Xanthii Fructus

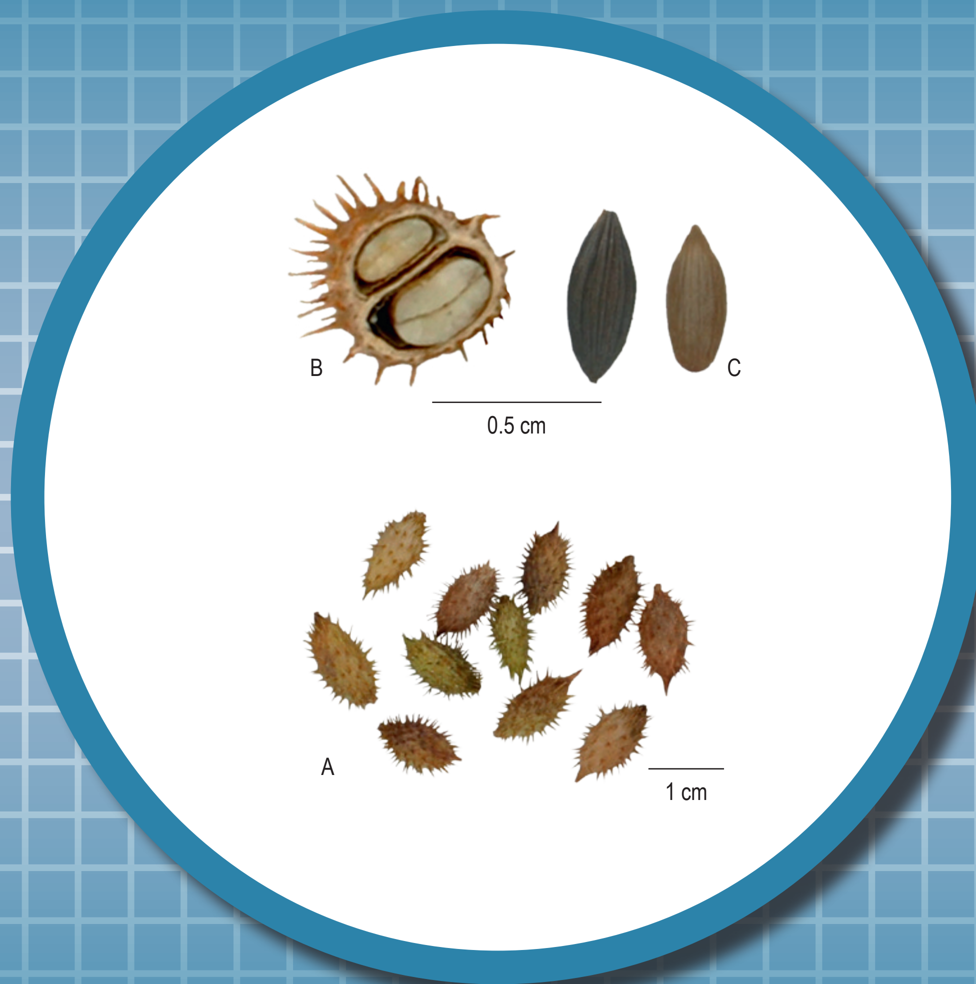

Figure 1 A photograph of Xanthii Fructus

A. Xanthii Fructus B. Transverse section C. Achene (left) and seed (right)

1. NAMES

Official Name: Xanthii Fructus

Chinese Name: 蒼耳子

Chinese Phonetic Name: Cang'erzi

2. SOURCE

Xanthii Fructus is the dried ripe fruit with involucre of *Xanthium sibiricum* Patr. (Asteraceae). The plant is collected in autumn when the fruit is ripe, and dried under the sun. The fruit with involucre gathered, foreign matter removed to obtain Xanthii Fructus.

3. DESCRIPTION

Fusiform to ovoid, 0.6-1.8 cm long, 3-9 mm in diameter. Externally greenish-yellow to brownish-yellow, with hooked spines throughout, and with 2 relatively thick spines at the apex and a fruit stalk scar at the base. Texture hard and tough. At the centre of transverse section, a septum separating 2 loculi clearly visible; each locule contains an achene. Achene ellipsoid to slightly fusiform, relatively even at one side, pericarp thin and fragile, greyish-black, with longitudinal wrinkles. Seed somewhat ellipsoid, beaked at the point of attachment to the pericarp. Testa membranous, yellowish-grey, cotyledons 2, oily. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermal cells of involucre subrectangular or subrounded. Parenchyma of involucre consists of several layers of cells. The outer involucre fibres arranged longitudinally in the lengthwise direction of the fruit, the inner involucre fibres arranged vertically, with vascular bundles scattered among them. Epidermis of pericarp consists of 1 layer of cells. A dark brown-pigment layer beneath pericarp. Several layers of pericarp fibres are arranged in a compact manner. Epidermis of testa consists of 1 layer of flat cells, with vascular bundles scattered underneath it. Endotesta consists of 1 layer of flat cells. Cotyledon cells filled with aleurone grains (Fig. 2).

Powder

Colour greyish-green to pale yellowish-brown. Epidermal cells of pericarp rectangular, often with adherent pigment and pericarp fibres on the lower layer. Epidermal cells of testa subrectangular or subrounded, wall relatively thick. Endotesta cells polygonal, with papillae. Involucre fibres often present in bundles, arranged in a crisscross pattern; single fibres fusiform, 5-25 µm in diameter, wall relatively thick, with pits visible; yellowish-white under the polarized microscope. Pericarp fibres mostly in bundles, single fibres fusiform, 5-24 µm in diameter, pits distinct; pale white under the polarized microscope. Cotyledon cells subrectangular, subrounded or polygonal, containing aleurone grains and oil droplets. Lignified parenchymatous cells subrectangular, with distinct pits. Vessels mainly spiral and reticulate, 3-20 µm in diameter. Non-glandular hairs occasionally found, multicellular (Fig. 3).

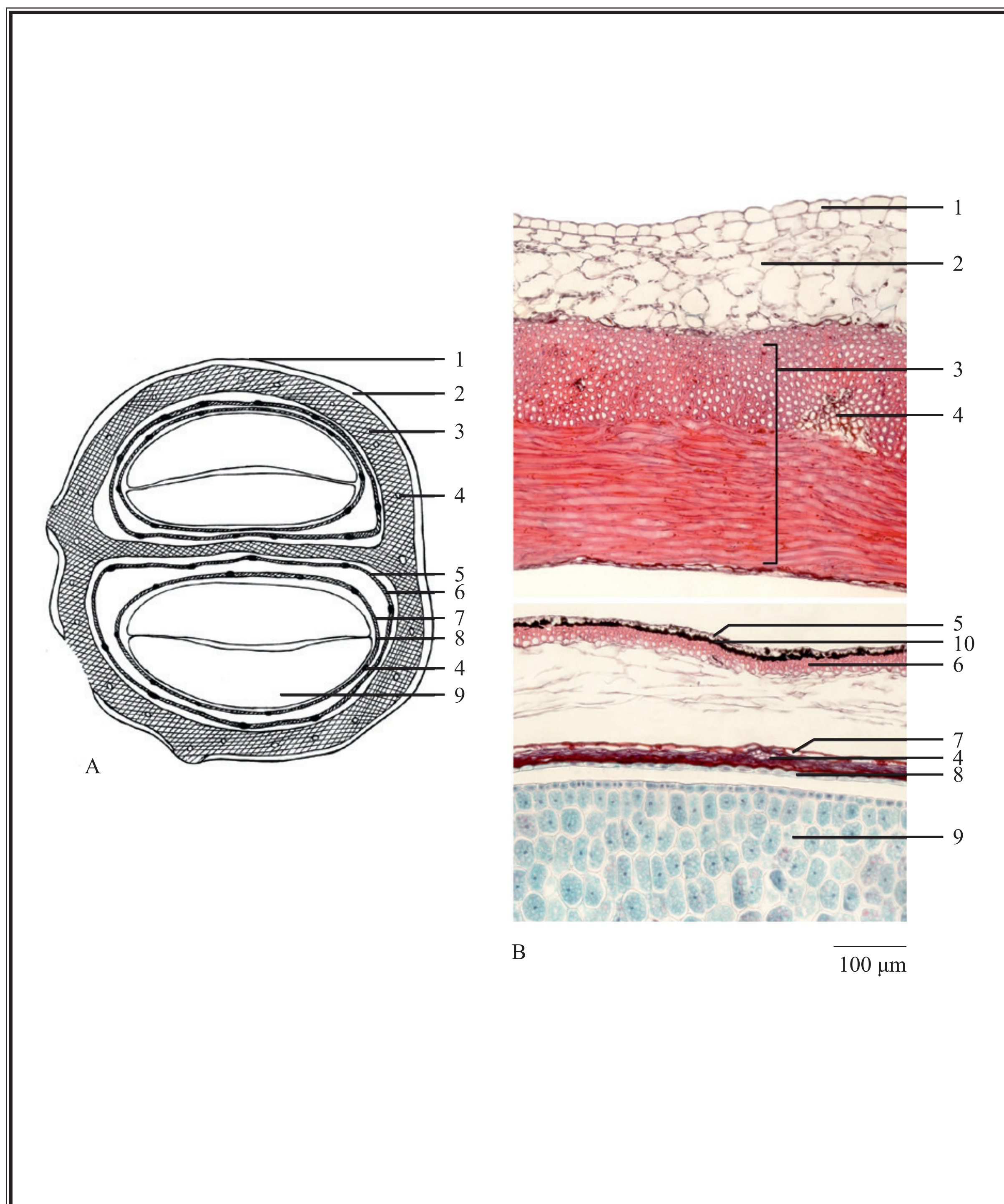

Figure 2 Microscopic features of transverse section of Xanthii Fructus

A. Sketch B. Section illustration

1. Epidermis of involucre 2. Involucre parenchyma 3. Involucre fibre
4. Vascular bundle 5. Epidermis of pericarp 6. Pericarp fibre 7. Epidermis of testa
8. Endotesta 9. Cotyledon 10. Pigment layer

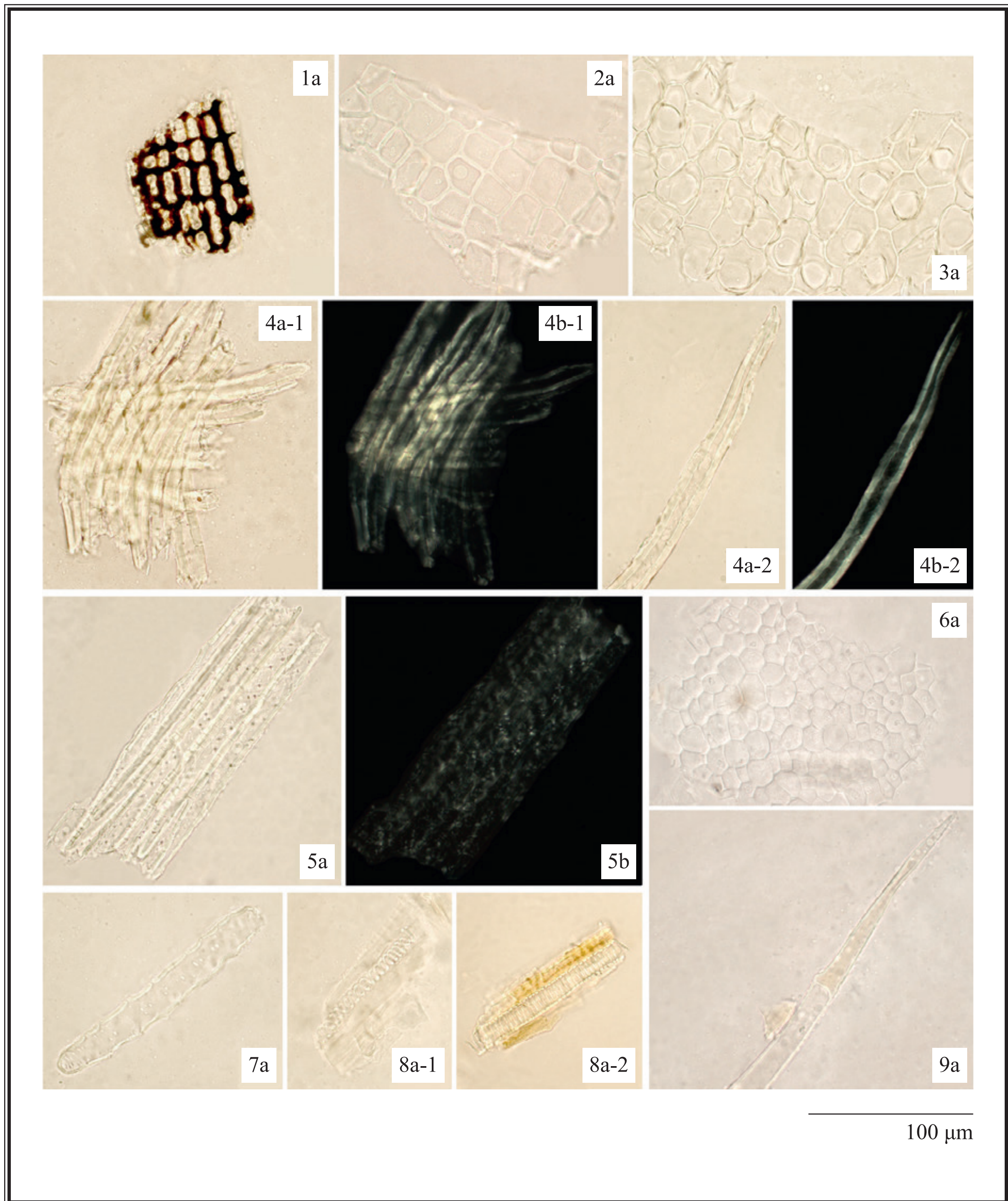

Figure 3 Microscopic features of powder of Xanthii Fructus

1. Epidermal cells of pericarp 2. Epidermal cells of testa 3. Endotesta cells 4. Involucre fibres 5. Pericarp fibres 6. Cotyledon cells 7. Lignified parenchymatous cell 8. Vessels (8-1 spiral vessel, 8-2 reticulate vessel) 9. Non-glandular hair

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

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

Standard solutions

Chlorogenic acid standard solution

Weigh 0.5 mg of chlorogenic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

1,5-Dicaffeoylquinic acid standard solution

Weigh 0.5 mg of 1,5-dicaffeoylquinic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of n-butyl acetate, water and formic acid (7:2.5:2.5, v/v). Freshly prepare and use the upper layer.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol (70%). 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 chlorogenic acid standard solution, 1,5-dicaffeoylquinic acid 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. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of chlorogenic acid and 1,5-dicaffeoylquinic acid.

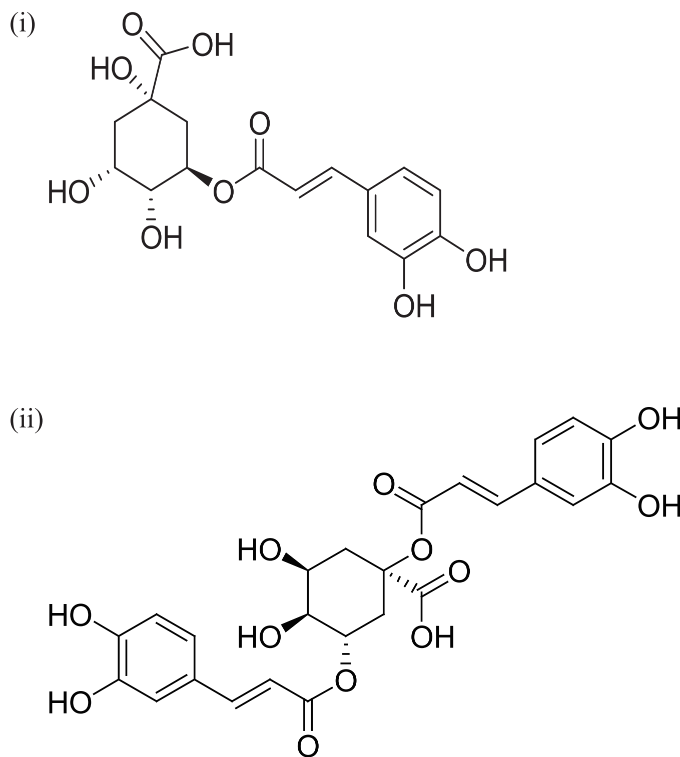

Figure 4 Chemical structures of (i) chlorogenic acid and (ii) 1,5-dicaffeoylquinic acid

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

Standard solutions

Chlorogenic acid standard solution for fingerprinting, Std-FP (80 mg/L)

Weigh 2.0 mg of chlorogenic acid CRS and dissolve in 25 mL of methanol (60%).

1,5-Dicaffeoylquinic acid standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of 1,5-dicaffeoylquinic acid CRS and dissolve in 50 mL of methanol (60%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (60%). Combine the solutions and make up to the mark with methanol (60%). 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 column temperature is maintained at 40 ºC 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 – 15	90 → 80	10 → 20	linear gradient
15 – 30	80 → 60	20 → 40	linear gradient
30 – 45	60	40	isocratic
45 – 60	60 → 20	40 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of chlorogenic acid Std-FP and 1,5-dicaffeoylquinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and 1,5-dicaffeoylquinic acid should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and 1,5-dicaffeoylquinic acid peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and 1,5-dicaffeoylquinic acid peaks should not be less than 70000 and 90000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject chlorogenic acid Std-FP, 1,5-dicaffeoylquinic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of chlorogenic acid and 1,5-dicaffeoylquinic acid peaks in the chromatograms of chlorogenic acid Std-FP, 1,5-dicaffeoylquinic acid Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify chlorogenic acid and 1,5-dicaffeoylquinic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chlorogenic acid Std-FP and 1,5-dicaffeoylquinic acid Std-FP. The retention times of chlorogenic acid and 1,5-dicaffeoylquinic acid peaks in the chromatograms of the test solution and the corresponding Std-FP 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 Xanthii Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Xanthii Fructus extract

Peak No.	RRT	Acceptable Range
1	0.62	± 0.05
2 (marker, chlorogenic acid)	1.00	-
3	1.07	± 0.03
4	1.53	± 0.03
5 (1,5-dicaffeoylquinic acid)	1.58	± 0.03

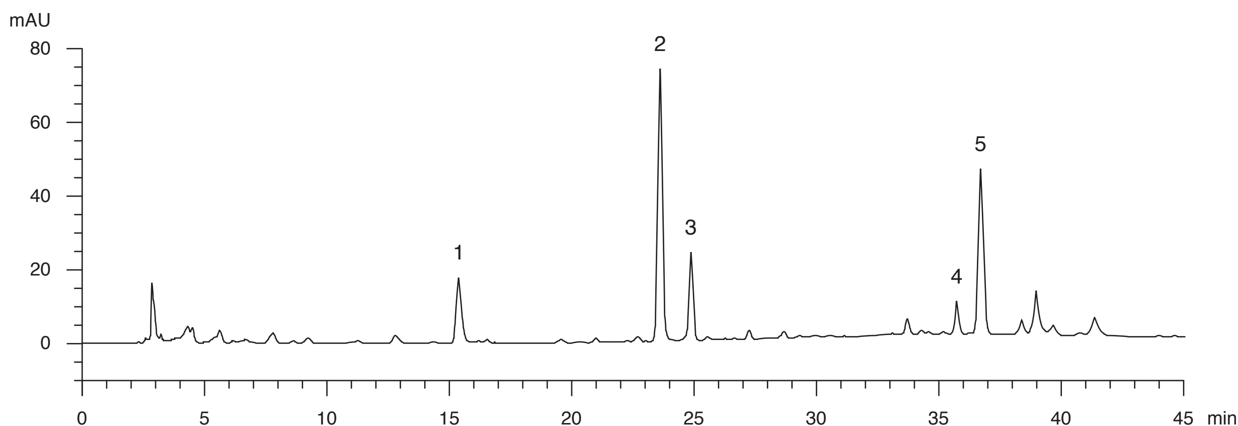

Figure 5 A reference fingerprint chromatogram of Xanthii Fructus extract

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. 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 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 7.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution

Mixed chlorogenic acid and 1,5-dicaffeoylquinic acid standard stock solution, Std-Stock (240 mg/L for chlorogenic acid and 80 mg/L for1,5-dicaffeoylquinic acid)

Weigh accurately 6.0 mg of chlorogenic acid CRS and 2.0 mg of 1,5-dicaffeoylquinic acid CRS, and dissolve in 25 mL of methanol (60%).

Mixed chlorogenic acid and 1,5-dicaffeoylquinic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed chlorogenic acid and 1,5-dicaffeoylquinic acid Std-Stock, dilute with methanol (60%) to produce a series of solutions of 15, 30, 60, 120, 240 mg/L for chlorogenic acid and 5, 10, 20, 40, 80 mg/L for 1,5-dicaffeoylquinic acid.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (60%). Combine the solutions and make up to the mark with methanol (60%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (327 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 40 ºC 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 – 15	90 → 80	10 → 20	linear gradient
15 – 30	80 → 60	20 → 40	linear gradient
30 – 45	60	40	isocratic
45 – 60	60 → 20	40 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed chlorogenic acid and 1,5-dicaffeoylquinic acid Std-AS (60 mg/L for chlorogenic acid and 20 mg/L for 1,5-dicaffeoylquinic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and 1,5-dicaffeoylquinic acid should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and 1,5-dicaffeoylquinic acid peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and 1,5-dicaffeoylquinic acid peaks should not be less than 70000 and 90000 theoretical plates respectively.

The *R* value between chlorogenic acid peak and the closest peak; and the *R* value between 1,5-dicaffeoylquinic acid 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 chlorogenic acid and 1,5-dicaffeoylquinic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of chlorogenic acid and 1,5-dicaffeoylquinic acid against the corresponding concentrations of the mixed chlorogenic acid and 1,5-dicaffeoylquinic acid 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 chlorogenic acid and 1,5-dicaffeoylquinic acid peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed chlorogenic acid and 1,5-dicaffeoylquinic acid Std-AS. The retention times of chlorogenic acid and 1,5-dicaffeoylquinic acid peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of chlorogenic acid and 1,5-dicaffeoylquinic acid in the test solution, and calculate the percentage contents of chlorogenic acid and 1,5-dicaffeoylquinic acid in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.43% of the total content of chlorogenic acid ($C_{16}H_{18}O_9$) and 1,5-dicaffeoylquinic acid ($C_{25}H_{24}O_{12}$), calculated with reference to the dried substance.