Celosiae Cristatae Flos

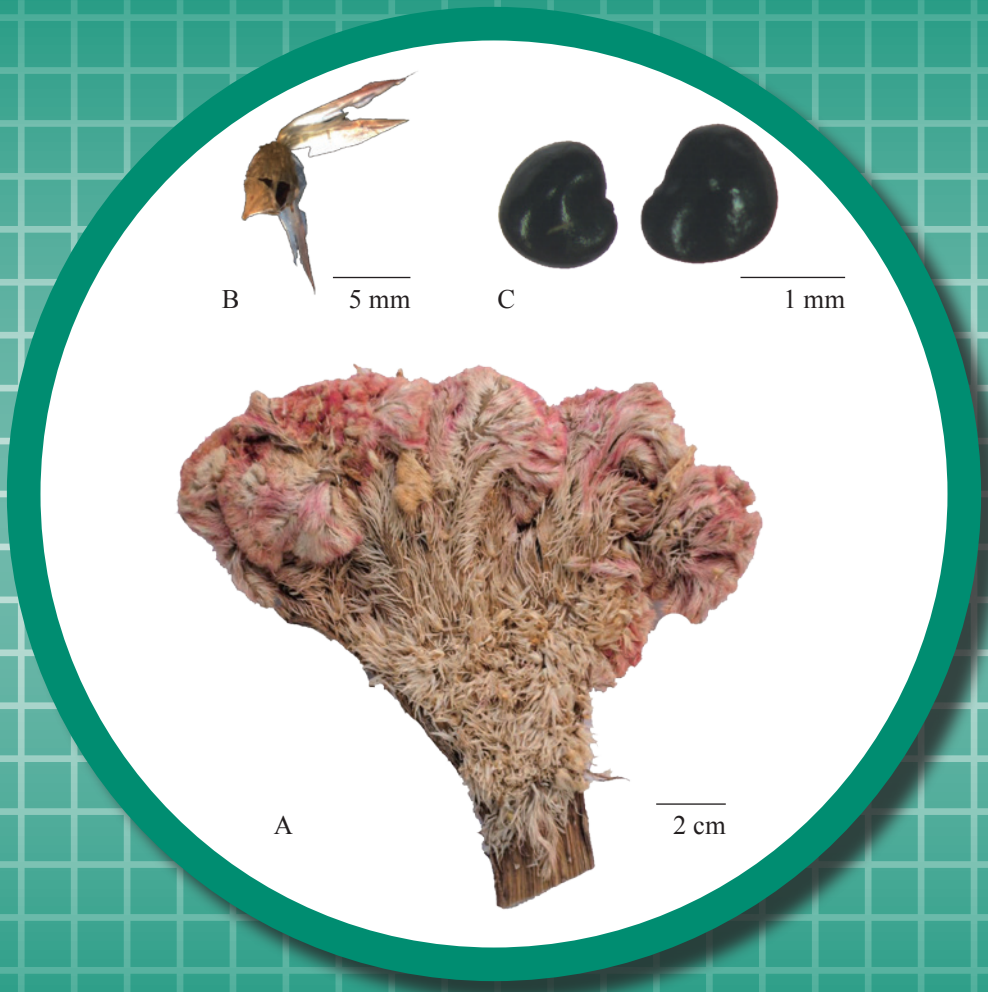


Figure 1 A photograph of Celosiae Cristatae Flos

A. Celosiae Cristatae Flos B. Magnified single flower
C. Magnified seeds

1. NAMES

Official Name: Celosiae Cristatae Flos

Chinese Name: 雞冠花

Chinese Phonetic Name: Jiguanhua

2. SOURCE

Celosiae Cristatae Flos is the dried capitulum of *Celosia cristata* L. (Amaranthaceae). The capitulum is collected in autumn, then dried under the sun to obtain Celosiae Cristatae Flos.

3. DESCRIPTION

Terminal inflorescence, mostly flatten and fleshy, cockscomb-shaped, 8-25 cm long, 5-22 cm wide. The upper margin broad, with wrinkles and densely linear scales, narrowed towards the base and usually with remnants of flat stem. Externally red, purplish-red or yellowish-white, with numerous florets aggregated below the middle part, persistent bracts and perianth of the florets membranous. Fruit an utricle, seeds black, oblate and reniform, petty, about 1 mm in diameter, lustrous. Texture pliable and light in weight. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Peduncle: Epidermis consists of 1 layer of cells. Cortex narrow, with parenchymatous cells occasionally contain microcrystals of calcium oxalate. Phloem relatively narrow. Cambium distinct. Xylem well developed, composed of vessels, xylem fibres and xylem parenchymatous cells. Vessels scattered singly or in groups. Pith broad, amphivasal vascular bundles scattered in the outer side of the pith area, microcrystals of calcium oxalate occasionally visible in the parenchymatous cells (Fig. 2).

Powder

Colour greyish-brown to brown. Pollen grains subglobular, 13-32 μm in diameter, exine with small protrusions. Epidermal cells of peduncle subsquare or subrectangular, walls thin. Microcrystals of calcium oxalate present in parenchymatous cells or scattered, slightly arrowhead-like in shape, extremely minute; bright white to polychromatic under the polarized microscope. Epidermal cells of testa polygonal, pits thickened. Anther cells annular thickened. Vessels mainly scalariform, 6-28 μm in diameter. Fibres mostly in bundles, 5-22 μm in diameter (Fig. 3).

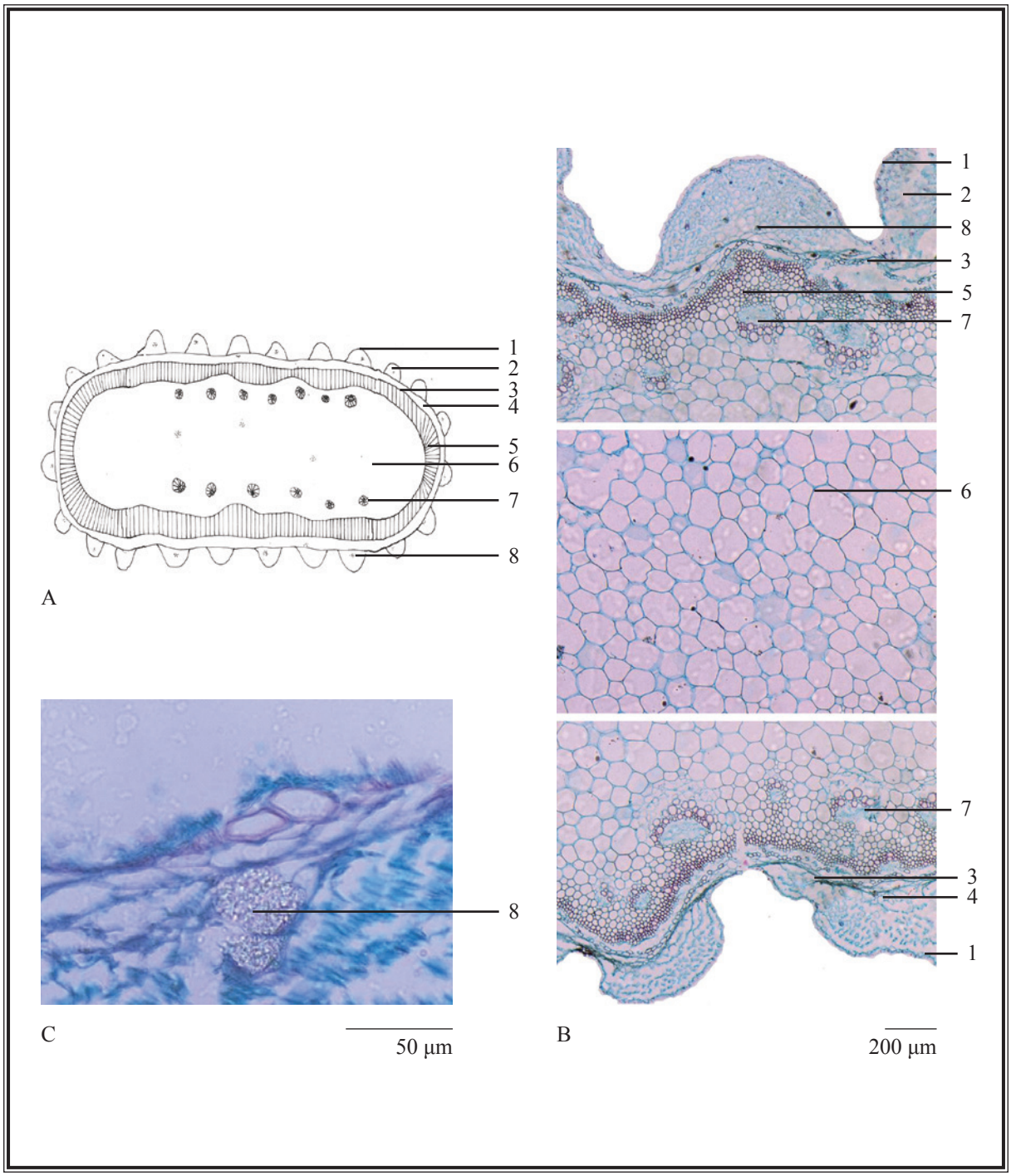


Figure 2 Microscopic features of transverse section of peduncle of Celosiae Cristatae Flos

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

1. Epidermis 2. Cortex 3. Cambium 4. Phloem 5. Xylem
6. Pith 7. Amphivasal vascular bundle 8. Microcrystals of calcium oxalate

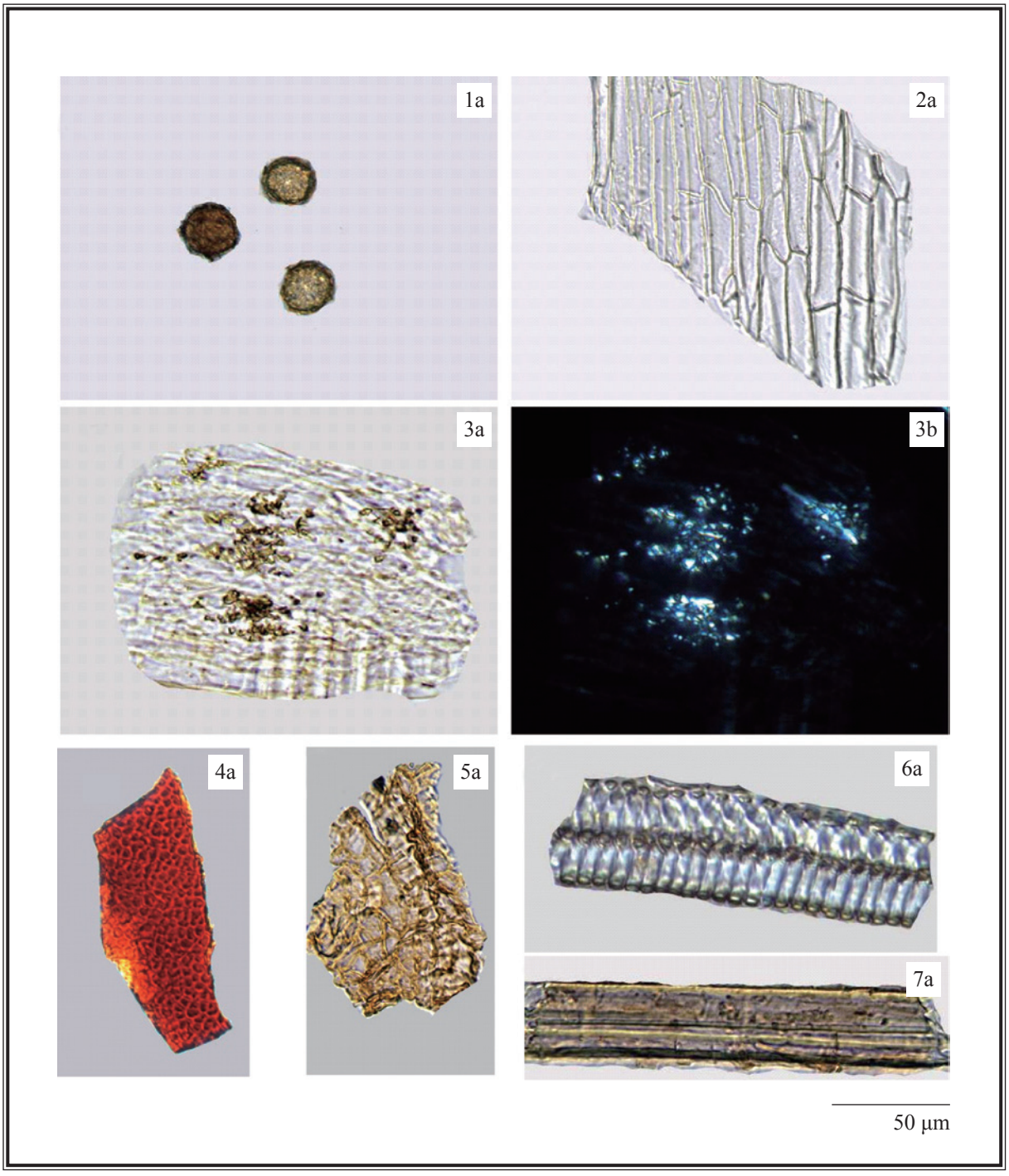


Figure 3 Microscopic features of powder of Celosiae Cristatae Flos

1. Pollen grains 2. Epidermal cells of peduncle
3. Microcrystals of calcium oxalate in parenchymatous cells
4. Epidermal cells of testa 5. Anther cells 6. Vessels 7. Fibres

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

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

Standard solution

Kaempferol standard solution

Weigh 0.5 mg of kaempferol CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of *n*-hexane, ethyl acetate, formic acid and glacial acetic acid (10:6:0.5:0.5, v/v).

Spray reagent

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

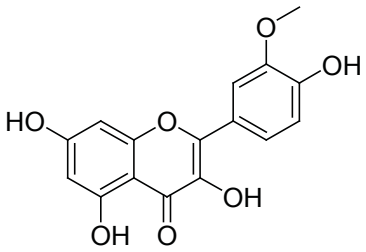
Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of a mixture of ethanol, water and hydrochloric acid (50:20:8, v/v). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 4000 × *g* for 10 min. Transfer 10 mL of 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 methanol and then 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 kaempferol standard solution (1.5 μL) and the test solution (2 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 8.5 cm. After the development, remove the plate from the chamber, mark the solvent front and heat at about 105ºC (about 1 min). Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 2 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

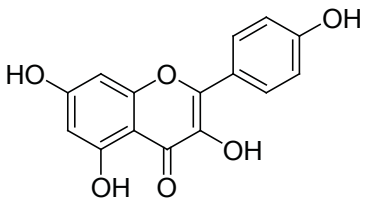


Figure 4 Chemical structures of (i) isorhamnetin and (ii) kaempferol

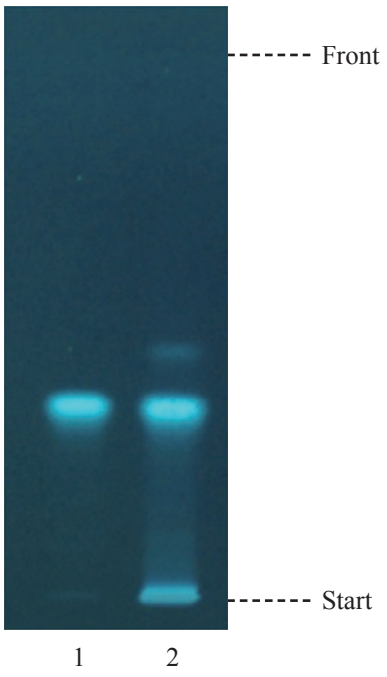


Figure 5 A reference HPTLC chromatogram of Celosiae Cristatae Flos extract observed under UV light (366 nm) after staining

1. Kaempferol 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 kaempferol (Fig. 5).

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

Standard solution

Kaempferol standard solution for fingerprinting, Std-FP (16 mg/L)

Weigh 0.16 mg of kaempferol CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of a mixture of ethanol, water and hydrochloric acid (50:20:8, v/v). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (365 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 27ºC 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)	0.2% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 30	60 → 40	40 → 60	linear gradient
30 – 40	40	60	isocratic
40 – 60	40 → 25	60 → 75	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of kaempferol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of kaempferol should not be more than 5.0%; the RSD of the retention time of kaempferol peak should not be more than 2.0%; the column efficiency determined from kaempferol peak should not be less than 50000 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. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Celosiae Cristatae Flos extract

Peak No.	RRT	Acceptable Range
1	0.88	± 0.03
2 (marker, kaempferol)	1.00	-
3 (isorhamnetin)	1.05	± 0.03
4	1.64	± 0.03

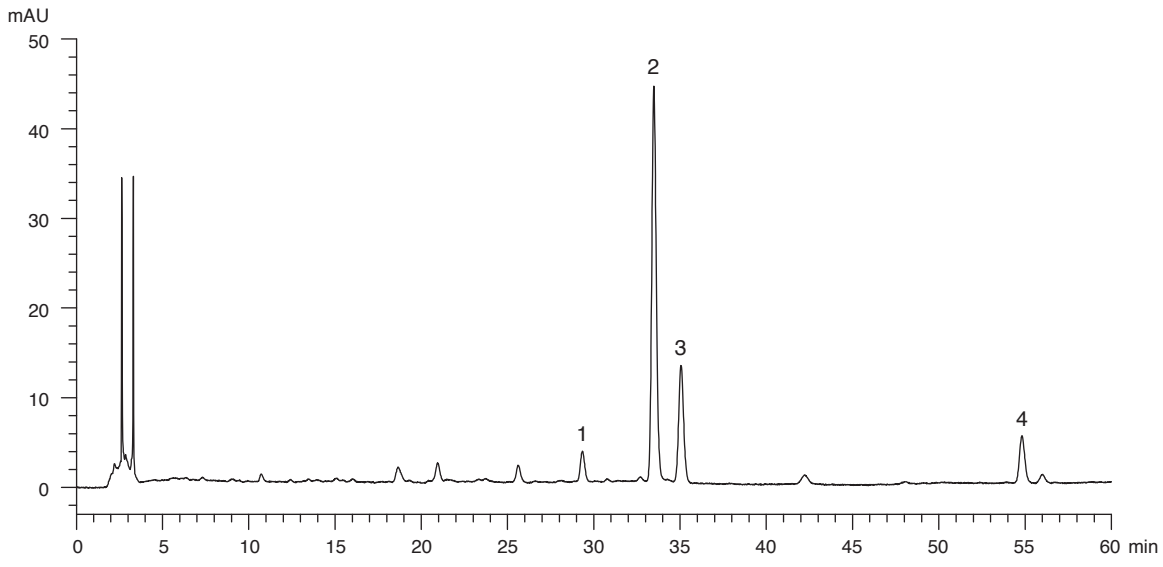


Figure 6 A reference fingerprint chromatogram of Celosiae Cristatae Flos extract

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 *(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 11.0%.
Acid-insoluble ash: not more than 2.5%.

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 21.0%.
Ethanol-soluble extractives (hot extraction method): not less than 16.0%.

7. ASSAY

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

Standard solution
Mixed isorhamnetin and kaempferol standard stock solution, Std-Stock (100 mg/L for isorhamnetin and 400 mg/L for kaempferol)
Weigh accurately 1.0 mg of isorhamnetin CRS (Fig. 4) and 4.0 mg of kaempferol CRS, and dissolve in 10 mL of ethanol.

Mixed isorhamnetin and kaempferol standard solution for assay, Std-AS

Measure accurately the volume of the mixed isorhamnetin and kaempferol Std-Stock, dilute with ethanol to produce a series of solutions of 1, 2.5, 5, 7.5, 15 mg/L for isorhamnetin and 4, 10, 20, 30, 60 mg/L for kaempferol.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of a mixture of ethanol, water and hydrochloric acid (50:20:8, v/v). Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol. Combine the solutions and make up to the mark with ethanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (365 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 27ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.2% phosphoric acid and methanol (45:55, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed isorhamnetin and kaempferol Std-AS (5 mg/L for isorhamnetin and 20 mg/L for kaempferol). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of isorhamnetin and kaempferol should not be more than 5.0%; the RSD of the retention times of isorhamnetin and kaempferol peaks should not be more than 2.0%; the column efficiencies determined from isorhamnetin and kaempferol peaks should not be less than 7000 theoretical plates.

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

Limits

The sample contains not less than 0.15% of the total content of isorhamnetin ($C_{16}H_{12}O_7$) and kaempferol ($C_{15}H_{10}O_6$), calculated with reference to the dried substance.