Potentillae Chinensis Herba

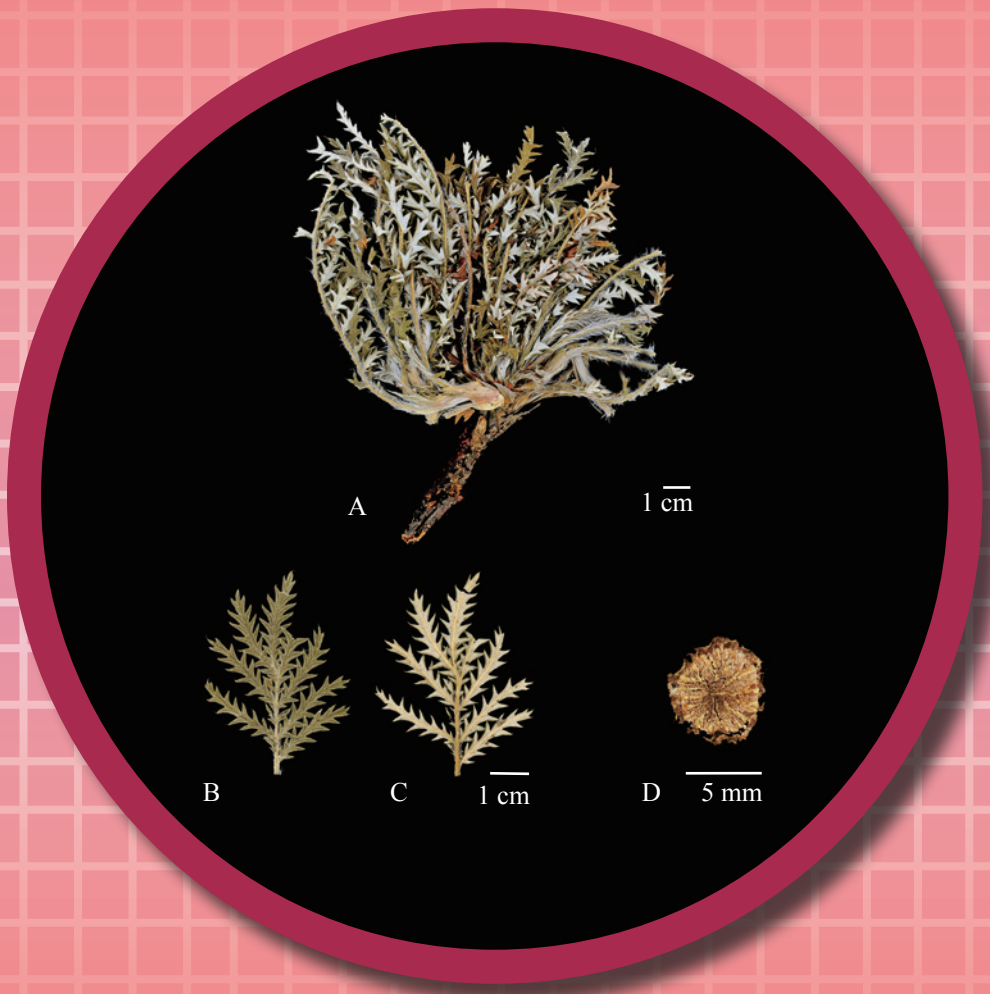

Figure 1 A photograph of Potentillae Chinensis Herba

A. Potentillae Chinensis Herba

B. The upper surface of leaves

C. The lower surface of leaves

D. Magnified image of transverse section of root

1. NAMES

Official Name: Potentillae Chinensis Herba

Chinese Name: 委陵菜

Chinese Phonetic Name: Weilingcai

2. SOURCE

Potentillae Chinensis Herba is the dried herb of *Potentilla chinensis* Ser. (Rosaceae). The whole plant is collected in spring before sprouting of stems, soil removed, then dried under the sun to obtain Potentillae Chinensis Herba.

3. DESCRIPTION

Roots cylindrical or subconical, slightly twisted, sometimes branched, occasionally broken, 3-14 cm long, up to 10 mm in diameter; externally dark brown to dark purplish-red, striated longitudinally, the outer bark coarse, easily exfoliated, root vertex slightly swollen; texture hard, easily broken; transverse section of root bark thin, dark brown, easily separated from wood, rays arranged radially. Compound leaves basal, odd-pinnate, petiolate; leaflets narrowly long-elliptic, margins pinnatipartite, the lower surface and petiole densely covered with greyish-white pubescence. Odour slight; taste astringent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of 4 to more than 10 layers of tangentially elongated cells, outer layer heavily stainned. Cortical cells subrounded or irregular, arranged loosely, not obvious. Cleft frequently located on the outer part of phloem. Phloem narrow. Cambium distinct, arranged in undulate ring. Xylem broad, vessels radially arranged. Rays distinct, broad. Clusters of calcium oxalate abundant, mainly scattered in xylem rays [Fig. 2(i)].

Leaf: Upper epidermis consists of 1 layer of subsquare cells, covered with cuticle. Palisade tissue consists of 2-3 layers of cells, sometimes scattered with clusters of calcium oxalate. Spongy tissue consists of several layers of subrounded cells. Collenchyma consists of 2-4 layers of cells underneath phloem, and below the upper and lower epidermis. Vascular bundles collateral, xylem half-moon shaped, phloem crescent-shaped. Clusters of calcium oxalate occasionally found in palisade tissue and parenchyma. Lower epidermal cells relatively small, tangentially elongated. Non-glandular hair unicellular mostly curved, raised from the upper and lower epidermis, abundant on the lower epidermis [Fig. 2(ii)].

Powder

Colour greyish-brown. Non-glandular hairs numerous, unicellular, 2 types, first type with thinner cell wall, long and slender, 4-7 μm in diameter, always twisted into mass; second type with thicker cell wall, varing in length, short one bended, in hook-shaped or straight; long one mostly broken, straight or slightly bended, 4-45 μm in diameter. Clusters of calcium oxalate in rosette shape, singly scattered or distributed among parenchymatous cell, 4-52 μm in diameter, and small prisms of calcium oxalate occasionally visible; polychromatic under the polarized microscope. Lower epidermal cells with stomata mostly covered with non-glandular hair, occasionally visible; stomata anomocytic. Cork cells subpolygonal or flat-rectangular, containing yellowish-brown contents. Xylem fibres long fusiform, 7-20 μm in diameter, walls slightly thickened and pit canals distinct. Vessels mainly spiral, reticulate or bordered-pitted (Fig. 3).

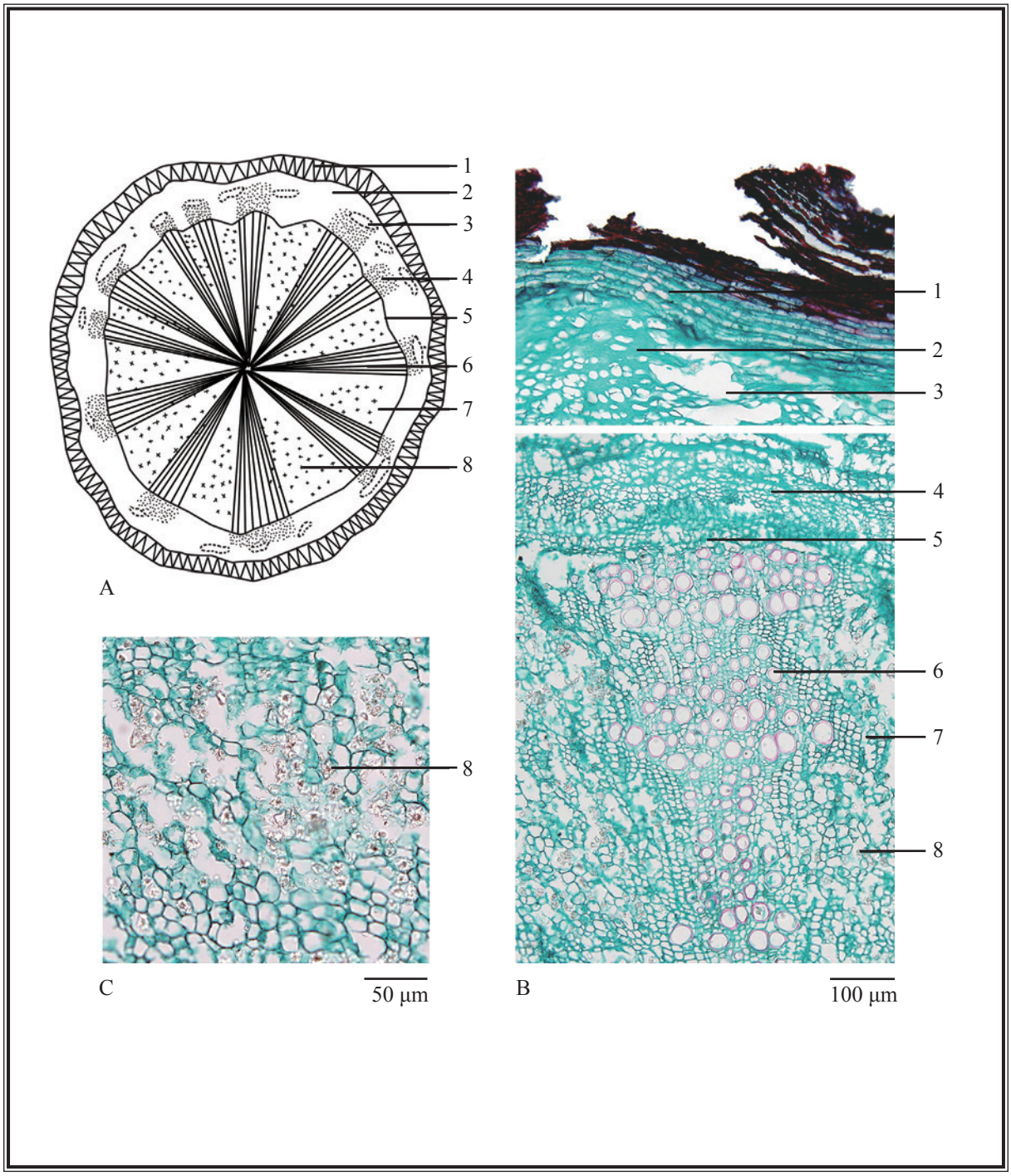

Figure 2 (i) Microscopic features of transverse section of root of Potentillae Chinensis Herba

A. Sketch B. Section illustration C. Clusters of calcium oxalate

1. Cork 2. Cortex 3. Cleft 4. Phloem 5. Cambium
6. Xylem 7. Ray 8. Cluster of calcium oxalate

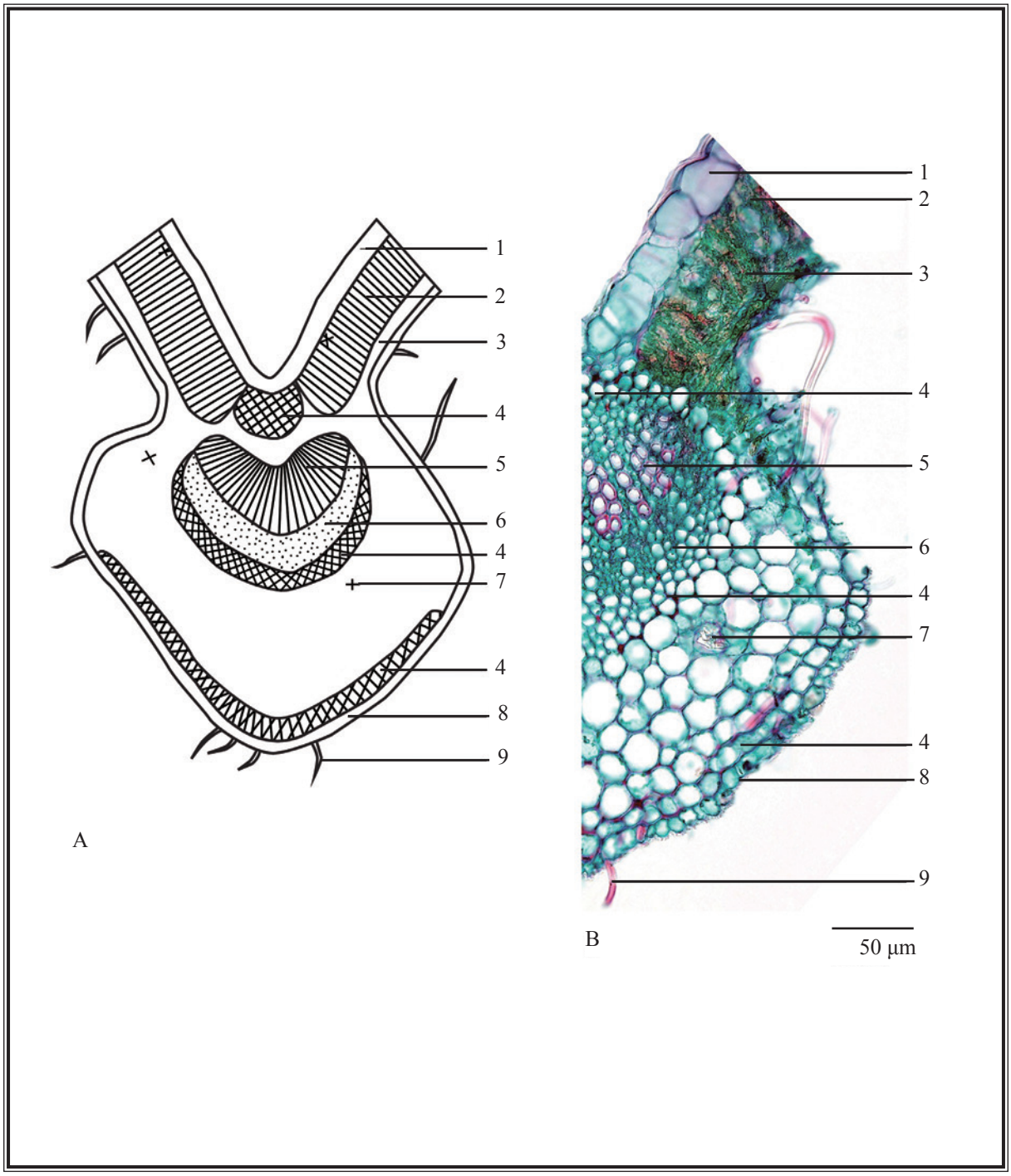

Figure 2 (ii) Microscopic features of transverse section of leaf of Potentillae Chinensis Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Collenchyma 5. Xylem
6. Phloem 7. Cluster of calcium oxalate 8. Lower epidermis 9. Non-glandular hair

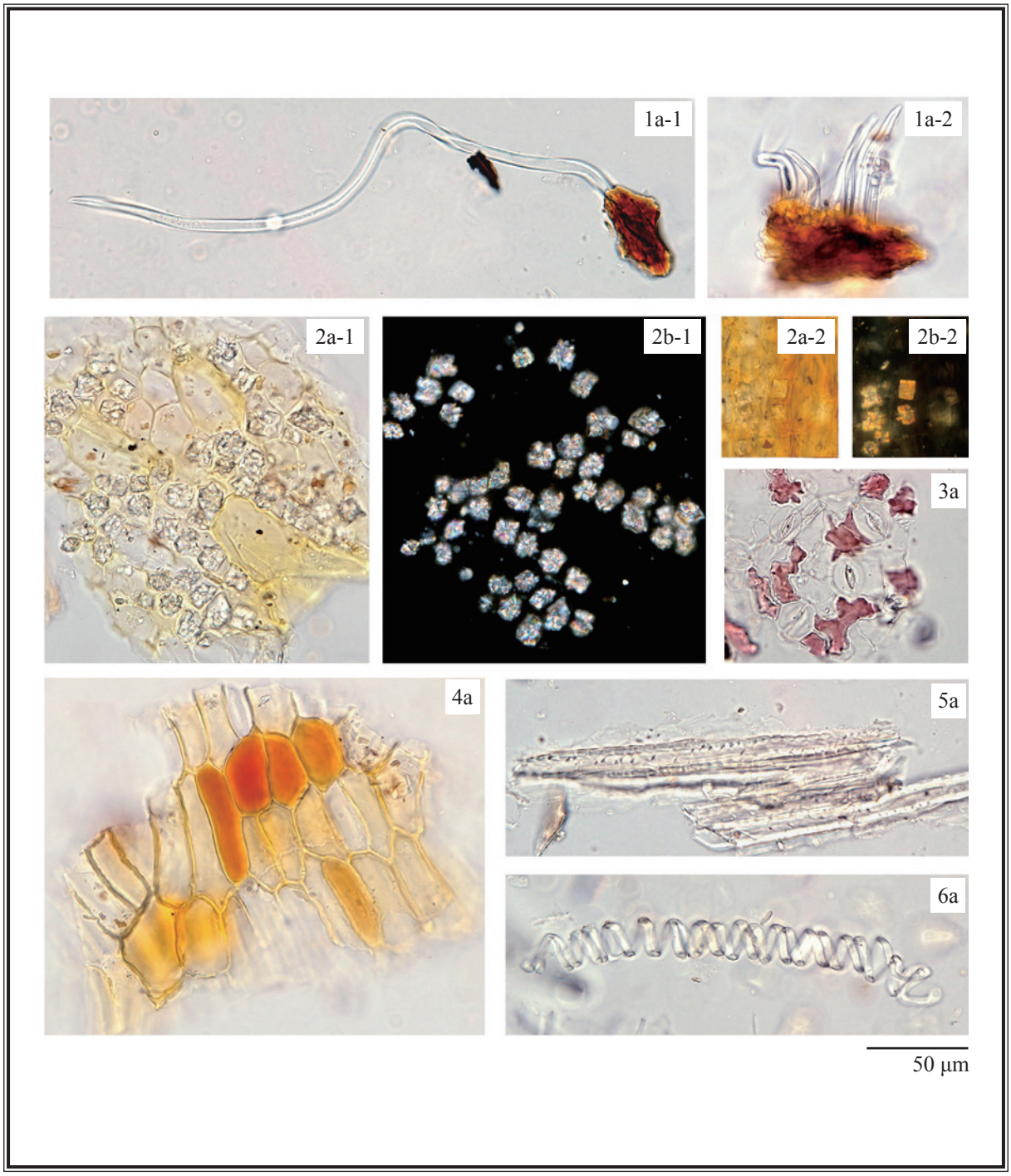

Figure 3 Microscopic features of powder of Potentillae Chinensis Herba

1. Non-glandular hairs (1-1 with thinner cell wall, 1-2 with thicker cell wall)
2. Crystals of calcium oxalate (2-1 clusters of calcium oxalate, 2-2 prisms of calcium oxalate)
3. Lower epidermal cells with stomata 4. Cork cells 5. Xylem fibres 6.Spiral vessel

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

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

Standard solutions

Apigenin 7-O-β-D-glucuronide standard solution

Weigh 1.0 mg of apigenin 7-*O*-β-D-glucuronide CRS (Fig. 4) and dissolve in 5 mL of methanol.

Luteolin 7-O-β-D-glucuronide standard solution

Weigh 1.0 mg of luteolin 7-*O*-β-D-glucuronide CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, methanol, water and formic acid (40:5:5:2, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-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 round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

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 apigenin 7-*O*-β-D-glucuronide standard solution (2 μL), luteolin 7-*O*-β-D-glucuronide standard solution (2 μL) and the test solution (1 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. 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 values by using the equation as indicated in Appendix IV (A).

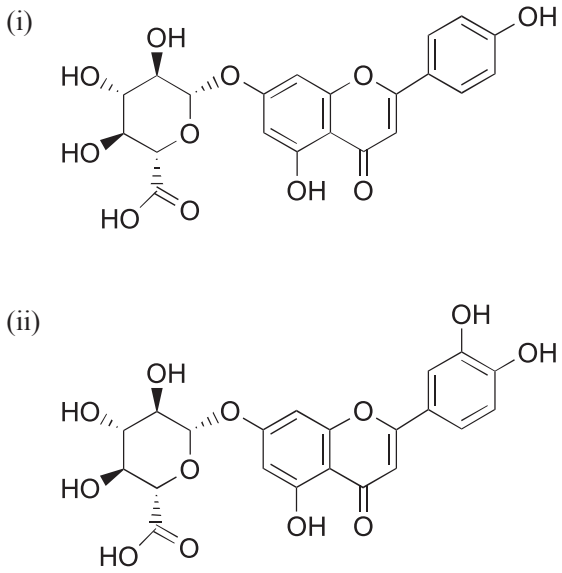

Figure 4 Chemical structures of (i) apigenin 7-*O*-β-D-glucuronide and (ii) luteolin 7-*O*-β-D-glucuronide

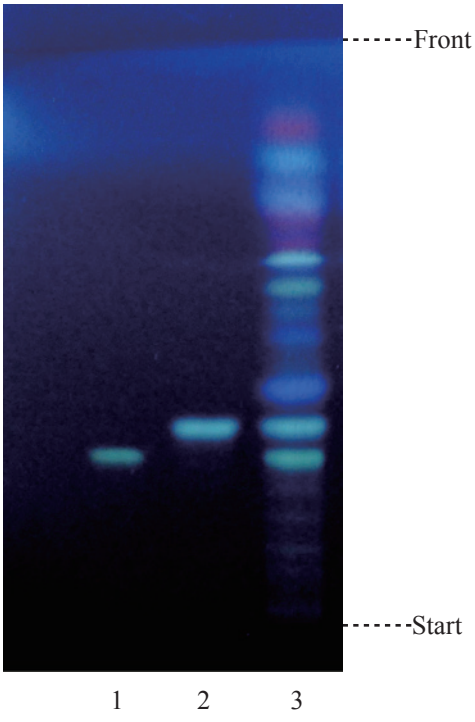

Figure 5 A reference HPTLC chromatogram of Potentillae Chinensis Herba extract observed under UV light (366 nm) after staining

1. Luteolin 7-*O*-β-D-glucuronide standard solution
2. Apigenin 7-*O*-β-D-glucuronide standard solution 3. Test solution

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 apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide (Fig. 5).

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

Standard solutions

Apigenin 7-O-β-D-glucuronide standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of apigenin 7-*O*-β-D-glucuronide CRS and dissolve in 10 mL of methanol (70%).

Luteolin 7-O-β-D-glucuronide standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of luteolin 7-*O*-β-D-glucuronide CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (70%). Sonicate (180 W) the mixture for 30 min. Centrifuge at about 2330 × *g* for 10 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	85 → 75	15 → 25	linear gradient
30 – 60	75 → 70	25 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of apigenin 7-*O*-β-D-glucuronide Std-FP and luteolin 7-*O*-β-D-glucuronide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide should not be more than 5.0%; the RSD of the retention times of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide peaks should not be more than 2.0%; the column efficiencies determined from apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide peaks should not be less than 45000 and 30000 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 1.5 (Fig. 6).

Procedure

Separately inject apigenin 7-*O*-β-D-glucuronide Std-FP, luteolin 7-*O*-β-D-glucuronide Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide peaks in the chromatograms of apigenin 7-*O*-β-D-glucuronide Std-FP, luteolin 7-*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 apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of apigenin 7-*O*-β-D-glucuronide Std-FP and luteolin 7-*O*-β-D-glucuronide Std-FP. The retention times of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide 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 Potentillae Chinensis Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, luteolin 7-*O*-β-D-glucuronide)	1.00	-
2 (apigenin 7-*O*-β-D-glucuronide)	1.30	± 0.03
3 (luteolin)	2.08	± 0.03
4 (tiliroside)	2.18	± 0.03

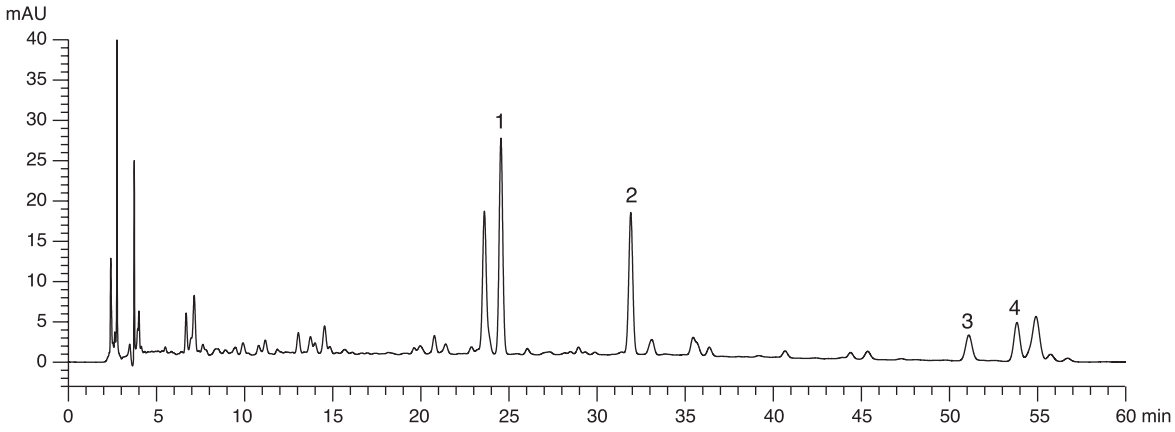

Figure 6 A reference fingerprint chromatogram of Potentillae Chinensis Herba extract

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 12.0%.
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

Mixed apigenin 7-O-β-D-glucuronide and luteolin 7-O-β-D-glucuronide standard stock solution, Std-Stock (100 mg/L each)

Weigh accurately 0.5 mg of apigenin 7-*O*-β-D-glucuronide CRS and 0.5 mg of luteolin 7-*O*-β-D-glucuronide CRS, and dissolve in 5 mL of methanol (70%).

Mixed apigenin 7-O-β-D-glucuronide and luteolin 7-O-β-D-glucuronide standard solution for assay, Std-AS

Measure accurately the volume of the mixed apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide Std-Stock, dilute with methanol (70%) to produce a series of solutions of 1, 2, 5, 10, 20 mg/L for both apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of methanol (70%). Sonicate (180 W) the mixture for 30 min. Centrifuge at about 2330 × *g* for 10 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with 10 mL of methanol (70%). Combine the solutions and make up to the mark with methanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	85 → 75	15→ 25	linear gradient
30 – 60	75 → 70	25 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide Std-AS (5 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide should not be more than 5.0%; the RSD of the retention times of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide peaks should not be more than 2.0%; the column efficiencies determined from apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide peaks should not be less than 45000 and 30000 theoretical plates respectively.

The *R* value between apigenin 7-*O*-β-D-glucuronide peak and the closest peak; and the *R* value between luteolin 7-*O*-β-D-glucuronide 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 apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide against the corresponding concentrations of the mixed apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide 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 apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide Std-AS. The retention times of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide 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 apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide in the test solution, and calculate the percentage contents of apigenin 7-*O*-β-D-glucuronide and luteolin 7-*O*-β-D-glucuronide in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.16% of the total content of apigenin 7-*O*-β-D-glucuronide ($C_{21}H_{18}O_{11}$) and luteolin 7-*O*-β-D-glucuronide ($C_{21}H_{18}O_{12}$), calculated with reference to the dried substance.

Potentillae Chinensis Herba（委陵菜）

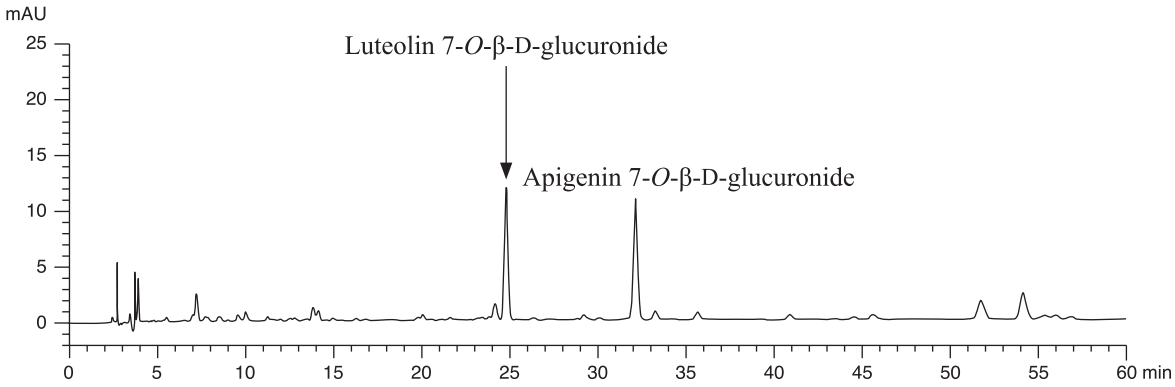

Figure 1 A reference assay chromatogram of Potentillae Chinensis Herba extract