Polygoni Perfoliati Herba

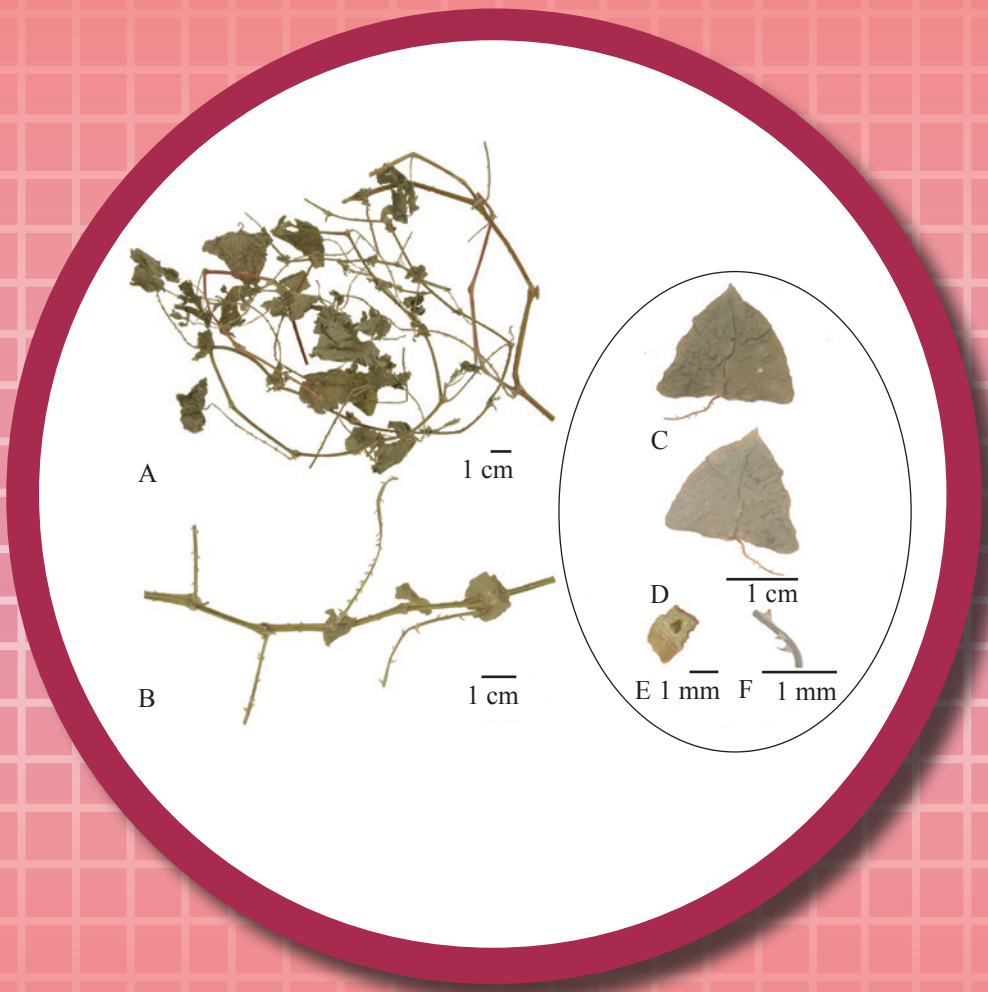

Figure 1 A photograph of Polygoni Perfoliati Herba

A. Polygoni Perfoliati Herba B. Magnified image of aerial part
C. Upper surface of leaf D. Lower surface of leaf
E. Magnified image of transverse section of stem
F. Magnified image of hooks

1. NAMES

Official Name: Polygoni Perfoliati Herba

Chinese Name: 杠板歸

Chinese Phonetic Name: Gangbangui

2. SOURCE

Polygoni Perfoliati Herba is the dried aerial part of *Polygonum perfoliatum* L. (Polygonaceae). The aerial part is collected during flowering period in summer, foreign matter removed, then dried under the sun to obtain Polygoni Perfoliati Herba.

3. DESCRIPTION

Stems slightly square, with ridges, mostly branched, up to 2.3 mm in diameter; externally purplish-red to purplish-brown, bearing reversed hooks on the ridges, nodes slightly swollen, internodes 2-6 cm long, fracture fibrous, yellowish-white, medullated or hollowed. Leaves simple, alternate, long petioled, peltate; lamina mostly crumpled, when whole, subequilateral triangle-shaped, greyish-green to reddish-brown, veins on the lower surface and petiole bear reversed hooks; stipular sheaths tightly surrounding the nodes or fallen off. Short spikes terminal or axillary at the upper part of stem, bracts rounded, flowers small, mostly withered or fallen off. Odour slight; taste bland for stem, and sour for leaf (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of cells. Collenchyma consists of multiple layers of rhombic, rectangular or subsquare cells, sometimes broken. Cortex narrow, consisting of 3-5 layers of cells. Epidermis and cortical cells sometimes contain reddish-brown contents. Pericyclic fibres arranged in a ring, wall thickened and lignified. Phloem consists of several layers of cells. Cambium visible. Xylem vessels large, singly or 3-5 in groups. Clusters of calcium oxalate scattered in parenchymatous cells. Pith cells large, sometimes hollowed in the centre [Fig. 2 (i)].

Leaf: Upper and lower epidermis consists of 1 layer of cells. Several layers of collenchymatous cells located under upper epidermis in the midrib and sometimes under lower epidermis. Phloem fibres in bundles. Clusters of calcium oxalate visible. Phloem consists of several layers of small subsquare parenchymatous cells. Xylem vessels slightly lignified. Reddish-brown contents scattered in mesophyll [Fig. 2 (ii)].

Powder

Colour yellowish-green to brownish-green. Clusters of calcium oxalate sometimes scattered, subrounded to rounded, 17-62 μm in diameter; polychromatic under the polarized microscope. Lower epidermal cells with sinuous anticlinal walls in surface view, stomata mostly paracytic or sometimes anisocytic, subsidiary cells 2-3. Vessels mainly spiral and bordered-pitted, 4-80 μm in diameter. Hook mostly broken, consisting of rhombic, rectangular or subsquare cells (Fig. 3).

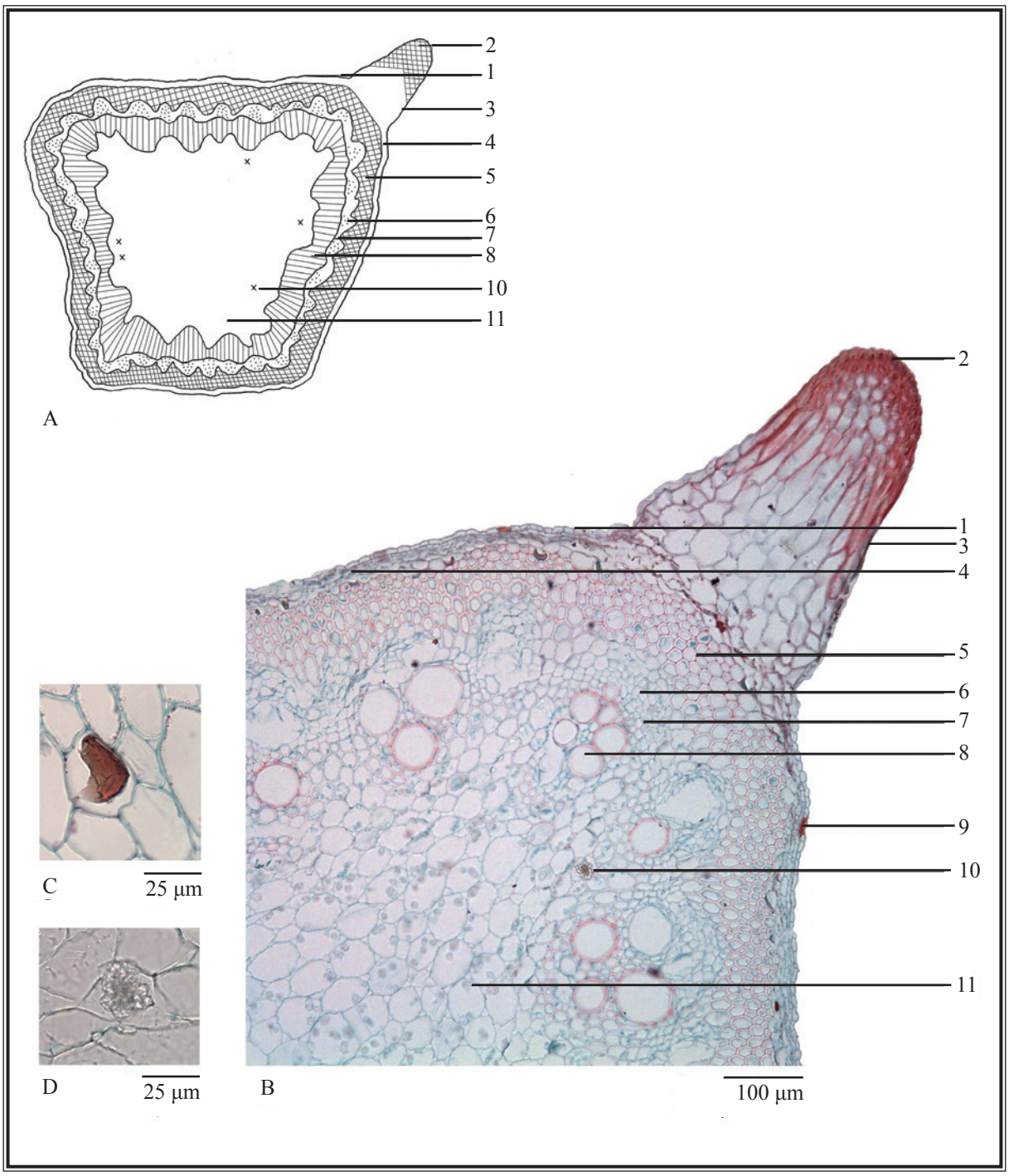

Figure 2 (i) Microscopic features of transverse section of stem of Polygoni Perfoliati Herba

A. Sketch B. Section illustration C. Reddish-brown content
D. Cluster of calcium oxalate

1. Epidermis 2. Collenchyma 3. Hook 4. Cortex 5. Pericyclic fibres 6. Phloem
7. Cambium 8. Xylem 9. Reddish-brown content 10. Cluster of calcium oxalate 11. Pith

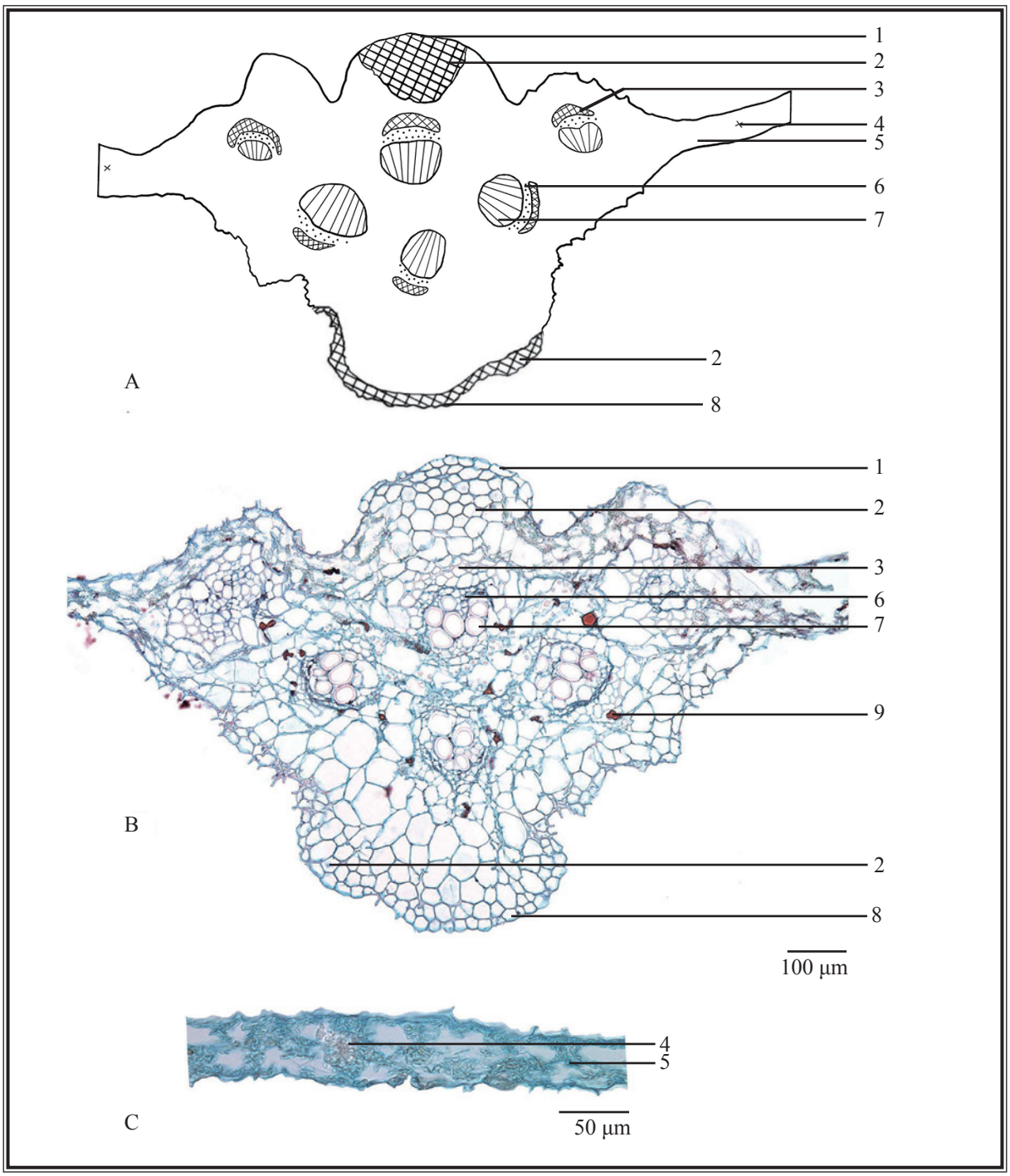

Figure 2 (ii) Microscopic features of transverse section of leaf of Polygoni Perfoliati Herba

A. Sketch B. Section illustration (midrib with blade) C. Magnified section of blade

1. Upper epidermis 2. Collenchyma 3. Phloem fibres 4. Cluster of calcium oxalate
5. Spongy tissue 6. Phloem 7. Xylem 8. Lower epidermis 9. Reddish-brown content

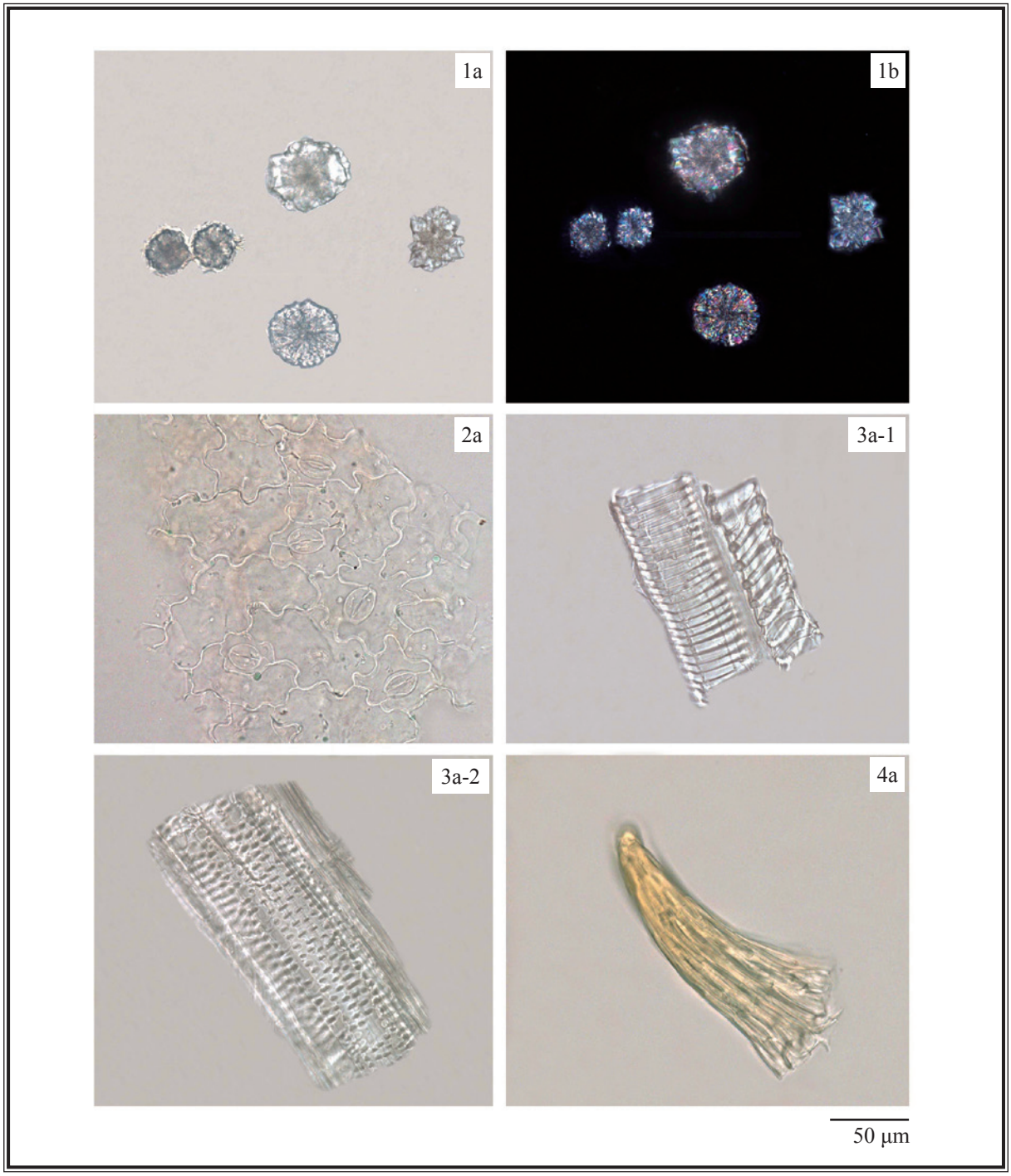

Figure 3 Microscopic features of powder of Polygoni Perfoliati Herba

1. Clusters of calcium oxalate 2. Lower epidermal cells with stomata
3. Vessels (3-1 spiral vessels, 3-2 bordered-pitted vessels) 4. Fragment of hook

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

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

Standard solution

Quercetin 3-O-β-D-glucuronide standard solution

Weigh 1.0 mg of quercetin 3-*O*-β-D-glucuronide CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, 2-butanone, methanol, water and formic acid (6:2:1:1:0.5, 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 20 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (70%).

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 quercetin 3-*O*-β-D-glucuronide standard solution (1 μL) and the test solution (0.5 μL) to the plate. Develop over a path of about 5 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 (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

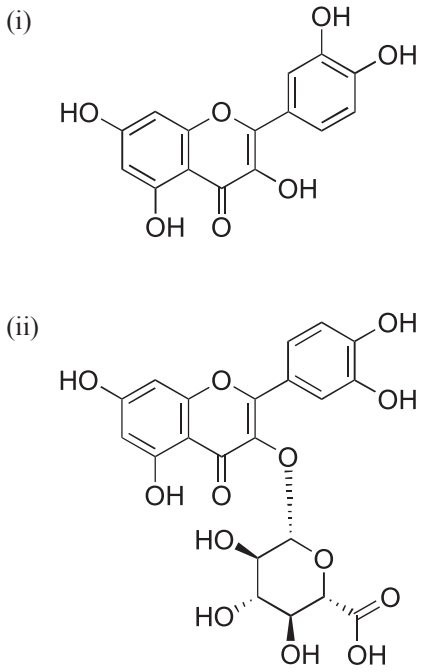

Figure 4 Chemical structures of (i) quercetin and (ii) quercetin 3-*O*-β-D-glucuronide

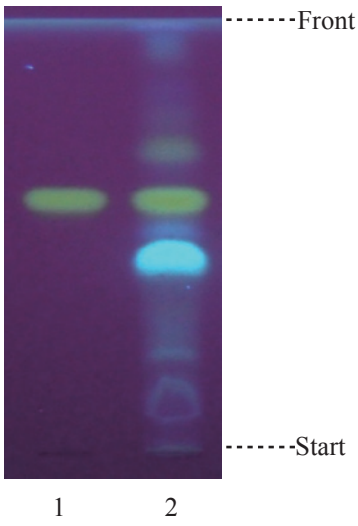

Figure 5 A reference HPTLC chromatogram of Polygoni Perfoliati Herba extract observed under UV light (366 nm) after staining

1. Quercetin 3-*O*-β-D-glucuronide standard solution 2. Test solution

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 quercetin 3-*O*-β-D-glucuronide (Fig. 5).

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

Standard solution

Quercetin 3-O-β-D-glucuronide standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 2.5 mg of quercetin 3-*O*-β-D-glucuronide CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (305 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)	Methanol (%, v/v)	0.5% Phosphoric acid (%, v/v)	Elution
0 – 45	30 → 68	70 → 32	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercetin 3-*O*-β-D-glucuronide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercetin 3-*O*-β-D-glucuronide should not be more than 5.0%; the RSD of the retention time of quercetin 3-*O*-β-D-glucuronide peak should not be more than 2.0%; the column efficiency determined from quercetin 3-*O*-β-D-glucuronide peak should not be less than 18000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject quercetin 3-*O*-β-D-glucuronide Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of quercetin 3-*O*-β-D-glucuronide peak in the chromatogram of quercetin 3-*O*-β-D-glucuronide Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify quercetin 3-*O*-β-D-glucuronide peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of quercetin 3-*O*-β-D-glucuronide Std-FP. The retention times of quercetin 3-*O*-β-D-glucuronide 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 Polygoni Perfoliati Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.38	± 0.03
2	0.53	± 0.03
3 (marker, quercetin 3-*O*-β-D-glucuronide)	1.00	-
4	2.00	± 0.05

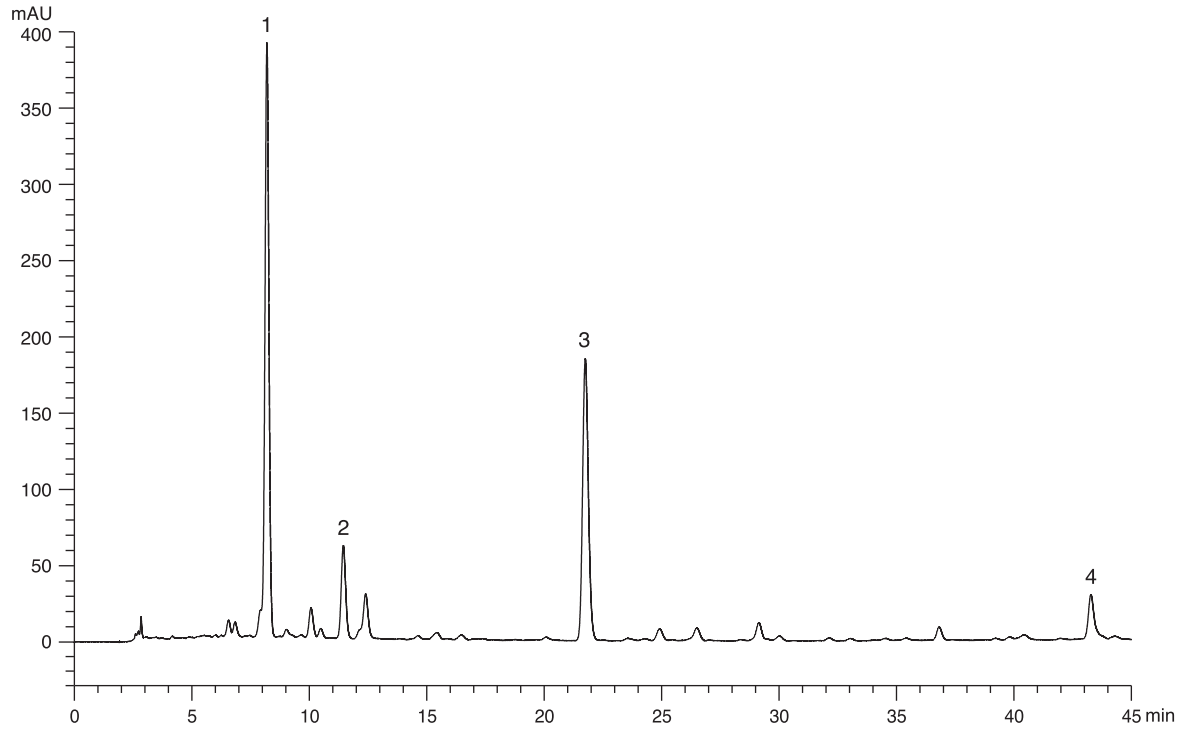

Figure 6 A reference fingerprint chromatogram of Polygoni Perfoliati Herba extract

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. 6).

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 9.0%.

Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 13.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

Quercetin standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of quercetin CRS (Fig. 4) and dissolve in 10 mL of methanol.

Quercetin standard solution for assay, Std-AS

Measure accurately the volume of the quercetin Std-Stock, dilute with methanol to produce a series of solutions of 2, 10, 50, 100, 150 mg/L for quercetin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 32 mL of methanol and 8 mL of hydrochloric acid. Reflux the mixture at about 90ºC for 2 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 8000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (370 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)	Methanol (%, v/v)	0.5% Phosphoric acid (%, v/v)	Elution
0 – 30	50 → 80	50 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of quercetin Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercetin should not be more than 5.0%; the RSD of the retention time of quercetin peak should not be more than 2.0%; the column efficiency determined from quercetin peak should not be less than 14000 theoretical plates.

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 curve

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

Limits

The sample contains not less than 0.20% of quercetin ($C_{15}H_{10}O_7$), calculated with reference to the dried substance.

Polygoni Perfoliati Herba（杠板歸）

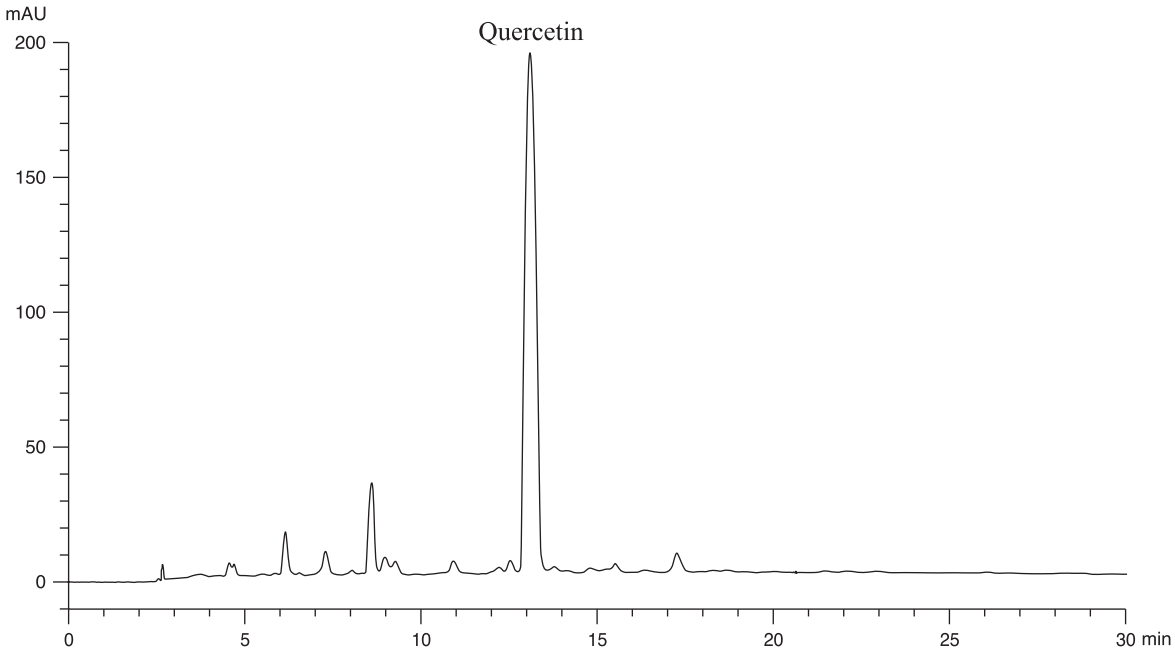

Figure 1 A reference assay chromatogram of Polygoni Perfoliati Herba extract