatous cells, 10-30 μm in diameter; polychromatic under the polarized microscope. Spiral vessels frequently found, 5-10 μm in diameter. Fibrous layer of anther reticulate, with thickened walls. Glandular hairs occasionally found, with disk-shaped or inverted conical heads [Fig. 3 (i)].

***Lonicera hypoglauca* Miq.:** Colour pale yellow. Pollen grains relatively numerous, rounded-triangular or subrounded, 50-90 μm in diameter. Thick-walled non-glandular hairs on outer surface of corolla straight, 15-40 μm in diameter, usually broken, with small and dense warty protuberances on the surface; non-glandular hairs on inner surface of corolla 15-30 μm in diameter. Clusters of calcium oxalate 12-30 μm in diameter; polychromatic under the polarized microscope. Spiral vessels 5-10 μm in diameter. Glandular hairs occasionally found, with shield-like and large heads [Fig. 3 (ii)].

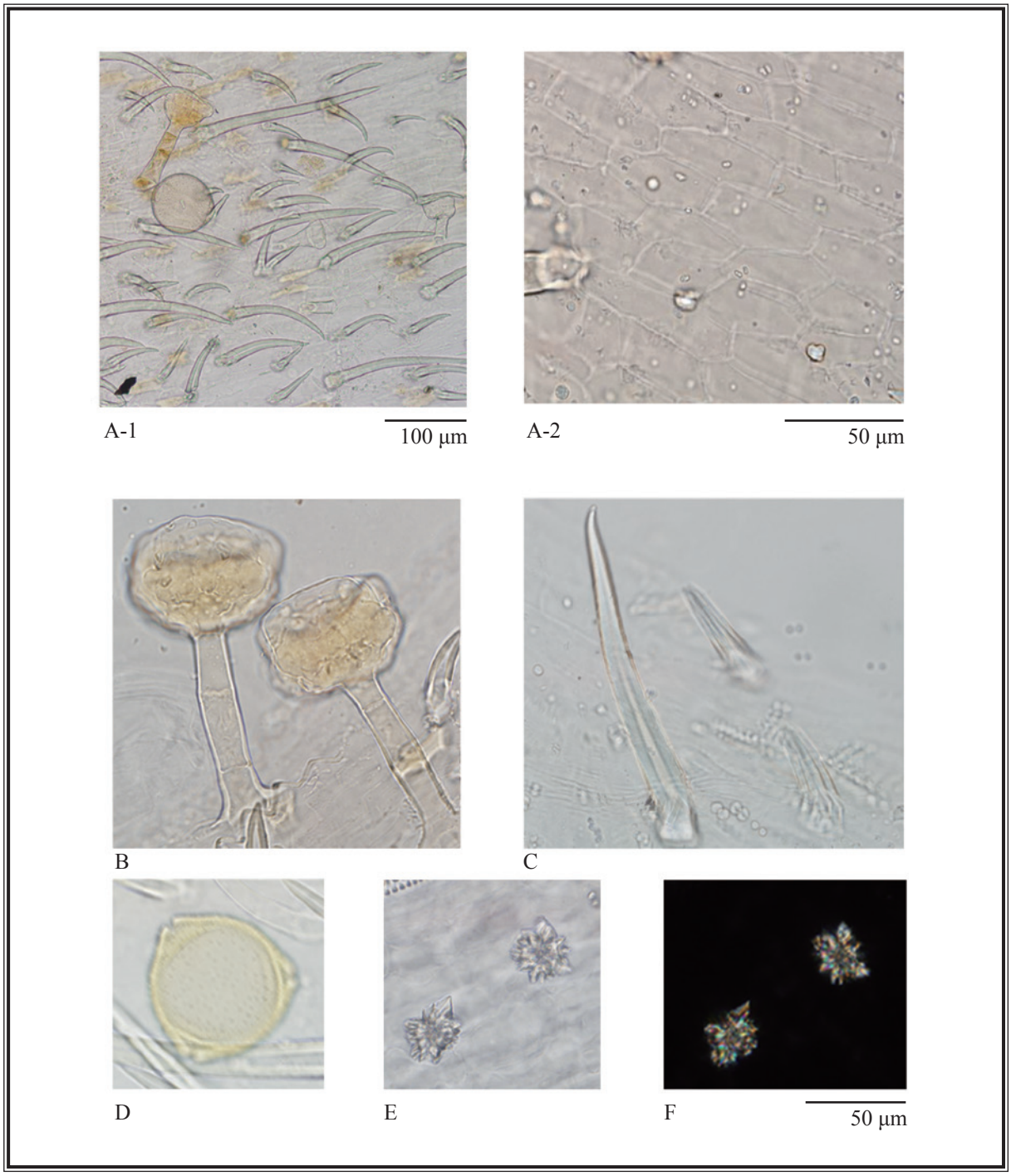

Figure 2 (i) Microscopic features of corolla surface of dried flower bud or with opening flower of *Lonicera macranthoides* Hand.-Mazz.

A-1. Corolla surface A-2. Epidermal cells B. Glandular hairs C. Non-glandular hairs
D. Pollen grain E. Clusters of calcium oxalate (under the light microscope)
F. Clusters of calcium oxalate (under the polarized microscope)

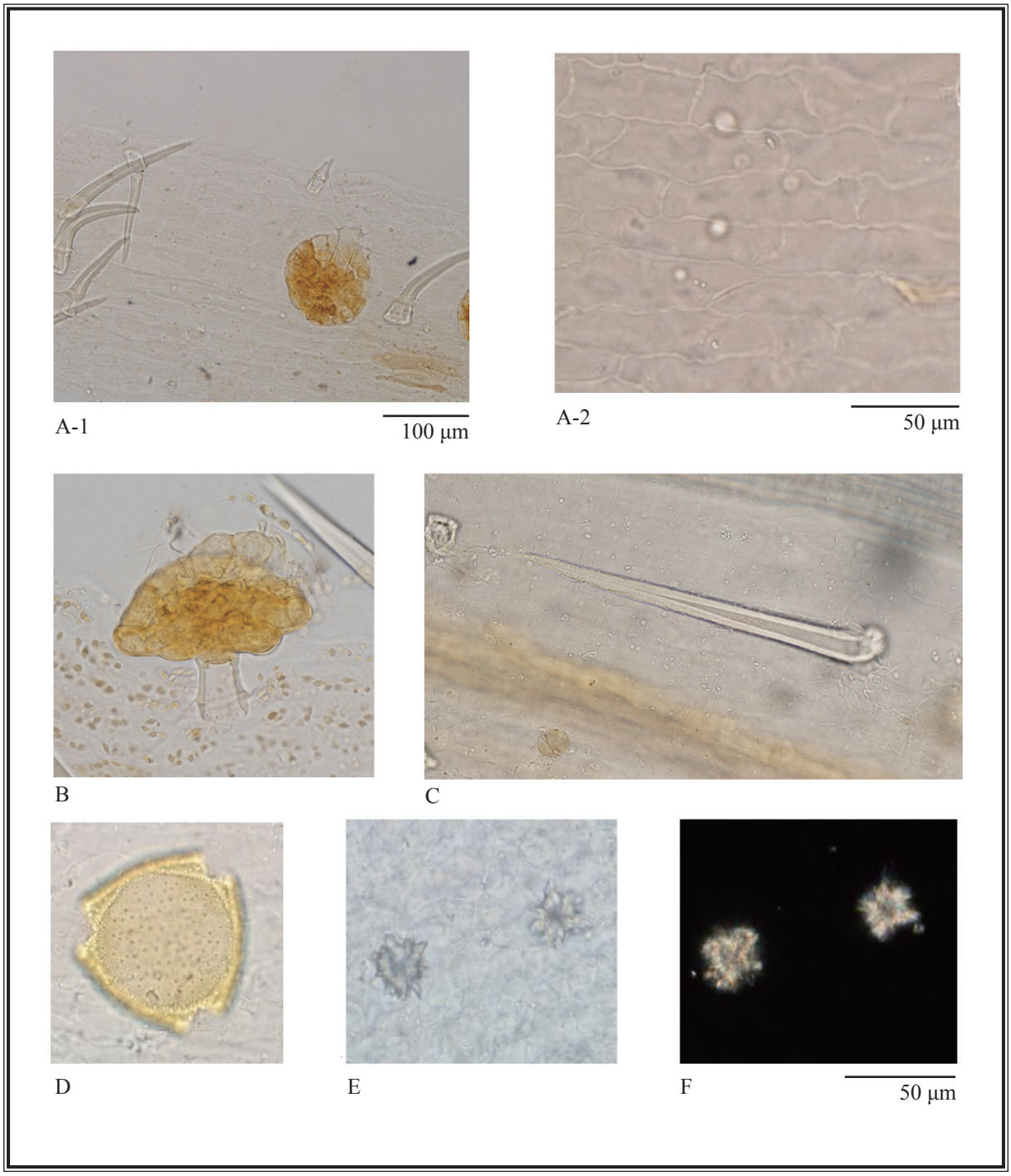

Figure 2 (ii) Microscopic features of corolla surface of dried flower bud or with opening flower of *Lonicera hypoglauca* Miq.

A-1. Corolla surface A-2. Epidermal cells B. Glandular hair C. Non-glandular hair
D. Pollen grain E. Clusters of calcium oxalate (under the light microscope)
F. Clusters of calcium oxalate (under the polarized microscope)

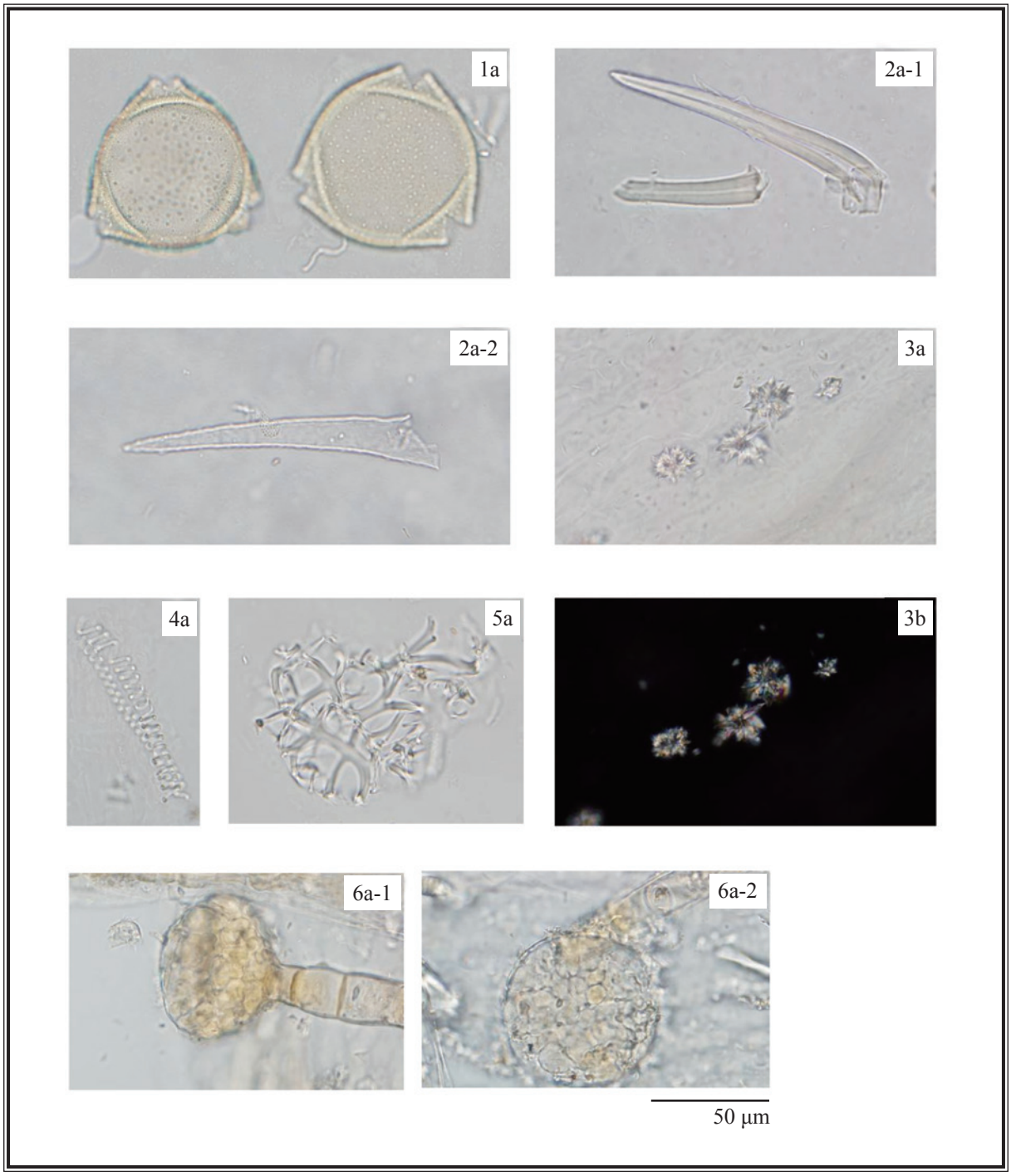

Figure 3 (i) Microscopic features of powder of dried flower bud or with opening flower of *Lonicera macranthoides* Hand.-Mazz.

1. Pollen grains 2. Non-glandular hairs (2-1 thick-walled, 2-2 thin-walled)
3. Clusters of calcium oxalate 4. Vessels 5. Fibrous layer of anther
6. Glandular hairs (6-1 in lateral view, 6-2 in top view)

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

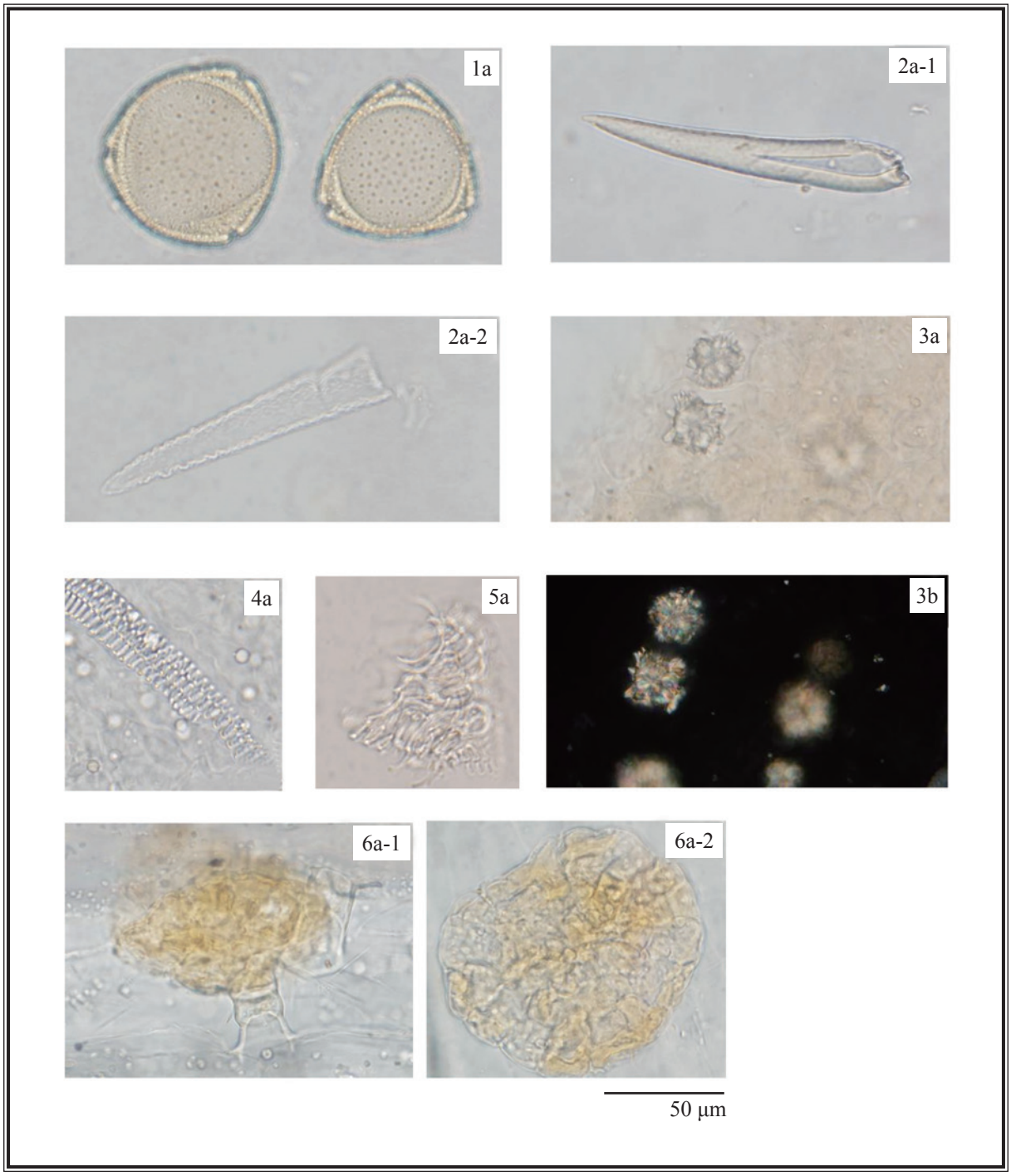

Figure 3 (ii) Microscopic features of powder of dried flower bud or with opening flower of *Lonicera hypoglauca* Miq.

1. Pollen grains 2. Non-glandular hairs (2-1 thick-walled, 2-2 thin-walled)
3. Clusters of calcium oxalate 4. Vessels 5. Fibrous layer of anther
6. Glandular hairs (6-1 in lateral view, 6-2 in top view)

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

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

(I) Identification of chlorogenic acid

Standard solution

Chlorogenic acid standard solution

Weigh 1.0 mg of chlorogenic acid CRS (Fig. 4) and dissolve in 1 mL of methanol. Keep at about 4ºC.

Developing solvent system

Prepare a mixture of *n*-butyl acetate, formic acid and water (7:2.5:2.5, v/v). Shake well and use the upper layer.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of methanol. Sonicate (250 W) the mixture for 20 min. Filter the mixture.

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

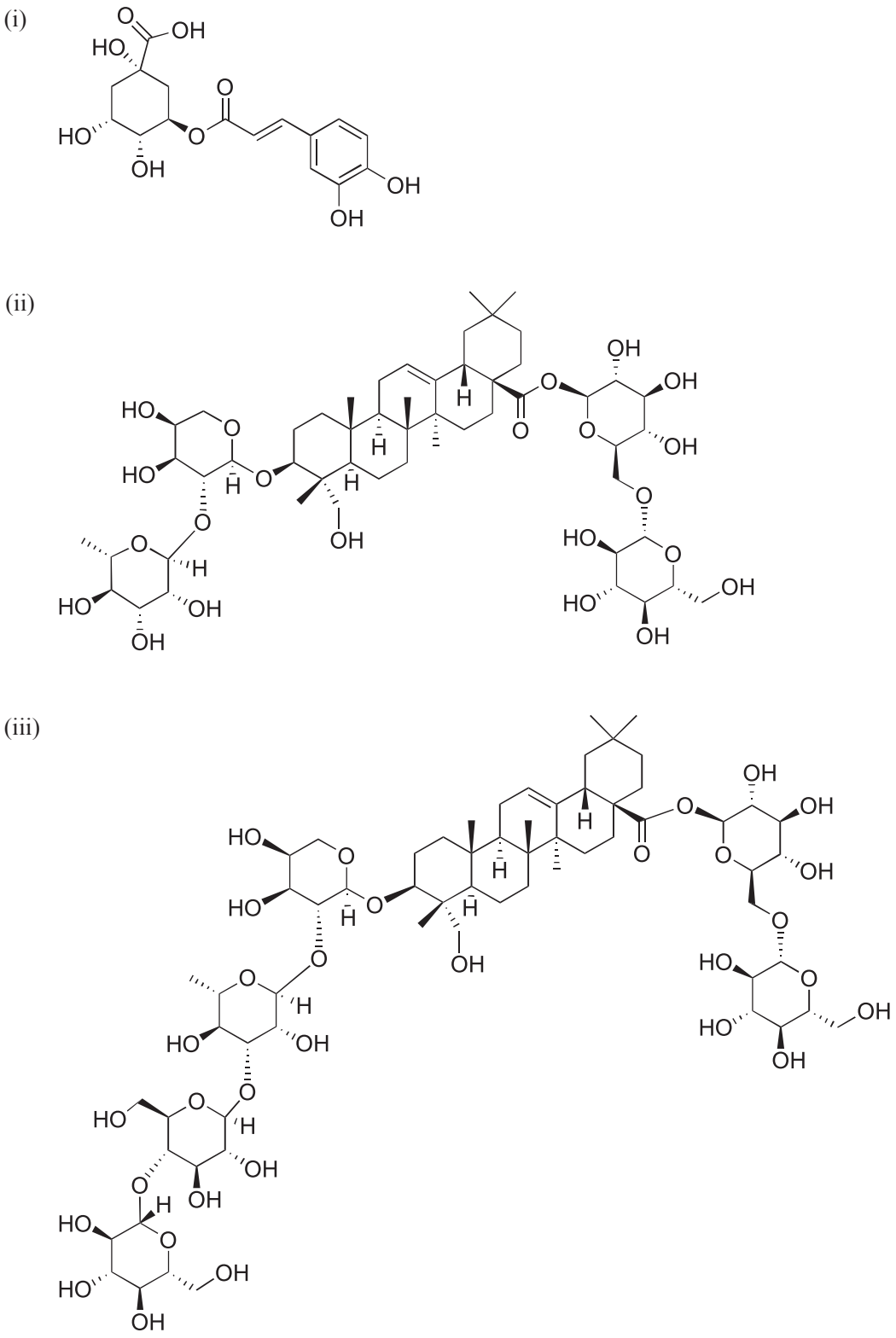

Figure 4 Chemical structures of (i) chlorogenic acid (ii) dipsacoside B and (iii) macranthoidin B

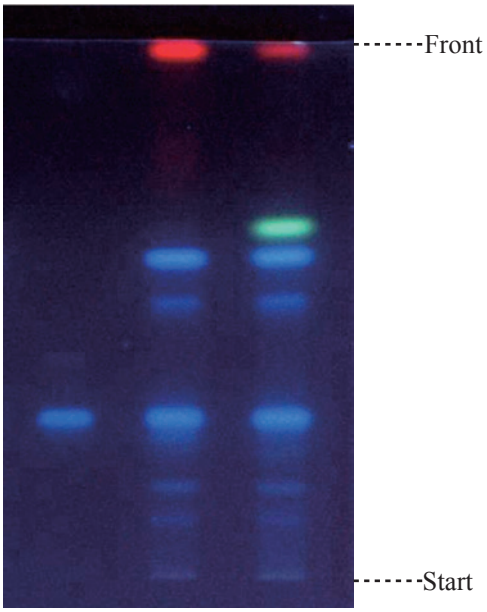

Figure 5 A reference HPTLC chromatogram of Lonicerae Flos extract observed under UV light (366 nm)

1. Chlorogenic acid standard solution
2. Test solution of
(i) dried flower bud or with opening flower of *Lonicera macranthoides* Hand.-Mazz.
(ii) dried flower bud or with opening flower of *Lonicera hypoglauca* Miq.

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. 5).

(II) Identification of dipsacoside B and macranthoidin B

Standard solutions

Dipsacoside B standard solution

Weigh 1.0 mg of dipsacoside B CRS (Fig. 4) and dissolve in 1 mL of methanol. Keep at about 4ºC.

Macranthoidin B standard solution

Weigh 1.0 mg of macranthoidin B CRS (Fig. 4) and dissolve in 1 mL of methanol. Keep at about 4ºC.

Developing solvent system

Prepare a mixture of *n*-butyl acetate, methanol and formic acid (10:10:0.5, v/v).

Spray reagent

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

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of methanol. Sonicate (250 W) the mixture for 20 min. Filter the mixture.

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 dipsacoside B standard solution, macranthoidin B standard solution and the test solution (2 µL each) 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 110ºC (about 2 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

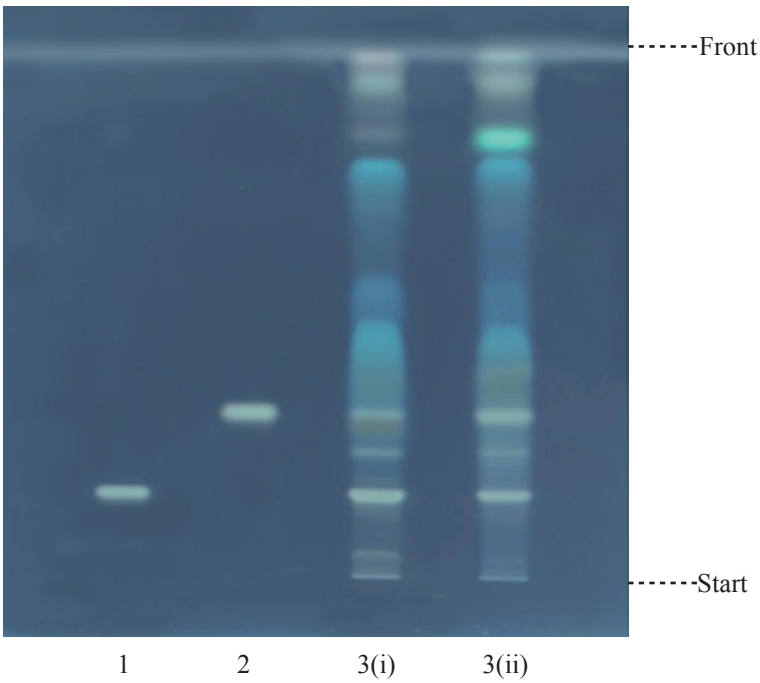

Figure 6 A reference HPTLC chromatogram of Lonicerae Flos extract observed under UV light (366 nm) after staining

1. Macranthoidin B standard solution
2. Dipsacoside B standard solution
3. Test solution of

(i) dried flower bud or with opening flower of *Lonicera macranthoides* Hand.-Mazz.

(ii) dried flower bud or with opening flower of *Lonicera hypoglauca* Miq.

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 dipsacoside B and macranthoidin B (Fig. 6).

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

Standard solutions

Chlorogenic acid standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol. Keep at about 4ºC.

Dipsacoside B standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of dipsacoside B CRS and dissolve in 10 mL of methanol. Keep at about 4ºC.

Macranthoidin B standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of macranthoidin B CRS and dissolve in 10 mL of methanol. Keep at about 4ºC.

Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (50%). Sonicate (300 W) the mixture for 40 min. Centrifuge at about 5000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Wash the residue with 7 mL of methanol (50%) and vortex the mixture for 1 min. Centrifuge at about 5000 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 60ºC; nebulizer gas (N_2) flow: 2.0 L/min] 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)	0.4% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	88.5→ 85	11.5 → 15	linear gradient
10 – 12	85 → 71	15 → 29	linear gradient
12 – 18	71 → 67	29 → 33	linear gradient
18 – 30	67 → 55	33 → 45	linear gradient

System suitability requirements

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

The *R* value between peak 1 and the closest peak; 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 1.5 [Fig. 7 (i) or (ii)].

Procedure

Separately inject chlorogenic acid Std-FP, dipsacoside B Std-FP, macranthoidin B Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of chlorogenic acid, dipsacoside B and macranthoidin B peaks in the chromatograms of chlorogenic acid Std-FP, dipsacoside B Std-FP, macranthoidin B Std-FP and the retention times of the four characteristic peaks [Fig. 7 (i) or (ii)] in the chromatogram of the test solution. Identify chlorogenic acid, dipsacoside B and macranthoidin B peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chlorogenic acid Std-FP, dipsacoside B Std-FP, macranthoidin B Std-FP. The retention times of chlorogenic acid, dipsacoside B and macranthoidin B 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 Lonicerae Flos extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Lonicerae Flos extract

Peak No.	RRT	Acceptable Range
1 (chlorogenic acid)	0.56	± 0.05
2	0.89	± 0.03
3 (marker, macranthoidin B)	1.00	-
4 (dipsacoside B)	1.10	± 0.03

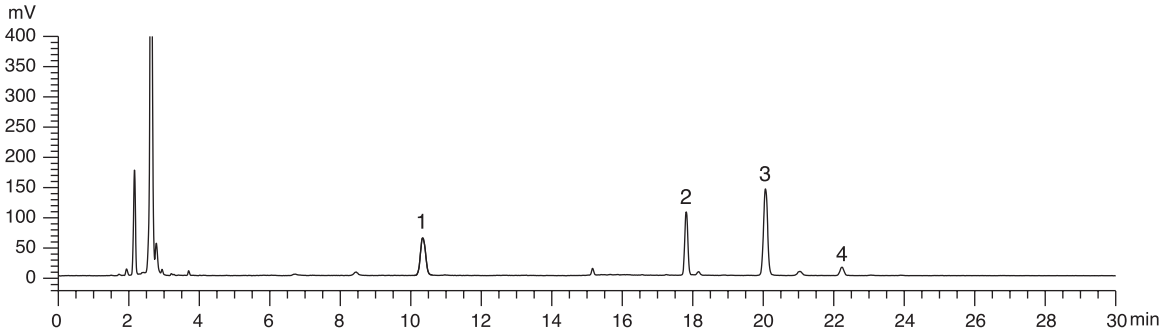

Figure 7 (i) A reference fingerprint chromatogram of dried flower bud or with opening flower of *Lonicera macranthoides* Hand.-Mazz. extract

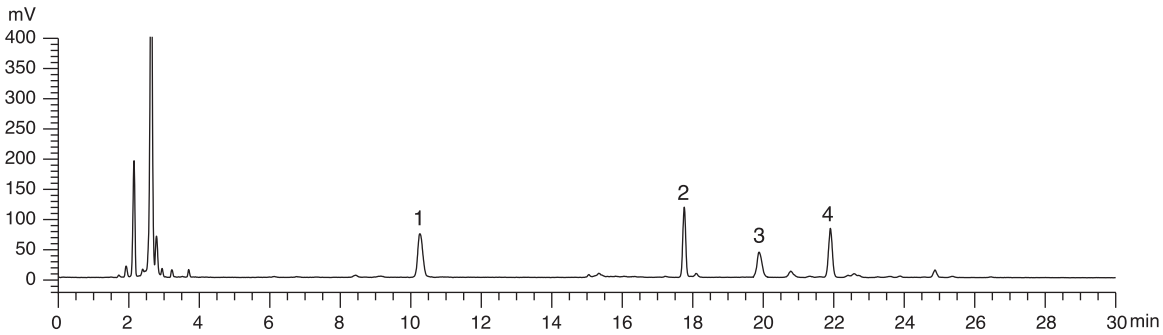

Figure 7 (ii) A reference fingerprint chromatogram of dried flower bud or with opening flower of *Lonicera hypoglauca* Miq. extract

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. 7 (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 – Aflatoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 5.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.0%.
Acid-insoluble ash: not more than 1.0%.

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 36.0%.
Ethanol-soluble extractives (hot extraction method): not less than 29.0%.

7. ASSAY

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

Standard solution

Mixed chlorogenic acid, dipsacoside B and macranthoidin B standard stock solution, Std-Stock (1000 mg/L for chlorogenic acid, 1000 mg/L for dipsacoside B and 2000 mg/L for macranthoidin B)

Weigh accurately 10.0 mg of chlorogenic acid CRS, 10.0 mg of dipsacoside B CRS and 20.0 mg of macranthoidin B CRS, and dissolve in 10 mL of methanol. Keep at about 4ºC.

Mixed chlorogenic acid, dipsacoside B and macranthoidin B standard solution for assay, Std-AS

Measure accurately the volume of the mixed chlorogenic acid, dipsacoside B and macranthoidin B Std-Stock, dilute with methanol to produce a series of solutions of 50, 100, 200, 400, 800 mg/L for chlorogenic acid, 10, 20, 100, 200, 400 mg/L for dipsacoside B and 50, 100, 200, 500, 1000 mg/L for macranthoidin B. Keep at about 4ºC.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (50%). Sonicate (300 W) the mixture for 40 min. Centrifuge at about 5000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Wash the residue with 7 mL of methanol (50%) and vortex the mixture for 1 min. Centrifuge at about 5000 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 60ºC; nebulizer gas (N_2) flow: 2.0 L/min] 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)	0.4% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	88.5→ 85	11.5 → 15	linear gradient
10 – 12	85 → 71	15 → 29	linear gradient
12 – 18	71 → 67	29 → 33	linear gradient
18 – 30	67 → 55	33 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed chlorogenic acid, dipsacoside B and macranthoidin B Std-AS (200 mg/L for chlorogenic acid, 100 mg/L for dipsacoside B and 200 mg/L for macranthoidin B). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid, dipsacoside B and macranthoidin B should not be more than 5.0%; the RSD of the retention times of chlorogenic acid, dipsacoside B and macranthoidin B peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid, dipsacoside B and macranthoidin B peaks should not be less than 20000 theoretical plates.

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

Limits

The sample contains not less than 2.6% of chlorogenic acid ($C_{16}H_{18}O_9$); and not less than 5.0% of the total content of dipsacoside B ($C_{53}H_{86}O_{22}$) and macranthoidin B ($C_{65}H_{106}O_{32}$), calculated with reference to the dried substance.

Lonicerae Flos（山銀花）

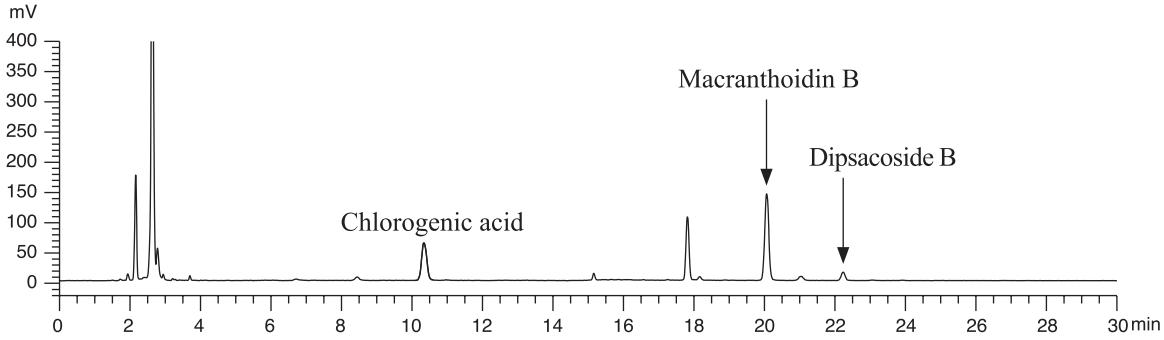

Figure 1 (i) A reference assay chromatogram of dried flower bud or with opening flower of *Lonicera macranthoides* Hand.-Mazz. extract

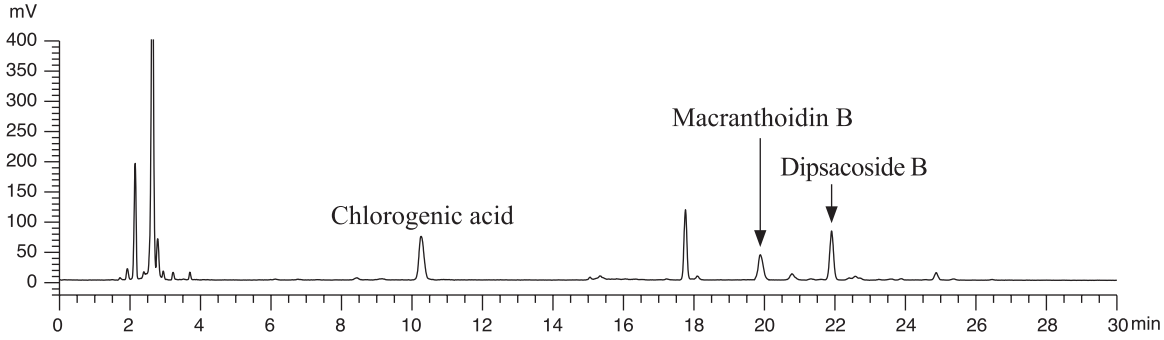

Figure 1 (ii) A reference assay chromatogram of dried flower bud or with opening flower of *Lonicera hypoglauca* Miq. extract