Agrimoniae Herba

Figure 1 A photograph of Agrimoniae Herba

A. Agrimoniae Herba B. A branch with leaves
C. Magnified image of upper part of stem
D. Magnified image of transverse section of upper part of stem
E. Magnified image of lower part of stem
F. Magnified image of transverse section of lower part of stem
G. Magnified image of inflorescence

1. NAMES

Official Name: Agrimoniae Herba

Chinese Name: 仙鶴草

Chinese Phonetic Name: Xianhecao

2. SOURCE

Agrimoniae Herba is the dried aerial part of *Agrimonia pilosa* Ledeb. (Rosaceae). The aerial part is collected in summer and autumn when foliage branch growing luxuriantly, foreign matter removed, then dried to obtain Agrimoniae Herba.

3. DESCRIPTION

Up to 93 cm long, covered with white pubescence. The lower part of stem cylindrical, 2-7 mm in diameter, reddish-brown; the upper part squared, the four sides slightly sunken, greenish-brown, with longitudinal furrows and ridges, the nodes distinct; texture hard and light in weight, easily broken; fracture hollowed. Leaves odd-pinnately compound, alternate, dark green, crumpled and rolled; texture fragile, easily broken; leaflets of two sizes, the small leaflets interposed between the large ones along the rachis, the leaflet at the apex relatively large, when intact flattened out, ovate to elongated-elliptic, apex acute, base cuneate, margin serrate; stipules 2, amplexicaul, oblique-ovate. Racemes disposed on terminal, slender, pedicel short, the lower part of calyx tubular, 5-lobed at the apex, petals 5, yellow. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Non-glandular hairs occur commonly on the surface of the epidermis. Epidermis consists of 1 layer of subsquared cells, arranged orderly. Cortex consists of several layers of parenchymatous cells. Pericyclic fibres consist of 6-8 layers of cells, arranged in ring; fibres polygonal, lignified. Phloem relatively narrow, arranged in ring. Cambium indistinct. Xylem relatively broad, arranged radically, arranged in an undulated ring. Pith broad, occupying about 2/3 of the stem. Clusters of calcium oxalate scattered in the cortex and pith [Fig. 2 (i)].

Leaf: Non-glandular hairs occur on the surface of the upper and lower epidermis. Upper epidermis consists of 1 layer of cells, arranged orderly. Palisade tissue consists of 2 layers of cells, not passing through the midvein. Spongy tissue consists of 2-4 layers of cells. Phloem narrow. Xylem broad. Lower epidermis consists of 1 layer of cells, arranged orderly. Collenchyma located distinctly at prominent regions underneath the lower epidermis. Clusters of calcium oxalate located in the palisade tissue and parenchymatous cells of the midvein [Fig. 2 (ii)].

Powder

Colour dark green. Upper epidermal cells polygonal. The cell walls of lower epidermal cells sinuous, stomata anomocytic or anisocytic. Non-glandular hairs unicellular, varying in length, walls thickened and lignified, with warty protuberances and sometimes with spiral striations. Multicellular glandular hairs consist of 1- to 4-celled head, ovoid, with a 1- to 2-celled stalk. Glandular hairs occur occasionally, consisting of a unicellular head, 43-74 μm in diameter, containing volatile oil droplet, locating on the top of a unicellular stalk. Clusters of calcium oxalate abundant, 8-46 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

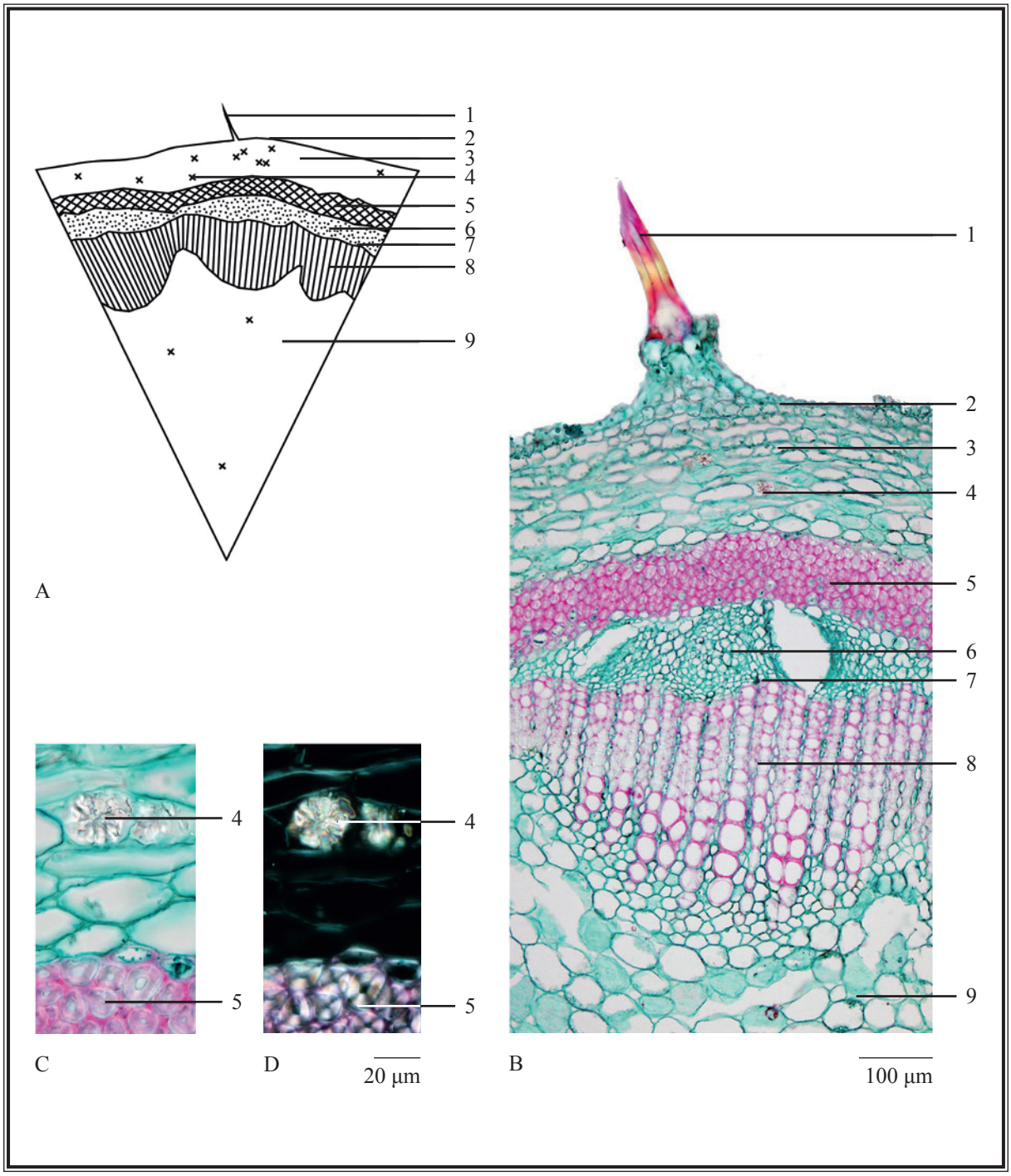

Figure 2 (i) Microscopic features of transverse section of stem of Agrimoniae Herba

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

1. Non-glandular hair 2. Epidermis 3. Cortex 4. Clusters of calcium oxalate
5. Pericyclic fibres 6. Phloem 7. Cambium 8. Xylem 9. Pith

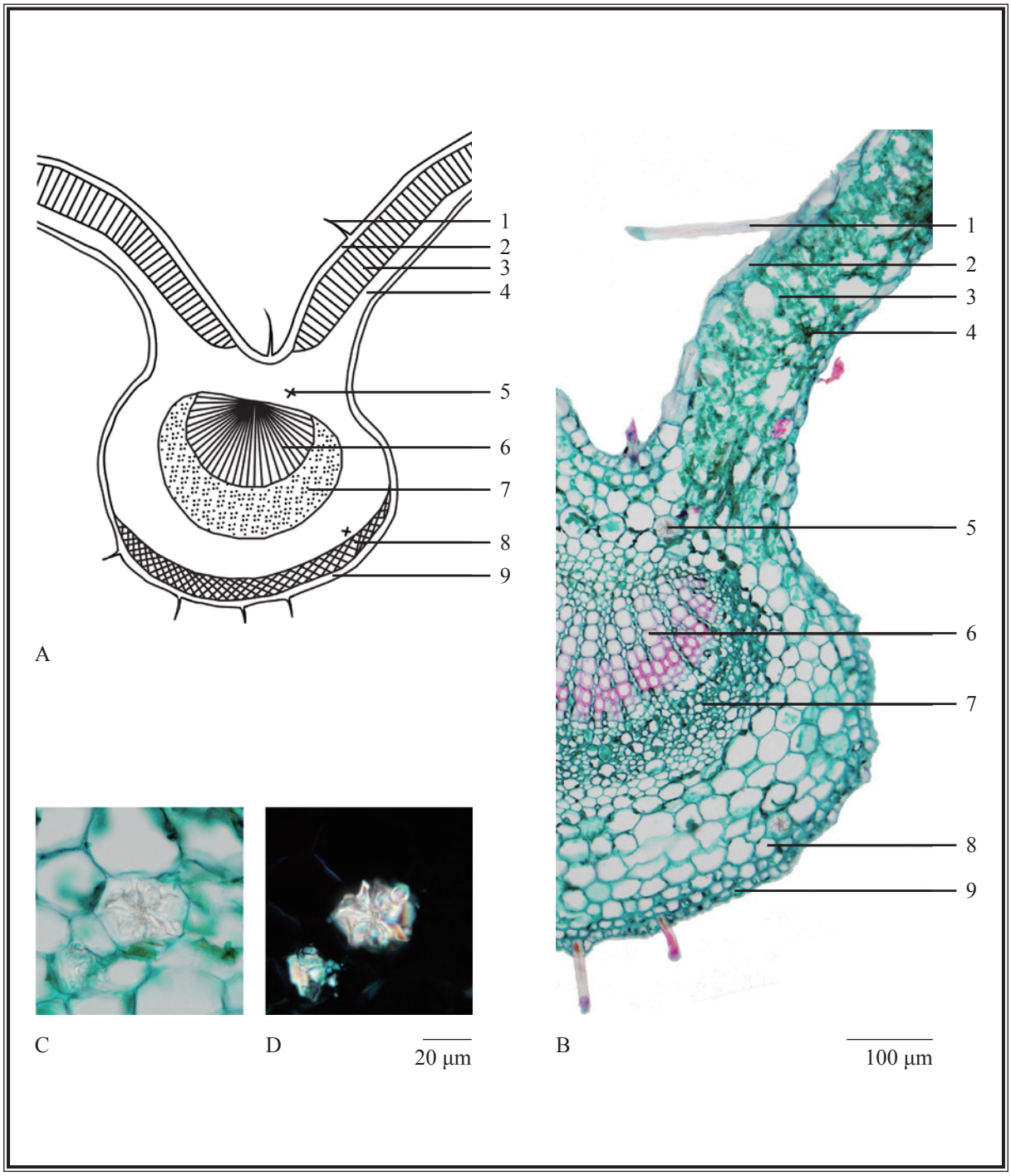

Figure 2 (ii) Microscopic features of transverse section of leaf of Agrimoniae Herba

A. Sketch B. Section illustration C. Clusters of calcium oxalate
D. Clusters of calcium oxalate (under the polarized microscope)

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

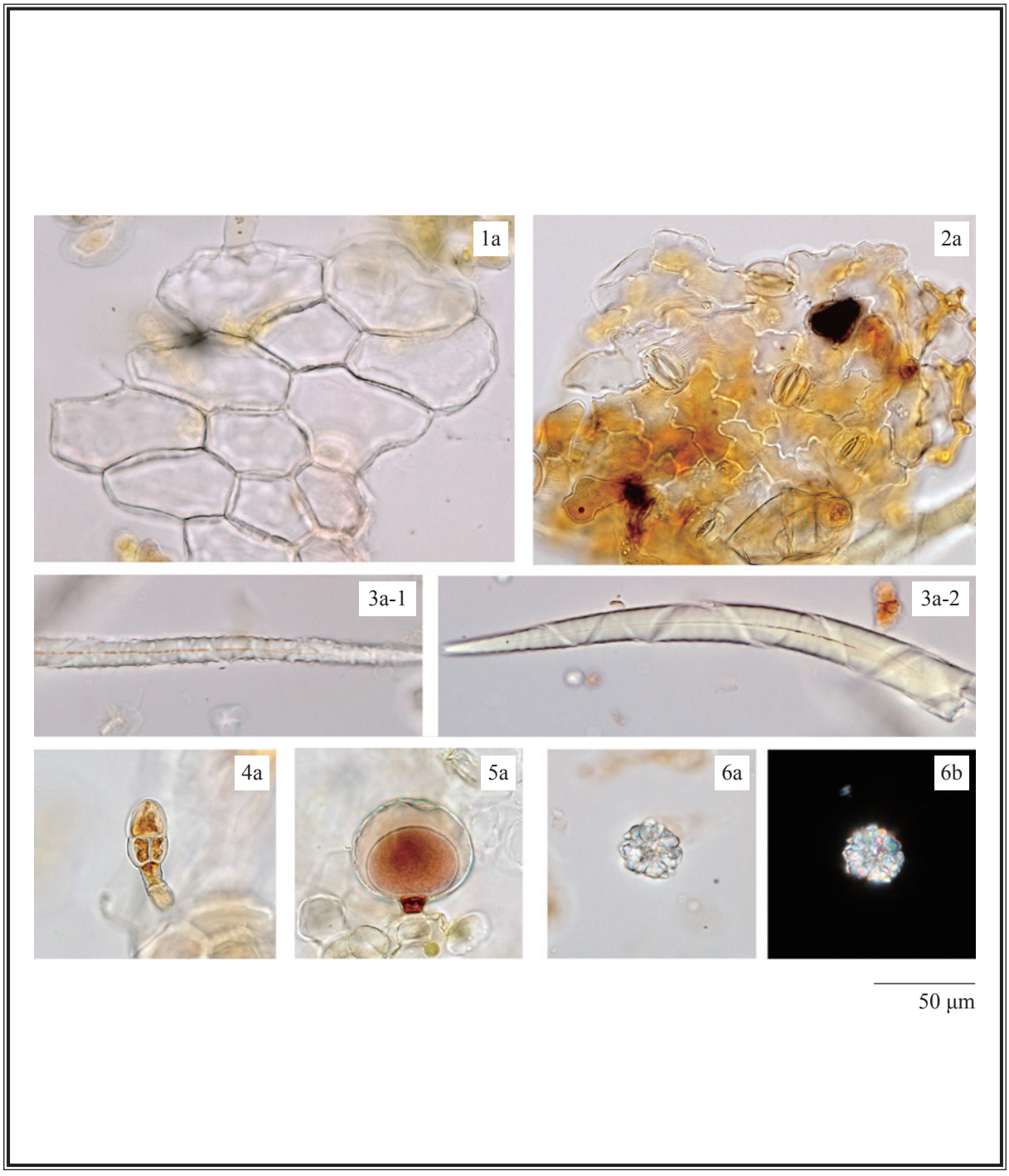

Figure 3 Microscopic features of powder of Agrimoniae Herba

1. Upper epidermal cells 2. Lower epidermal cells with stomata
3. Non-glandular hair (3-1 with warty protuberances, 3-2 with spiral striations)
4. Multicellular glandular hair 5. Glandular hair 6. Cluster of calcium oxalate

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

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

Standard solution

Quercitrin standard solution

Weigh 1.0 mg of quercitrin CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, formic acid and water (25:2:2:1, 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. 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 and a freshly prepared developing solvent system as described above. Apply separately quercitrin standard solution and the test solution (2 µL each) 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 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).

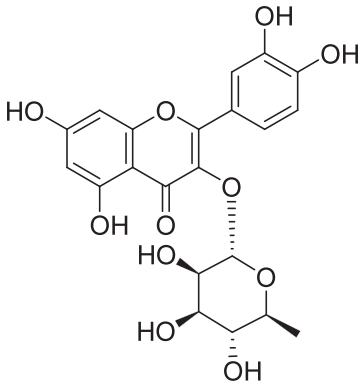

Figure 4 Chemical structure of quercitrin

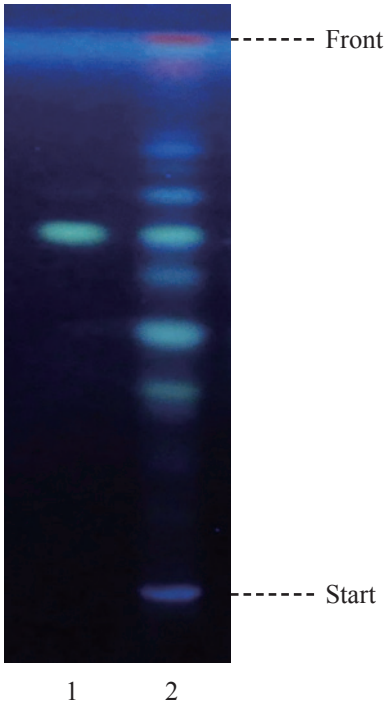

Figure 5 A reference HPTLC chromatogram of Agrimoniae Herba extract observed under UV light (366 nm) after staining

1. Quercitrin 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 quercitrin (Fig. 5).

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

Standard solution

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

Weigh 0.5 mg of quercitrin CRS and dissolve in 10 mL of methanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (50%). Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Make up to the mark with methanol (50%). 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	90 → 65	10 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of quercitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercitrin should not be more than 5.0%; the RSD of the retention time of quercitrin peak should not be more than 2.0%; the column efficiency determined from quercitrin peak should not be less than 80000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject quercitrin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of quercitrin peak in the chromatogram of quercitrin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify quercitrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of quercitrin Std-FP. The retention times of quercitrin 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 five characteristic peaks of Agrimoniae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.82	± 0.03
2	0.99	± 0.03
3 (marker, quercitrin)	1.00	-
4	1.05	± 0.03
5	1.13	± 0.03

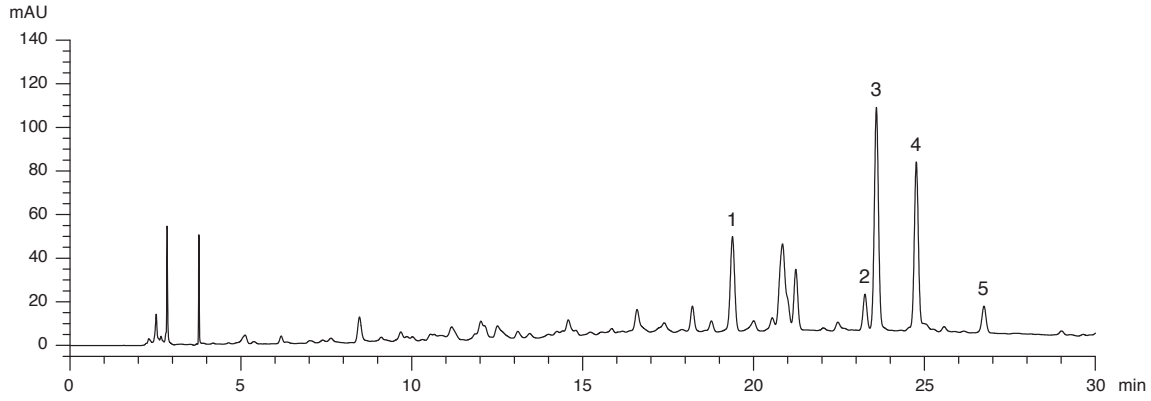

Figure 6 A reference fingerprint chromatogram of Agrimoniae Herba extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 3.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 14.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

Quercitrin standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of quercitrin CRS and dissolve in 10 mL of methanol (50%).

Quercitrin standard solution for assay, Std-AS

Measure accurately the volume of the quercitrin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 1, 2.5, 5, 10, 15 mg/L for quercitrin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (50%). Sonicate (180 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 15 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for three more times. Combine the filtrates and make up to the mark with methanol (50%). 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. The mobile phase is a mixture of 0.1% phosphoric acid and acetonitrile (77:23, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercitrin Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercitrin should not be more than 5.0%; the RSD of the retention time of quercitrin peak should not be more than 2.0%; the column efficiency determined from quercitrin peak should not be less than 7000 theoretical plates.

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

Limits

The sample contains not less than 0.11% of quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.