Rosae Chinensis Flos

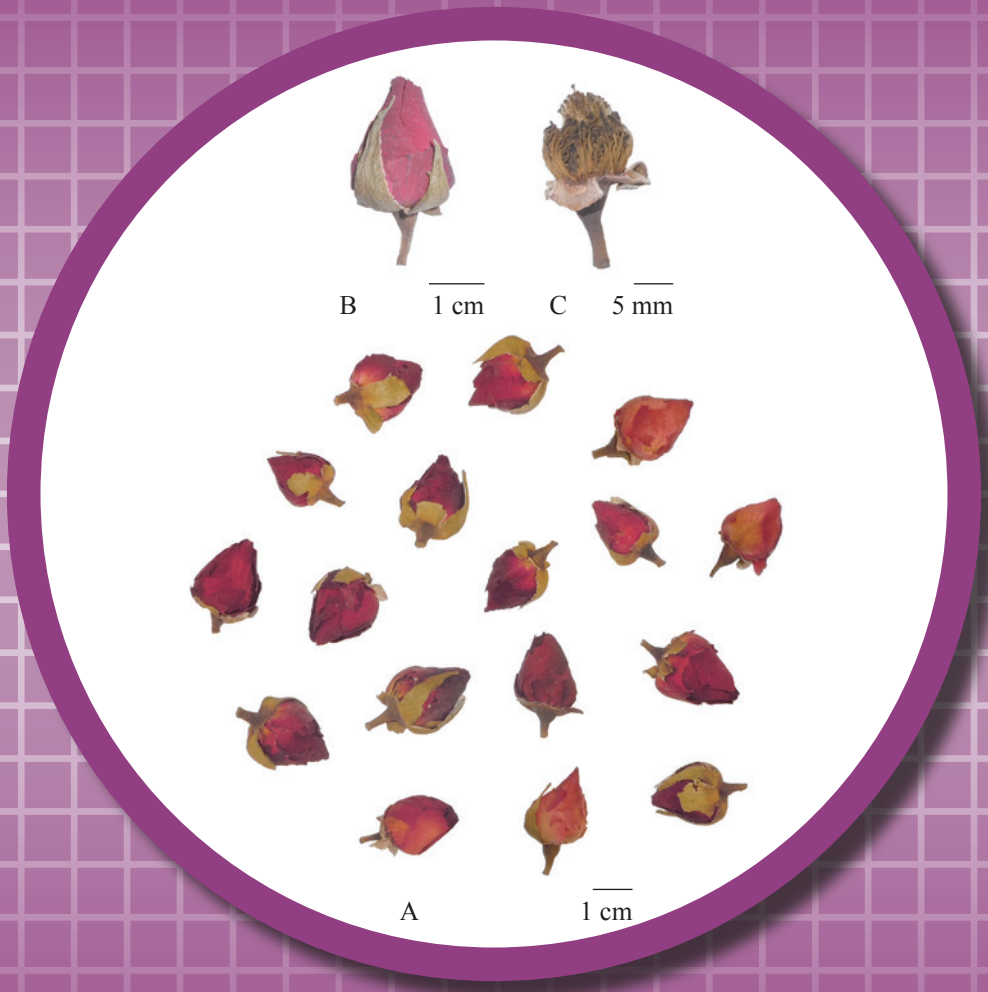

Figure 1 A photograph of Rosae Chinensis Flos

A. Rosae Chinensis Flos B. Magnified image of flower
C. Magnified image of stamens and pistils

1. NAMES

Official Name: Rosae Chinensis Flos

Chinese Name: 月季花

Chinese Phonetic Name: Yuejihua

2. SOURCE

Rosae Chinensis Flos is the dried flower of *Rosa chinensis* Jacq. (Rosaceae). The flower is collected in summer and autumn when it is slightly open, then dried in a shaded area or at low temperature (below 60°C) to obtain Rosae Chinensis Flos.

3. DESCRIPTION

Ovoid or subspherical, 1-2.4 cm in diameter. Receptacle obconical or oblong, often with pedicel. Sepals 5, yellowish-green to dark green, apex acute, inner surface densely covered with white pubescence. Petals purplish-red or dark purplish-red, double, imbricate, with prominent veins. Stamens numerous, filaments yellowish-brown. Pistils numerous, longer than stamens, with golden-yellow villi. Texture fragile and light in weight. Odour delicately fragrant; taste slightly bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pedicel: Epidermis consists of 1 layer of cells, covered with cuticle. Cortex consists of parenchymatous cells, several layers of cells on the outer side with relatively thickened walls. Vascular bundles collateral, arranged into 2 rings; the vascular bundles in the outer ring sparsely arranged; the vascular bundles in the inner ring relatively smaller, closely packed. Pith consists of parenchymatous cells. Clusters of calcium oxalate scattered in parenchymatous cells (Fig. 2).

Powder

Colour pale purplish-red. Non-glandular hairs of pistil unicellular, mostly broken, pale yellow to pale yellowish-brown, extremely long, 10-74 μm in diameter, apex acute or obtuse, base similar to stone cells in shape, lumens large; yellow under the polarized microscope. Non-glandular hairs of calyx unicellular, long, often curved, colourless to pale yellow, 8-23 μm in diameter, apex acute, lumens linear; bright yellow under the polarized microscope. Pollen grains subspherical, 13-59 μm in diameter, with 3 furrows, exine scabrous, immature pollen grains also found. Epidermal cells of corolla subrounded to subpolygonal in surface view, outer walls papillary protruded, with wavy striations on the surface. Cells of endothecium subrounded to subpolygonal in surface view, walls thickened, coarsely striate or reticulate. Clusters of calcium oxalate scattered or present in parenchymatous cells, 8-46 μm in diameter; polychromatic under the polarized microscope. Prisms of calcium oxalate mostly present in parenchymatous cells, small, about 5 μm in diameter; orange or polychromatic under the polarized microscope (Fig. 3).

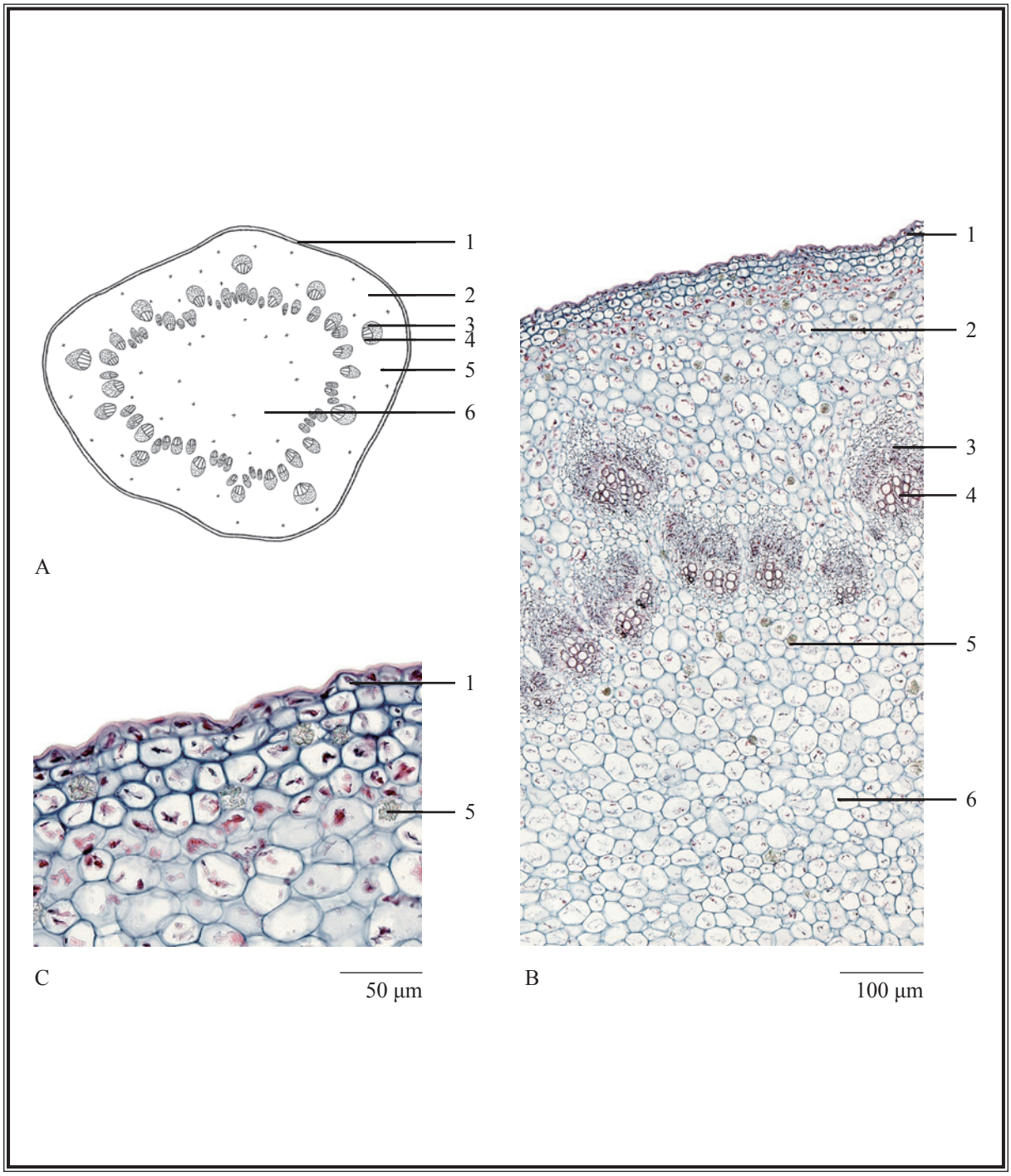

Figure 2 Microscopic features of transverse section of pedicel of Rosae Chinensis Flos

A. Sketch B. Section illustration C. Epidermis and clusters of calcium oxalate

1. Epidermis 2. Cortex 3. Phloem 4. Xylem 5. Clusters of calcium oxalate 6. Pith

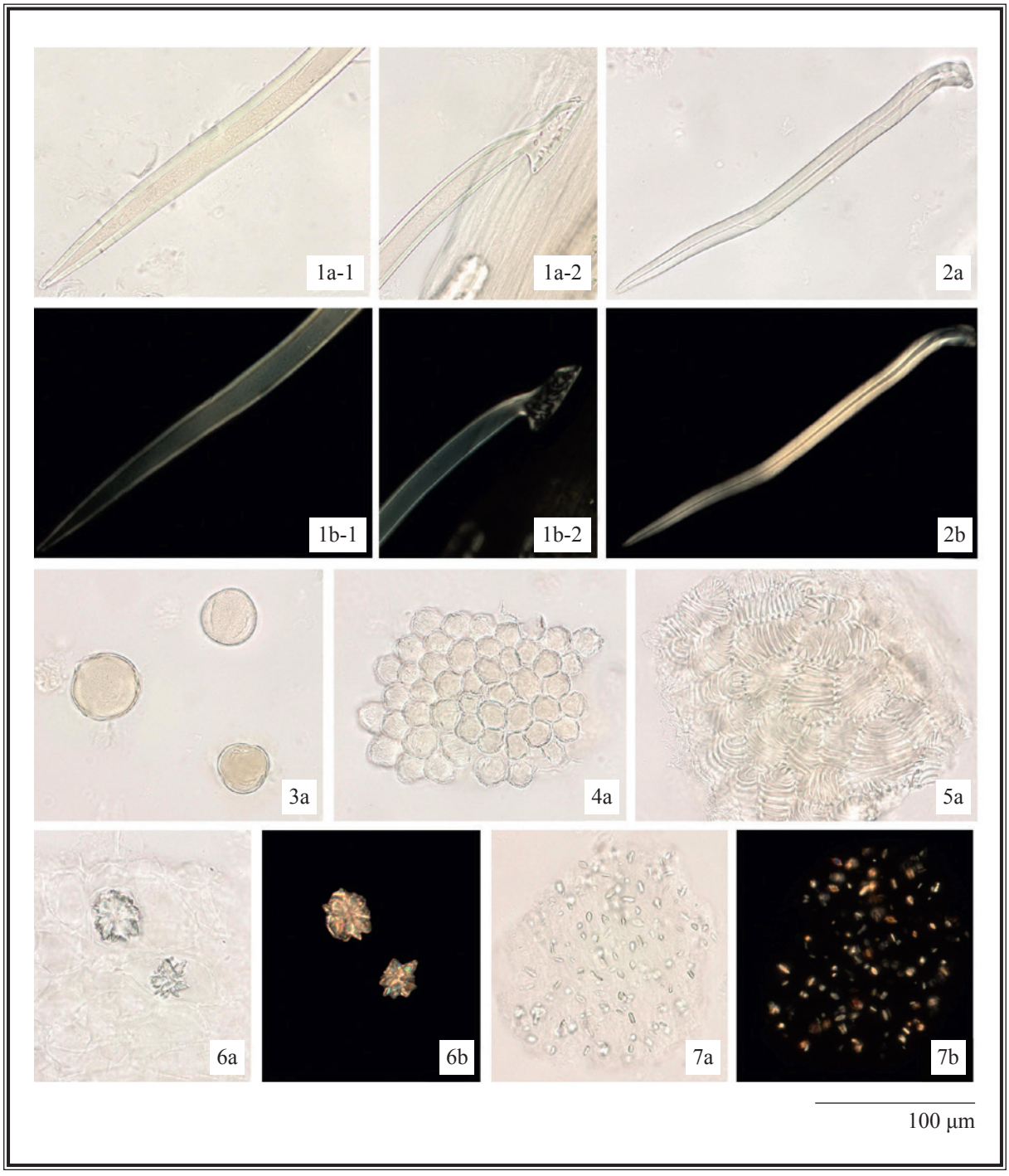

Figure 3 Microscopic features of powder of Rosae Chinensis Flos

1. Non-glandular hair of pistil (1-1 apex, 1-2 base) 2. Non-glandular hair of calyx
3. Pollen grains 4. Epidermal cells of corolla with papillae 5. Cells of endothecium
6. Clusters of calcium oxalate 7. Prisms 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 0.5 mg of quercitrin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, methanol, formic acid and water (25:1:1:1, v/v).

Spray reagent

Weigh 2.5 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 centrifuge tube, then add 10 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of water. Transfer the solution to a 50-mL centrifuge tube. Add 10 mL of ethyl acetate. Centrifuge at about 2800 × *g* for 10 min. Transfer the upper layer 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. Filter through a 0.45-μm nylon filter.

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 quercitrin standard solution (0.5 μL) and the test solution (1 μL) 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 6 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 85ºC (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

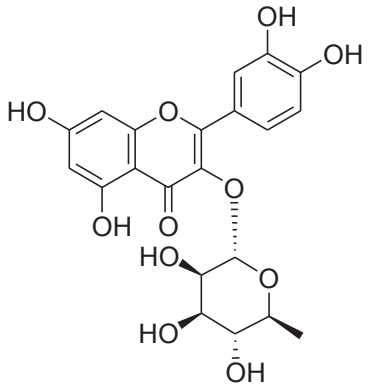

Figure 4 Chemical structure of quercitrin

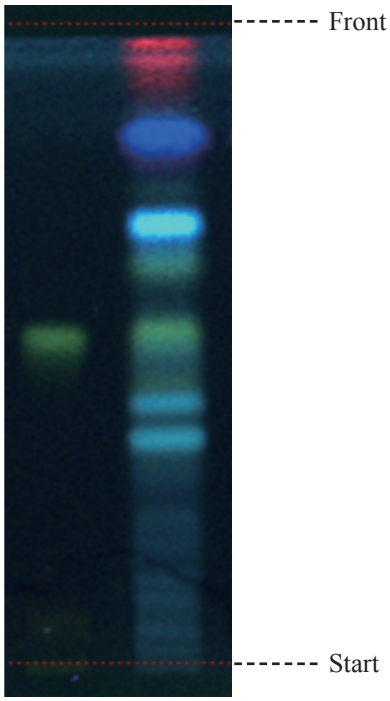

Figure 5 A reference HPTLC chromatogram of Rosae Chinensis Flos extract observed under UV light (366 nm) after staining

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 (8 mg/L)

Weigh 0.2 mg of quercitrin CRS and dissolve in 25 mL of methanol.

Test solution

Weigh 0.4 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 1.5 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 2800 × *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 PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (349 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 40ºC during the separation. 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% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	90 → 79	10 → 21	linear gradient
20 – 55	79 → 74	21 → 26	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 20000 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 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 six 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 six characteristic peaks of Rosae Chinensis Flos extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Rosae Chinensis Flos extract

Peak No.	RRT	Acceptable Range
1 (ellagic acid)	0.78	± 0.03
2	0.98	± 0.03
3 (marker, quercitrin)	1.00	-
4	1.04	± 0.04
5	1.11	± 0.03
6	1.27	± 0.03

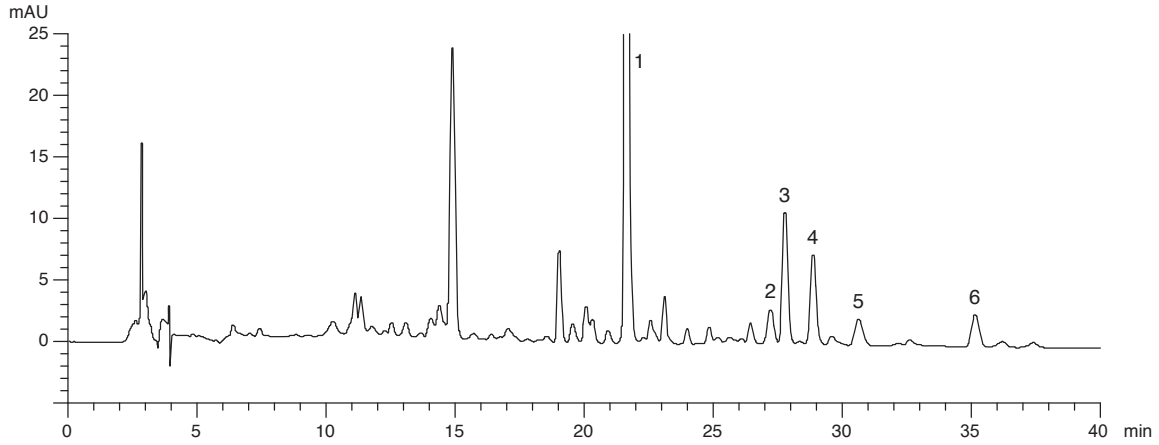

Figure 6 A reference fingerprint chromatogram of Rosae Chinensis Flos extract

For positive identification, the sample must give the above six 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 *(Appendix VII)*: meet the requirements.

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

5.5 Foreign Matter *(Appendix VIII):* not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 44.0%.
Ethanol-soluble extractives (hot extraction method): not less than 43.0%.

7. ASSAY

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

Standard solution

Quercitrin standard stock solution, Std-Stock (50 mg/L)
Weigh accurately 0.5 mg of quercitrin CRS and dissolve in 10 mL of methanol.
Quercitrin standard solution for assay, Std-AS
Measure accurately the volume of the quercitrin Std-Stock, dilute with methanol to produce a series of solutions of 1, 2, 5, 8, 10 mg/L for quercitrin.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 1.5 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 2800 × *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 PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (349 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 40ºC during the separation. 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% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	90 → 79	10 → 21	linear gradient
20 – 55	79 → 74	21 → 26	linear gradient

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 20000 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 0.039% of quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.