Albiziae Flos

Figure 1 A photograph of Albiziae Flos

A. Albiziae Flos B. Magnified image of flowers
C. Magnified image of inflorescence with flower
D. Magnified image of inflorescence with flower buds

1. NAMES

Official Name: Albiziae Flos

Chinese Name: 合歡花

Chinese Phonetic Name: Hehuanhua

2. SOURCE

Albiziae Flos is the dried inflorescence of *Albizia julibrissin* Durazz. (Fabaceae). The inflorescence is collected in summer at flowering on sunny days, foreign matter removed, then dried under the sun in time to obtain Albiziae Flos.

3. DESCRIPTION

Capitulum crumpled to masses. Peduncle 3-4 cm long, sometimes detached from inflorescence, yellowish-green, with longitudinal striations, covered with sparse tomenta. Flowers densely covered with tomenta, slender and curved, pale yellow to pale yellowish-brown, sessile or short-pedicellate. Calyx and corolla 0.7-1 cm long. Calyx tubular, 5-toothed at the apex. Corolla tube about 2 times in length of calyx tube, 5-lobed at the apex; lobes lanceolate. Stamens numerous, filaments slender, yellowish-brown, connate at the lower part and separated at the upper part, extended over corolla tube. Flower buds occasionally visible, club shaped, calyx tubular, corolla unopened. Odour slightly fragrant; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour greyish-yellow. Compound pollen grains oblate, 16-polysomic, 70-105 μm in diameter, the central 8 monosomic arranged in cruciform and surrounded by the other 8 monosomic; individual monosomic subsquare or long-spherical. Non-glandular hairs unicellular, 81-447 μm long. Prisms of calcium oxalate frequently occur in parenchymatous cells, 3-21 μm in diameter; polychromatic under the polarized microscope. Vessels mainly spiral or bordered-pitted. Epidermal cells of filament elongated rectangular, with cuticle striations in surface view (Fig. 2).

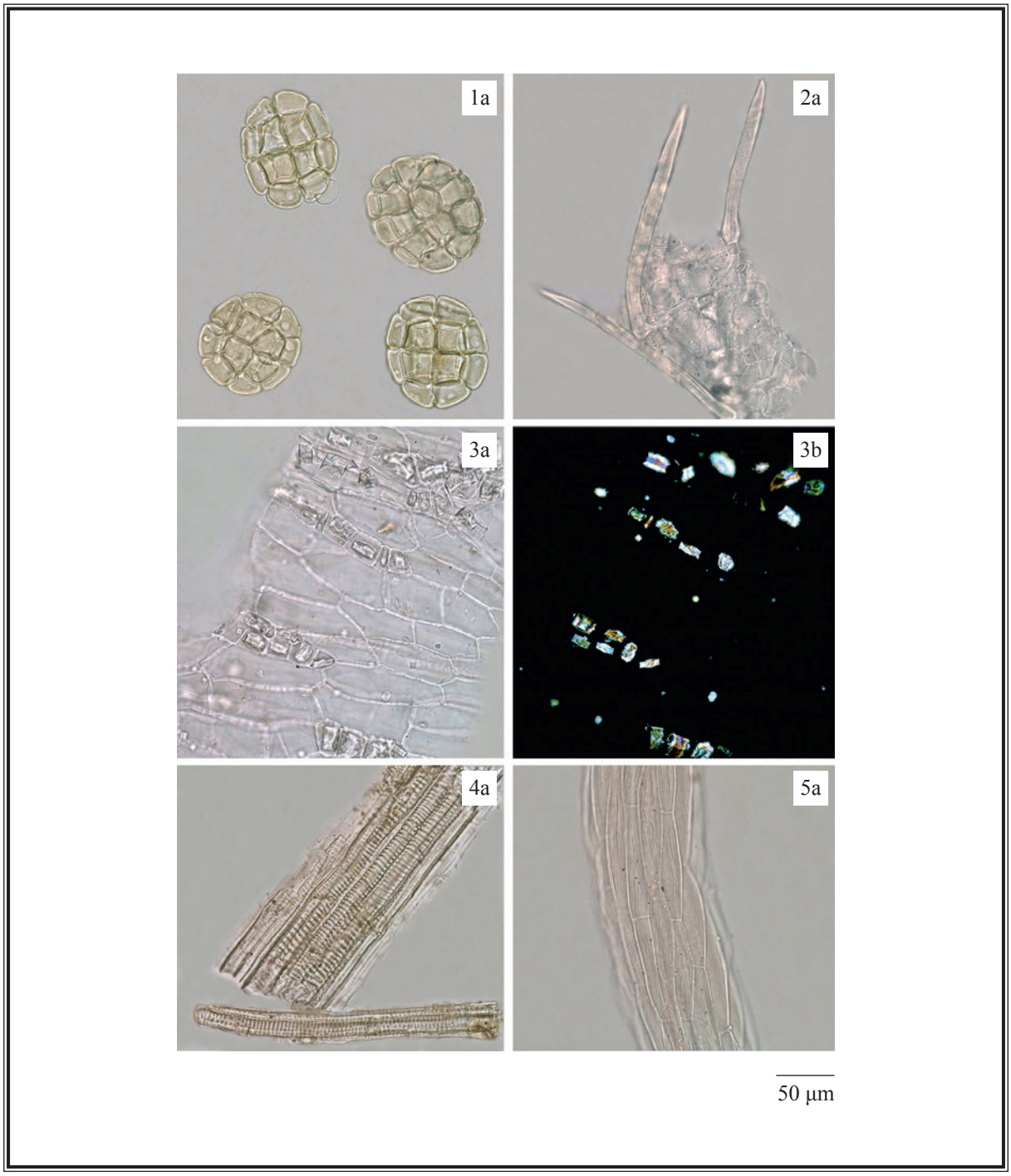

Figure 2 Microscopic features of powder of Albiziae Flos

1. Compound pollen grains 2. Non-glandular hairs 3. Prisms of calcium oxalate
4. Vessels 5. Epidermal cells of filament

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. 3) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (15:0.5:0.5, v/v).

Spray reagent

Weigh 0.5 g of aluminium trichloride and dissolve in 50 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (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, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately quercitrin 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 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 70ºC (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).

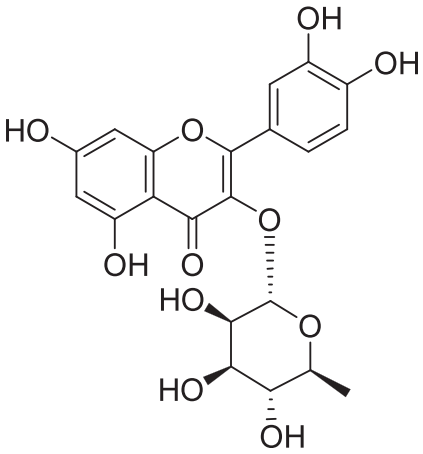

Figure 3 Chemical structure of quercitrin

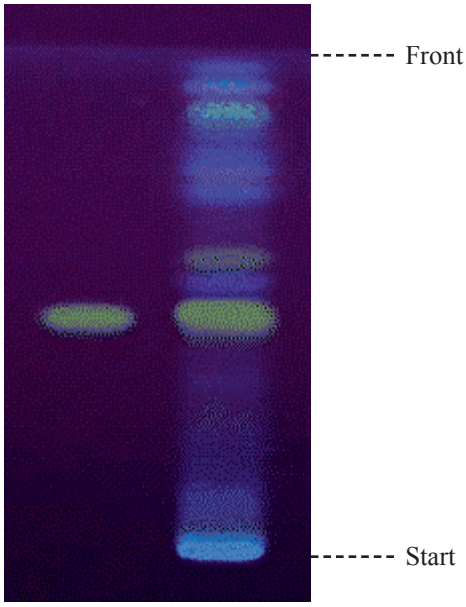

Figure 4 A reference HPTLC chromatogram of Albiziae Flos extract observed under UV light (366 nm) after staining

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. 4).

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

Standard solution

Quercitrin standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 3.0 mg of quercitrin CRS and dissolve in 50 mL of ethanol (70%).

Test solution

Weigh 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (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 ethanol (70%). Filter through a 0.45-μm RC 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% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	85 → 60	15 → 40	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 26000 theoretical plates.

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. 5).

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 three characteristic peaks (Fig. 5) 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 three characteristic peaks of Albiziae Flos extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Albiziae Flos extract

Peak No.	RRT	Acceptable Range
1	0.76	± 0.03
2 (marker, quercitrin)	1.00	-
3	1.18	± 0.03

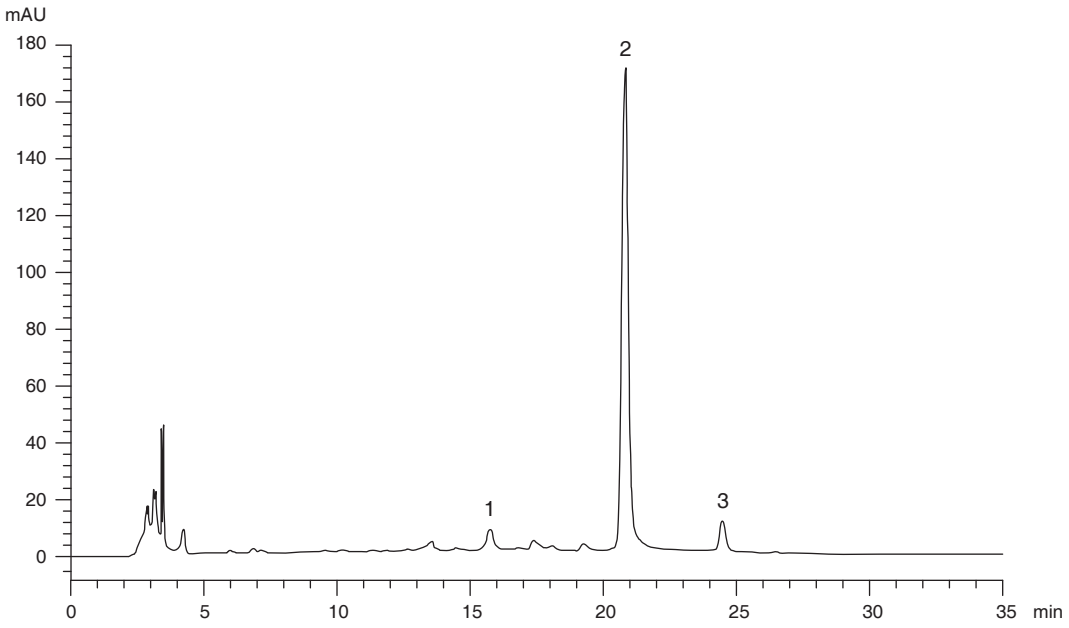

Figure 5 A reference fingerprint chromatogram of Albiziae Flos extract

For positive identification, the sample must give the above three characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

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 10.0%.
Acid-insoluble ash: not more than 3.0%.

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 24.0%.
Ethanol-soluble extractives (cold extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

Quercitrin standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of quercitrin CRS and dissolve in 5 mL of ethanol (70%).

Quercitrin standard solution for assay, Std-AS

Measure accurately the volume of the quercitrin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 20, 60, 100, 150, 300 mg/L for quercitrin.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (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 ethanol (70%). Filter through a 0.45-μm RC 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% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	85 → 60	15 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of quercitrin Std-AS (100 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 26000 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 1.0% of quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.