Carthami Flos

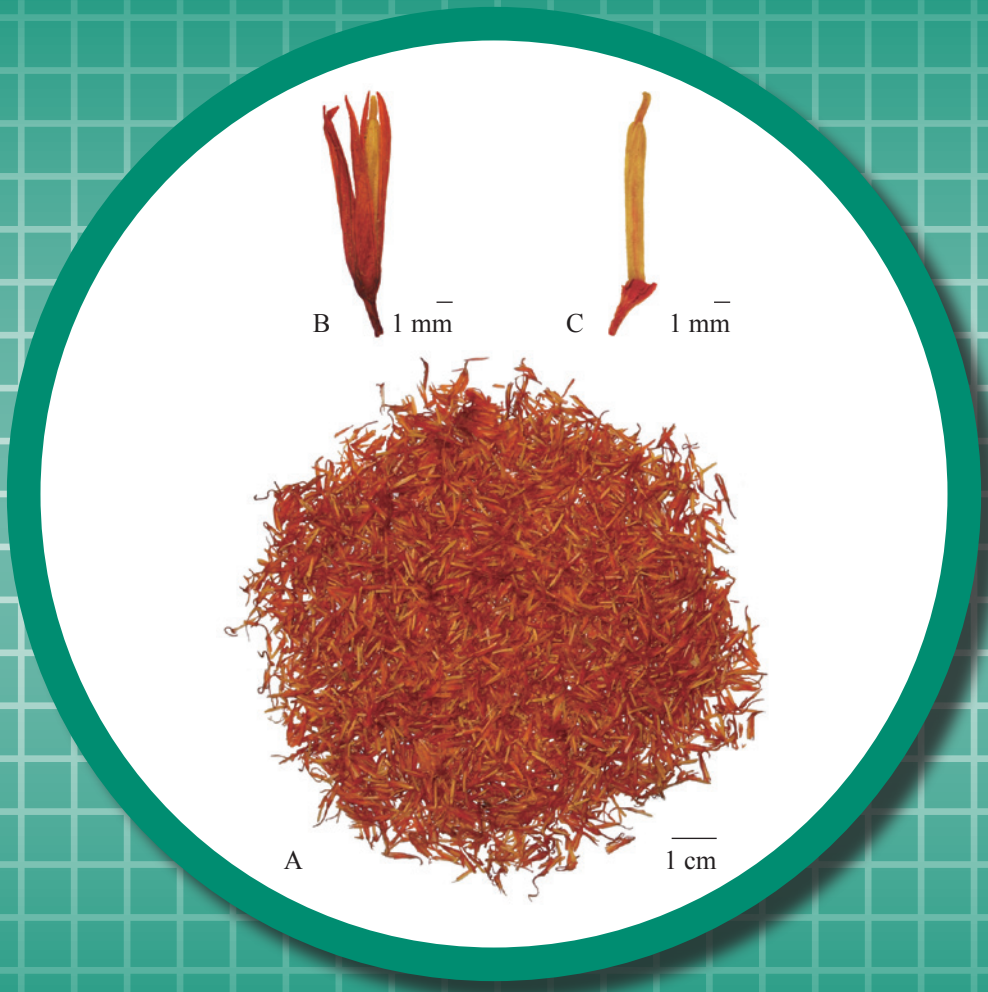

Figure 1 A photograph of Carthami Flos

A. Carthami Flos B. Magnified flower
C. Magnified stamens and pistil

1. NAMES

Official Name: Carthami Flos

Chinese Name: 紅花

Chinese Phonetic Name: Honghua

2. SOURCE

Carthami Flos is the dried flower of *Carthamus tinctorius* L. (Asteraceae). The flower is collected in summer when it turns from yellow to red, then dried in a shaded area or under the sun to obtain Carthami Flos.

3. DESCRIPTION

Tubular flowers without ovaries, 6-23 mm long. Corolla tube orange-red to red, slender, 5-lobed at the apex, lobes linear. Stamens 5, anthers yellow, fused into a tube. Pistil 1, style slender, extending beyond anther tube. Texture soft. Odour slightly fragrant, taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour orange-red. Pollen grains elliptical or spherical, 27-73 μm in diameter (occasionally up to 86 μm), with 3 germinal pores, exine dentate-spinose. Secretory ducts long tubular, 4-49 μm in diameter, filling with yellowish-brown or reddish-brown secretions, generally accompanied by spiral vessels. Epidermal cells of stigma differentiated into conical unicellular hairs, straight or slightly curved. Epidermal cells of corolla tip with slightly thickened walls, outer walls with papillary protrusions. Epidermal cells of corolla subrectangular or long strip-shaped in surface view, anticlinal walls wavy. Cells of endothecium subrectangular in surface view, cross wall beaded-thickened. Cells of anther base subsquare or subrectangular, walls thickened. Prisms of calcium oxalate mainly present in parenchymatous cells, square, rectangular or cylindrical; white to yellowish-white under the polarized microscope (Fig. 2).

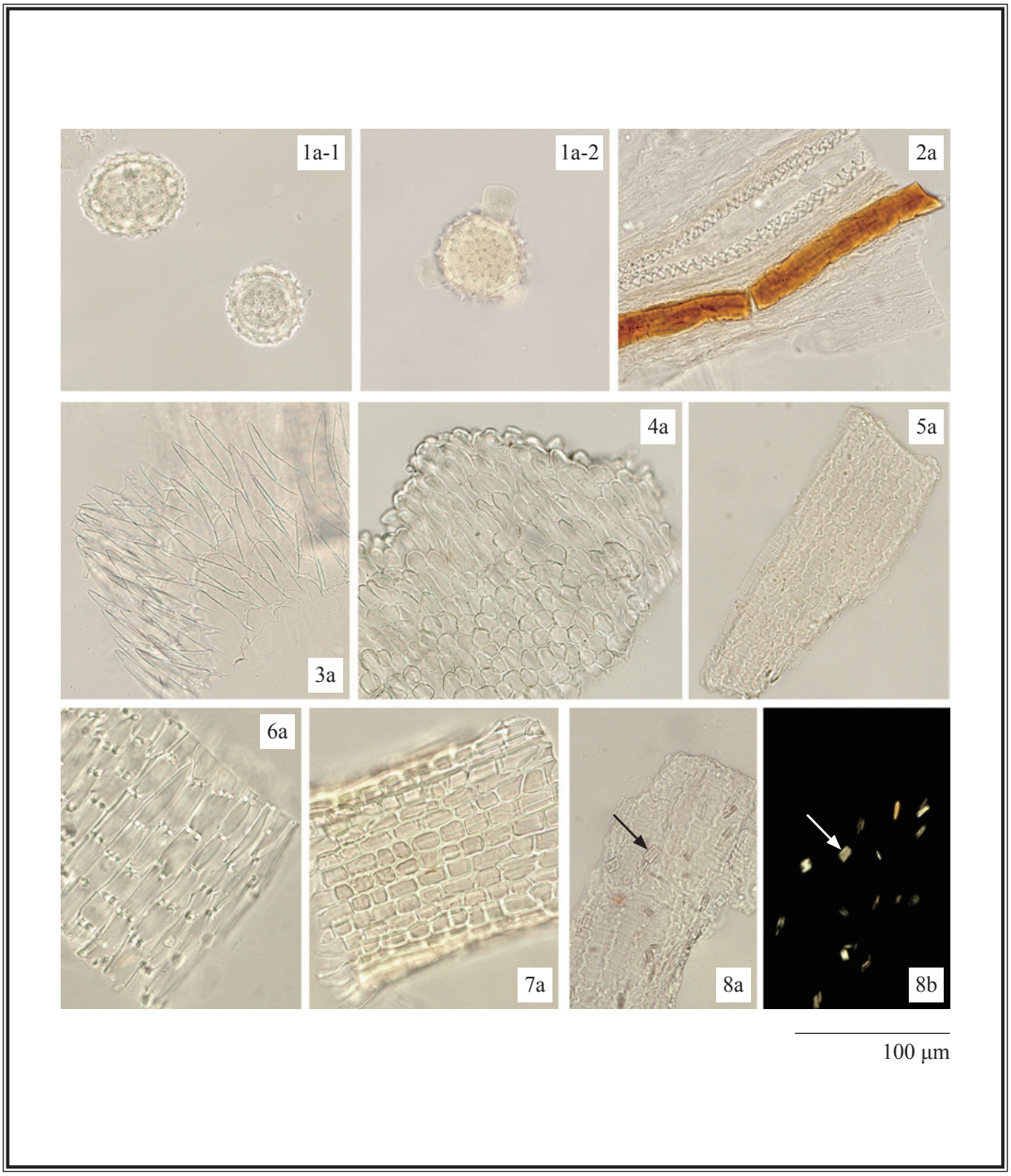

Figure 2 Microscopic features of powder of Carthami Flos

1. Pollen grains 2. Secretory ducts 3. Fragment of stigma
4. Epidermal cells of corolla tip 5. Epidermal cells of corolla 6. Fragment of endothecium
7. Fragment of anther base 8. 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

Hydroxysafflor yellow A standard solution

Weigh 5.0 mg of hydroxysafflor yellow A CRS (Fig. 3) and place it in a 5-mL amber glass volumetric flask. Make up to the mark with methanol. Freshly prepare the standard solution.

Developing solvent system

Prepare a mixture of ethyl acetate, water, formic acid and methanol (7:3:2:0.4, v/v).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 2-mL centrifuge tube, then add 1 mL of acetone (80%). Sonicate (140 W) the mixture for 15 min. Centrifuge at about 2500 × *g* for 1 min. Filter through a 0.45-μm nylon filter. Transfer 300 μL of the filtrate to a 2-mL centrifuge tube and add 300 μL of acetone. Centrifuge at about 2500 × *g* for 1 min. Collect the supernatant.

Procedure

Carry out the method by using a HPTLC silica gel $GHLF_{254}$ plate (150 μm thickness), a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately hydroxysafflor yellow A standard solution and the test solution (2 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. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

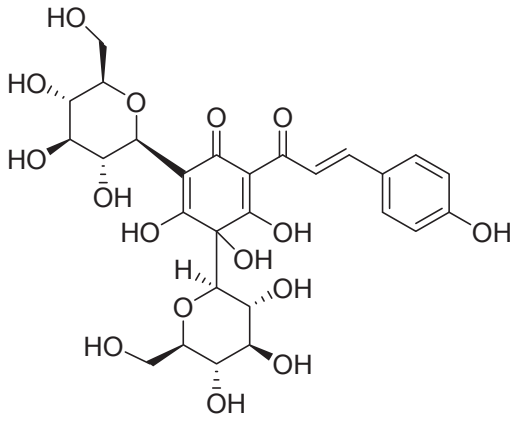

Figure 3 Chemical structure of hydroxysafflor yellow A

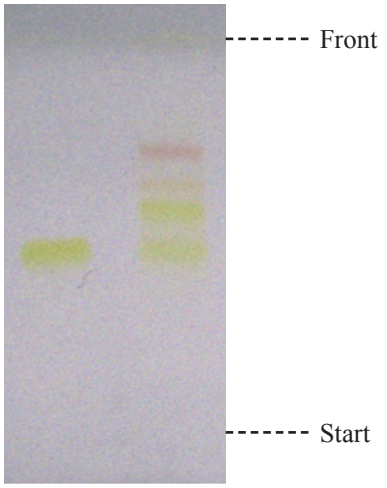

Figure 4 A reference HPTLC chromatogram of Carthami Flos extract observed under visible light

1. Hydroxysafflor yellow A 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 hydroxysafflor yellow A (Fig. 4).

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

Standard solution

Hydroxysafflor yellow A standard solution for fingerprinting, Std-FP (12 mg/L)

Weigh 0.3 mg of hydroxysafflor yellow A CRS and place it in a 25-mL amber glass volumetric flask. Make up to the mark with methanol (25%). Freshly prepare the standard solution.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (25%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol (25%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (403 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC 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% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 45	70 → 45	30 → 55	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of hydroxysafflor yellow A Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of hydroxysafflor yellow A should not be more than 5.0%; the RSD of the retention time of hydroxysafflor yellow A peak should not be more than 2.0%; the column efficiency determined from hydroxysafflor yellow A peak should not be less than 8000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject hydroxysafflor yellow A Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of hydroxysafflor yellow A peak in the chromatogram of hydroxysafflor yellow A Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify hydroxysafflor yellow A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of hydroxysafflor yellow A Std-FP. The retention times of hydroxysafflor yellow A 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 Carthami Flos extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, hydroxysafflor yellow A)	1.00	-
2	1.34	± 0.03
3	2.35	± 0.04
4	2.91	± 0.03

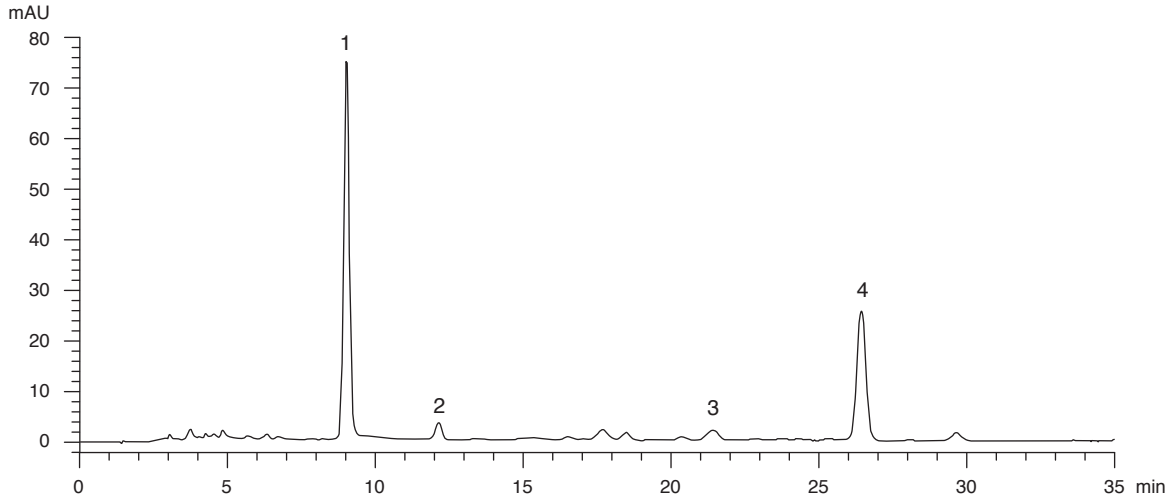

Figure 5 A reference fingerprint chromatogram of Carthami Flos extract

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. 5).

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 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 11.0%.

5.8 Absorbance *(Appendix XV)*

Red pigment: Weigh accurately 0.25 g of the powdered sample and place it in a 200-mL conical flask, then add 50 mL of acetone (80%). Heat the mixture on a water bath at 50ºC for 1.5 h. Cool down to room temperature. Filter through a sintered-glass filter (16-40 μm pore size) and transfer the filtrate to a 100-mL volumetric flask. Wash the residue with acetone (80%). Combine the solution and make up to the mark with acetone (80%). Measure the absorbance at 518 nm. The absorbance should not be less than 0.20.

Yellow pigment: Weigh accurately 0.1 g of the powdered sample and place it in a 200-mL conical flask, then add 150 mL of water. Stir for 1 h. Filter through a sintered-glass filter (16-40 μm pore size) and transfer the filtrate to a 500-mL volumetric flask. Wash the residue with water. Combine the solution and make up to the mark with water. Measure the absorbance at 401 nm. The absorbance should not be less than 0.40.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 32.0%.
Ethanol-soluble extractives (cold extraction method): not less than 26.0%.

7. ASSAY

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

Standard solution

Hydroxysafflor yellow A standard stock solution, Std-Stock (600 mg/L)

Weigh accurately 6.0 mg of hydroxysafflor yellow A CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol (25%). Freshly prepare the standard solution.

Hydroxysafflor yellow A standard solution for assay, Std-AS

Measure accurately the volume of the hydroxysafflor yellow A Std-Stock, dilute with methanol (25%) to produce a series of solutions of 3, 6, 12, 30, 60 mg/L for hydroxysafflor yellow A.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (25%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol (25%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (403 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 30ºC 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% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 45	70 → 45	30 → 55	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of hydroxysafflor yellow A Std-AS (12 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of hydroxysafflor yellow A should not be more than 5.0%; the RSD of the retention time of hydroxysafflor yellow A peak should not be more than 2.0%; the column efficiency determined from hydroxysafflor yellow A peak should not be less than 8000 theoretical plates.

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

Limits

The sample contains not less than 1.2% of hydroxysafflor yellow A ($C_{27}H_{32}O_{16}$), calculated with reference to the dried substance.