Potentillae Discoloris Herba

Figure 1 A photograph of Potentillae Discoloris Herba

A. Potentillae Discoloris Herba

B. Magnified image of lower surface of leaf

C. Magnified image of upper surface of leaf

D. Magnified image of fracture of tuberous root

1. NAMES

Official Name: Potentillae Discoloris Herba

Chinese Name: 翻白草

Chinese Phonetic Name: Fanbaicao

2. SOURCE

Potentillae Discoloris Herba is the dried whole plant of *Potentilla discolor* Bge. (Rosaceae). The whole plant is collected in summer and autumn before flowering, soil and foreign matter removed, then dried under the sun to obtain Potentillae Discoloris Herba.

3. DESCRIPTION

Tuberous root spindle-shaped or cylindrical, 2-13 cm long, 3-15 mm in diameter. Externally yellowish-brown to dark brown, irregularly twisted-furrowed; texture hard and brittle. Fracture flat, greyish-white to yellowish-white. Stem straight and brittle, the surface covered with white pubescence. Basal leaves tufted, odd-pinnate, petiole varies in length, mainly crumpled and bent; leaflets 5-9, petiole varies in length or sessile, oblong to long-elliptic, apical leaflets relatively larger, upper surface greyish-green to dark green, lower surface densely covered with white pubescence, margin coarse. Odour slight; taste sweet and slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Rhytidome broken, cell walls slightly thickened, suberized. Cork consists of 4 to more than 10 layers of flattened cells, cells subrounded or irregular in shape, loosely arranged. Phloem narrow, with clusters of calcium oxalate inside the cells of the outer layer. Cambium arranged in a wavy ring. Xylem broad, occupying about 4/5 of the central portion of the root, vessels arranged radially. Rays distinct, broad. Clusters of calcium oxalate abundant, mainly distributed in the xylem rays [Fig. 2 (i)].

Stem: Non-glandular hairs commonly found on the surface of the epidermis. Epidermis consists of 1-2 layers of cells, cells subrounded to subsquare. Cortex consists of several layers of parenchymatous cells. Pericycle fibres consist of 4-6 layers of cells, arranged in a ring; fibres small, cell walls thickened. Phloem relatively narrow, arranged in a ring. Cambium indistinct. Xylem relatively narrow, arranged radially. Pith broad, usually hollow in the centre, occupying 3/5 of the central portion of the stem, clusters of calcium oxalate occasionally found [Fig. 2 (ii)].

Leaf: Upper epidermis consists of 1 layer of subsquare to subrounded parenchymatous cells. Palisade tissue consists of 2-3 layers of cells, sometimes with clusters of calcium oxalate. Spongy tissue narrow, with clusters of calcium oxalate. Non-glandular hairs unicellular and mostly curved, found on the surfaces of epidermis, but more abundant on the lower epidermis. Vascular bundles collateral, xylem half-moon-shaped, phloem crescent-shaped. Lower epidermal cells relatively small, tangentially elongated [Fig. 2 (iii)].

Powder

Colour yellowish-brown. Non-glandular hairs numerous, found in stem and largely from lower epidermis of leaf, two types: one type with a thin cell wall, long, sinuous and slender, 4-17 μm in diameter, always twisted together; another type with a thicker cell wall, straight or slightly bent, 6-40 μm in diameter. Clusters of calcium oxalate aggregated in rosette shape and singly scattered, distributed in parenchymatous cells, 3-53 μm in diameter; small prisms of calcium oxalate occasionally found; polychromatic under the polarized microscope. Upper epidermal cells of leaf polygonal in surface view, anticlinal wall nearly straight, always showing cicatrix of non-glandular hairs. Fibres mostly in bundles; single fibres 5-38 μm in diameter, relatively long, walls thickened; polychromatic under the polarized microscope. Vessels mainly of bordered-pitted and spiral; reticulate vessels occasionally found. Starch granules mostly single, spherical or oblong in shape; black and cruciate-shaped under the polarized microscope (Fig. 3).

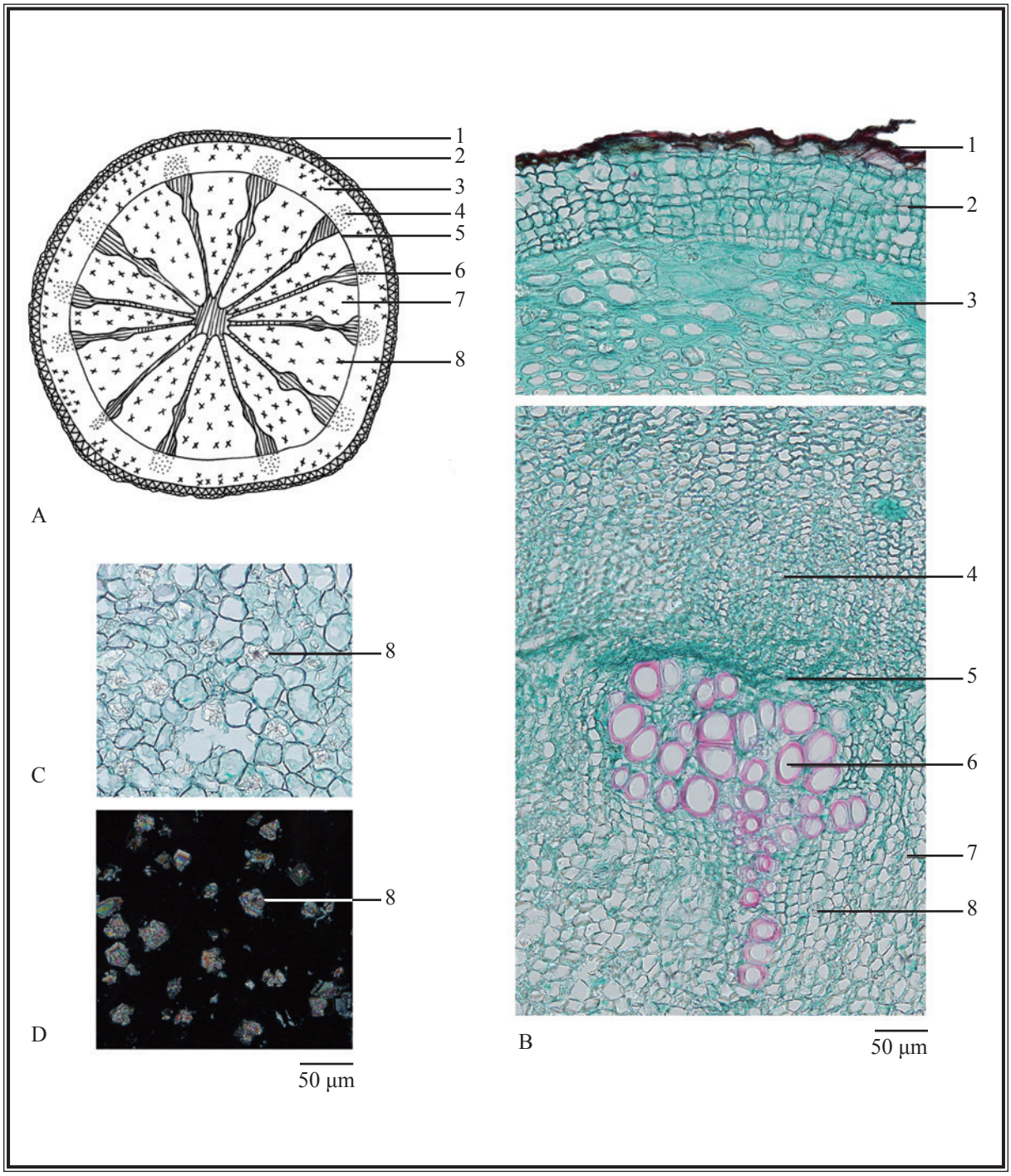


Figure 2 (i) Microscopic features of transverse section of tuberous root of Potentillae Discoloris Herba

A. Sketch B. Section illustration C. Section magnified (under the light microscope)
D. Section magnified (under the polarized microscope)

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

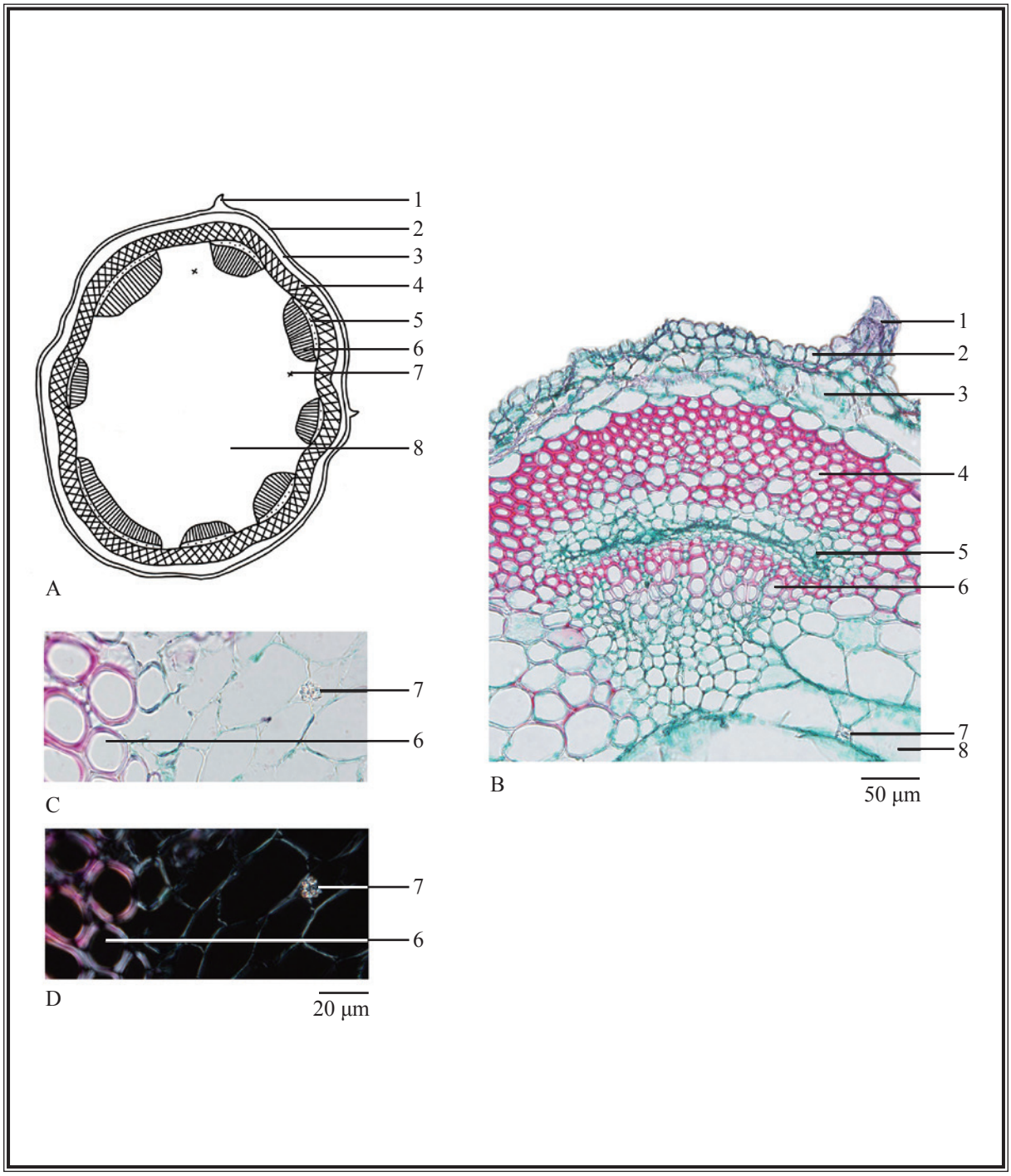


Figure 2 (ii) Microscopic features of transverse section of stem of Potentillae Discoloris Herba

A. Sketch B. Section illustration C. Section magnified (under the light microscope)
D. Section magnified (under the polarized microscope)

1. Non-glandular hair 2. Epidermis 3. Cortex 4. Pericycle fibres 5. Phloem 6. Xylem
7. Cluster of calcium oxalate 8. Pith

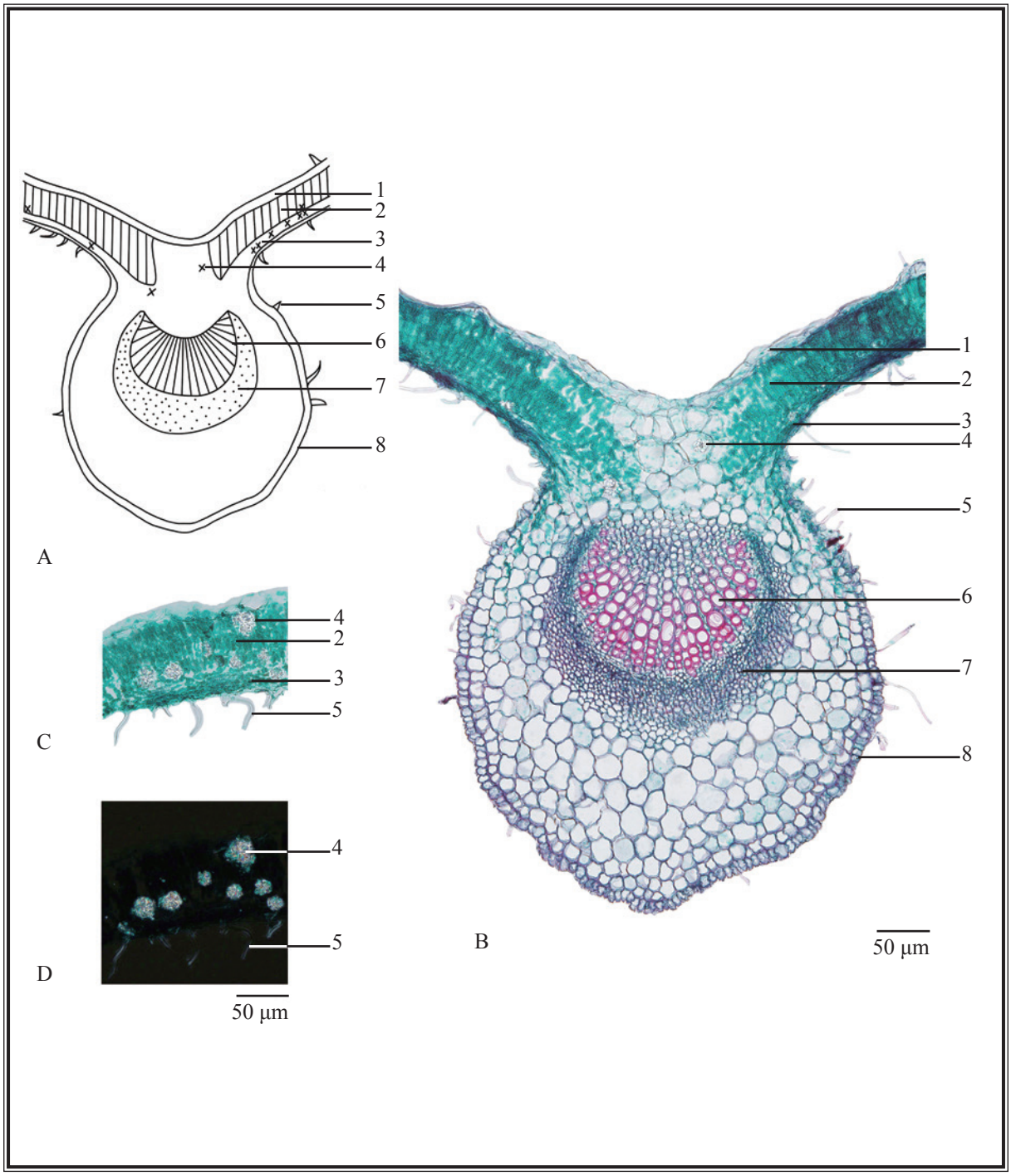


Figure 2 (iii) Microscopic features of transverse section of leaf of Potentillae Discoloris Herba

A. Sketch B. Section illustration C. Section magnified (under the light microscope)
D. Section magnified (under the polarized microscope)

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

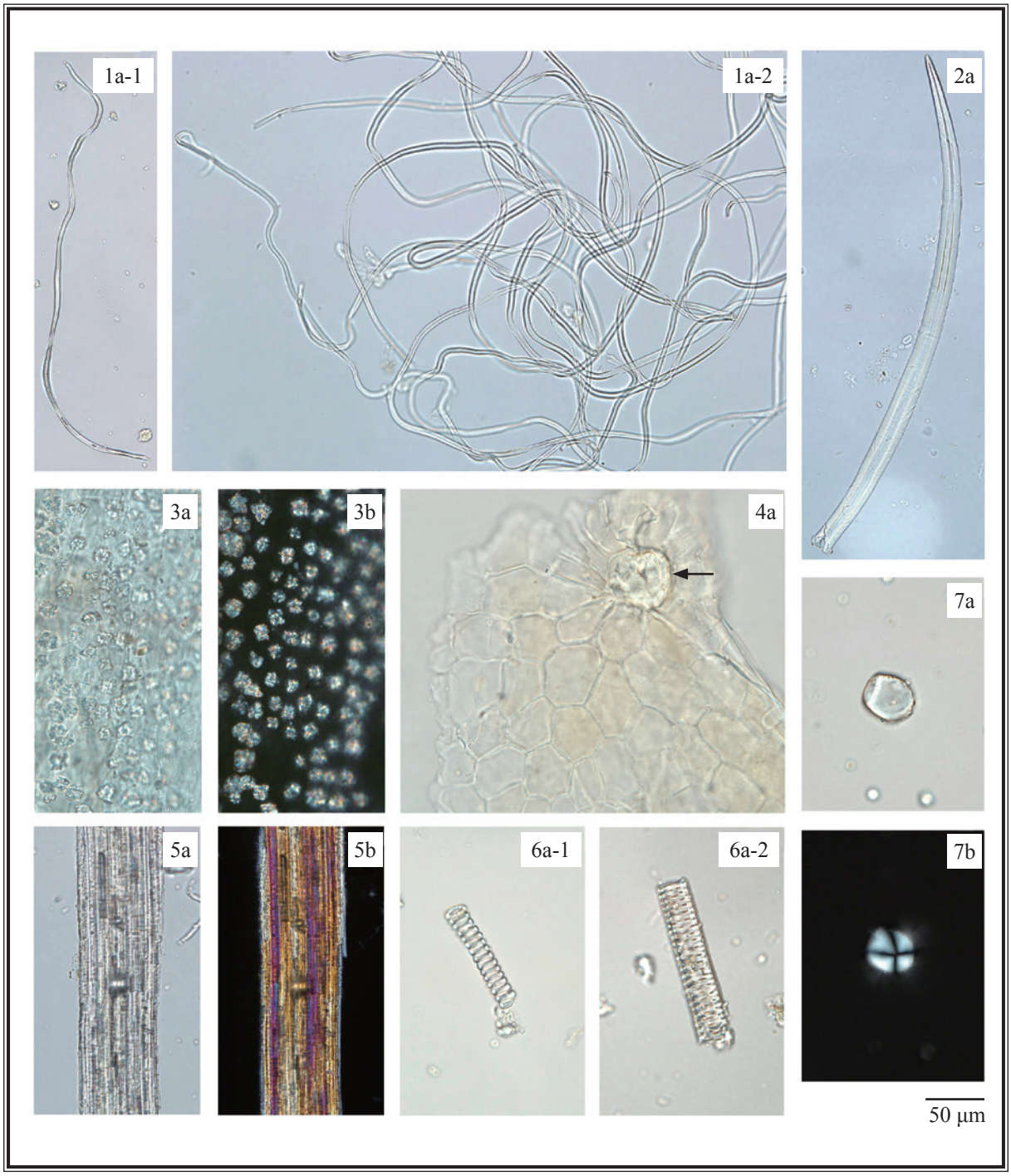


Figure 3 Microscopic features of powder of Potentillae Discoloris Herba

1. Non-glandular hairs with thin cell wall (1-1 single, 1-2 twisted together)
2. Non-glandular hair with a thick cell wall 3. Clusters of calcium oxalate
4. Upper epidermal cells of leaf with a cicatrix of non-glandular hair (⟶) 5. Fibre bundle
6. Vessels (6-1 spiral vessel, 6-2 bordered-pitted vessel) 7. Starch granule

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

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

Standard solutions

Ellagic acid standard solution

Weigh 1.5 mg of ellagic acid CRS (Fig. 4) and dissolve in 10 mL of methanol.

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

Weigh 1.5 mg of kaempferol 3-*O*-β-D-glucuronide CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, formic acid, ethyl acetate and water (12:6:4:1, v/v).

Spray reagent

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

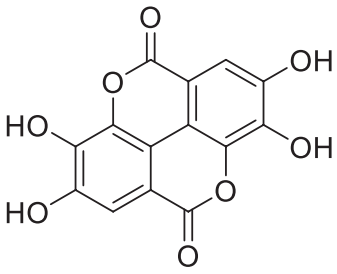
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol (70%). 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, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately ellagic acid standard solution, kaempferol 3-*O*-β-D-glucuronide standard solution and the test solution (1 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 30 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 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).

(i)



(ii)

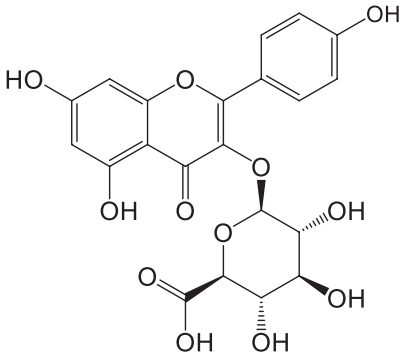


Figure 4 Chemical structures of (i) ellagic acid and (ii) kaempferol 3-*O*-β-D-glucuronide

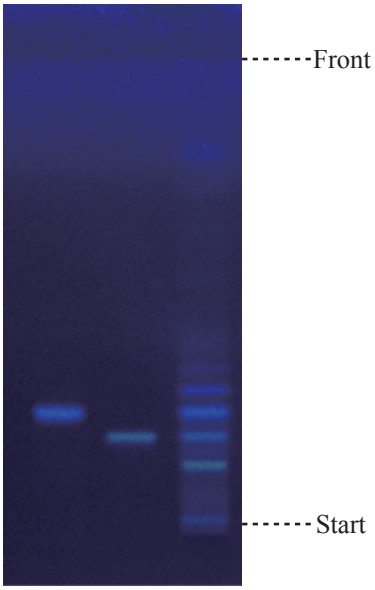


Figure 5 A reference HPTLC chromatogram of Potentillae Discoloris Herba extract observed under UV light (366 nm) after staining

1. Ellagic acid standard solution
2. Kaempferol 3-*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 ellagic acid and kaempferol 3-*O*-β-D-glucuronide (Fig. 5).

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

Standard solutions

Ellagic acid standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of ellagic acid CRS and dissolve in 10 mL of methanol.

Kaempferol 3-O-β-D-glucuronide standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.5 mg of kaempferol 3-*O*-β-D-glucuronide CRS and dissolve in 20 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of methanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Transfer the residue to a 50-mL conical flask, then add 15 mL of methanol (70%). Sonicate (180 W) the mixture for 15 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for two times each with 2 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 (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 – 5	82	18	isocratic
5 – 10	82 → 75	18 → 25	linear gradient
10 – 30	75	25	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ellagic acid Std-FP and kaempferol 3-*O*-β-D-glucuronide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ellagic acid and kaempferol 3-*O*-β-D-glucuronide should not be more

than 5.0%; the RSD of the retention times of ellagic acid and kaempferol 3-*O*-β-D-glucuronide peaks should not be more than 2.0%; the column efficiencies determined from ellagic acid and kaempferol 3-*O*-β-D-glucuronide peaks should not be less than 30000 and 20000 theoretical plates respectively.

The *R* value between peak 4 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject ellagic acid Std-FP, kaempferol 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 times of ellagic acid and kaempferol 3-*O*-β-D-glucuronide peaks in the chromatograms of ellagic acid Std-FP, kaempferol 3-*O*-β-D-glucuronide Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify ellagic acid and kaempferol 3-*O*-β-D-glucuronide peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of ellagic acid Std-FP and kaempferol 3-*O*-β-D-glucuronide Std-FP. The retention times of ellagic acid and kaempferol 3-*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 five characteristic peaks of Potentillae Discoloris Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Potentillae Discoloris Herba extract

Peak No.	RRT	Acceptable Range
1	0.82	± 0.03
2	0.90	± 0.03
3 (quercetin 3-*O*-β-D-glucuronide)	0.97	± 0.03
4 (marker, ellagic acid)	1.00	-
5 (kaempferol 3-*O*-β-D-glucuronide)	1.19	± 0.03

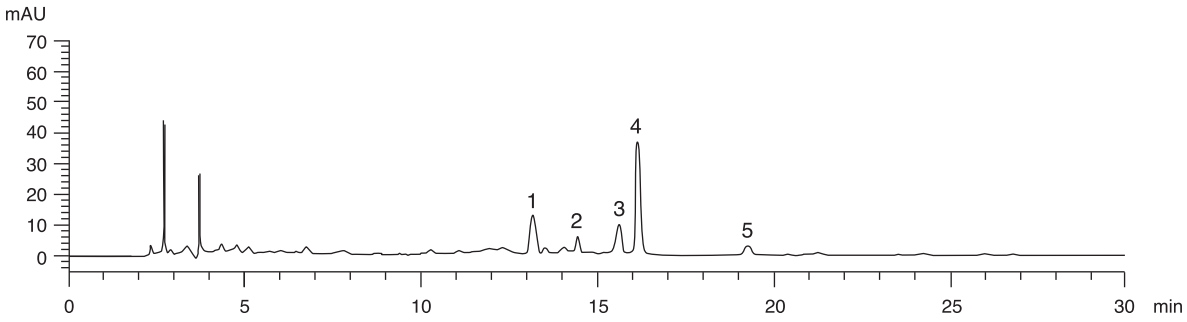


Figure 6 A reference fingerprint chromatogram of Potentillae Discoloris Herba extract

For positive identification, the sample must give the above five 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 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

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

Standard solution

Mixed ellagic acid and kaempferol 3-O-β-D-glucuronide standard stock solution, Std-Stock (50 mg/L for ellagic acid and 25 mg/L for kaempferol 3-O-β-D-glucuronide)

Weigh accurately 1.0 mg of ellagic acid CRS and 0.5 mg of kaempferol 3-*O*-β-D-glucuronide CRS, and dissolve in 20 mL of methanol.

Mixed ellagic acid and kaempferol 3-O-β-D-glucuronide standard solution for assay, Std-AS

Measure accurately the volume of the mixed ellagic acid and kaempferol 3-*O*-β-D-glucuronide Std-Stock, dilute with methanol to produce a series of solutions of 1, 2, 5, 10, 15 mg/L for ellagic acid and 0.5, 1, 2.5, 5, 7.5 mg/L for kaempferol 3-*O*-β-D-glucuronide.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of methanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Transfer the residue to a 50-mL conical flask, then add 15 mL of methanol (70%). Sonicate (180 W) the mixture for 15 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for two times each with 2 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 (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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	82	18	isocratic
5 – 10	82 → 75	18 → 25	linear gradient
10 – 30	75	25	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed ellagic acid and kaempferol 3-*O*-β-D-glucuronide Std-AS (5 mg/L for ellagic acid and 2.5 mg/L for kaempferol 3-*O*-β-D-glucuronide). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ellagic acid and kaempferol 3-*O*-β-D-glucuronide should not be more than 5.0%; the RSD of the retention times of ellagic acid and kaempferol 3-*O*-β-D-glucuronide peaks should not be more than 2.0%; the column efficiencies determined from ellagic acid and kaempferol 3-*O*-β-D-glucuronide peaks should not be less than 30000 and 20000 theoretical plates respectively.

The *R* value between ellagic acid peak and the closest peak; and the *R* value between kaempferol 3-*O*-β-D-glucuronide peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curves

Inject a series of the mixed ellagic acid and kaempferol 3-*O*-β-D-glucuronide Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of ellagic acid and kaempferol 3-*O*-β-D-glucuronide against the corresponding concentrations of the mixed ellagic acid and kaempferol 3-*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 ellagic acid and kaempferol 3-*O*-β-D-glucuronide peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed ellagic acid and kaempferol 3-*O*-β-D-glucuronide Std-AS. The retention times of ellagic acid and kaempferol 3-*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 ellagic acid and kaempferol 3-*O*-β-D-glucuronide in the test solution, and calculate the percentage contents of ellagic acid and kaempferol 3-*O*-β-D-glucuronide in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.18% of ellagic acid ($C_{14}H_6O_8$) and not less than 0.091% of kaempferol 3-*O*-β-D-glucuronide ($C_{21}H_{18}O_{12}$), calculated with reference to the dried substance.

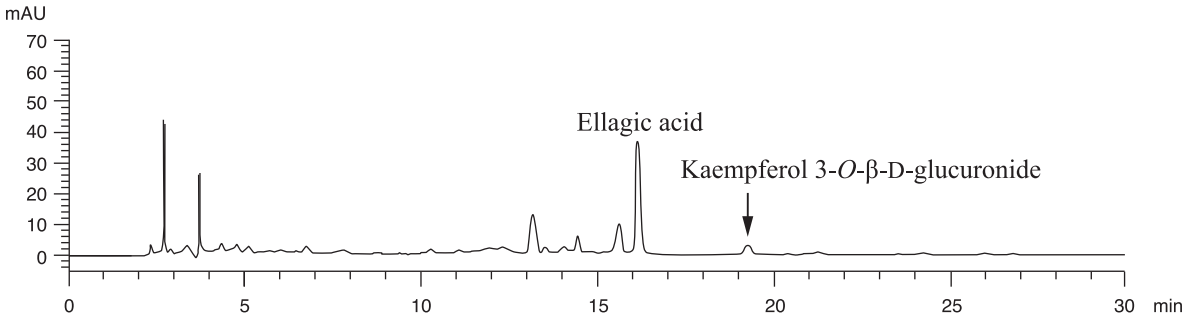


Figure 7 A reference assay chromatogram of Potentillae Discoloris Herba extract