Cnidii Fructus

Figure 1 A photograph of Cnidii Fructus

1. NAMES

Official Name: Cnidii Fructus

Chinese Name: 蛇床子

Chinese Phonetic Name: Shechuangzi

2. SOURCE

Cnidii Fructus is the dried ripe fruit of *Cnidium monnieri* (L.) Cuss. (Apiaceae). The ripe fruit is collected during summer or autumn, followed by removal of the foreign matter, then dried under the sun to obtain Cnidii Fructus.

3. DESCRIPTION

Cremocarp ellipsoid, 1.5-4 mm long, 1-3 mm in diameter. Externally greyish-yellow to greyish-brown or yellowish-brown; stylopoida 2, curved outward at the top; occasionally with a small fruit stalk at the base. Dorsal side of the mericarps consists of 5 thin and prominent longitudinal ridges; commissures flat, with 2 brown and slightly raised longitudinal ribs, each in a furrow. Pericarp brittle, easily fallen off when rubbed. Seeds small, greyish-brown, oleaginous. Odour fragrant; taste pungent, cool and tongue-numbing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of flat cells, in a degenerative state, covered with cuticles. Mesocarp relatively thick, ridges prominent, consisting of vittae, reticulated cells, and vascular bundles. Vascular bundles are located in the middle of the ridges, surrounded by reticulated cells. There are 6 vittae, including 1 vitta between each 2 ridges and 2 vittae at commissural surface; vittae relatively large, oblong in shape. Endocarp consists of 1-2 layers of flat inlaid cells, rectangle in shape. Testa consists of 1 layer of cells, in a degenerative state and a few rows of cells in commissure with a small vascular bundle in the raphe. Endosperm contains numerous aleurone granules and fine cluster crystals or subprisms of calcium oxalate (Fig. 2).

Powder

Colour yellowish-brown. Inlaid (endocarp) cells yellowish-brown to reddish-brown, subsquare to polygonal, cell walls bead-like thickened. Endosperm cells polygonal to ovate in shape, containing aleurone granules and small clusters of calcium oxalate, 3-6 μm in diameter; polychromatic under the polarized microscope. Reticulated cells colourless to pale yellow, subsquare to subrounded, 10-34 μm in diameter, with reticulated striations; walls slightly thickened, non-lignified to slightly lignified. Vessel elements are mainly spiral, 4-19 μm in diameter. Vittae mostly dismembered, fragments of vittae yellowish-brown to reddish-brown in colour; septa can be observed occasionally, cellular trace indistinct on the surface, with subglobular oil droplets inside (Fig. 3).

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

Standard solutions

Bergapten standard solution

Weigh 1.0 mg of bergapten CRS (Fig. 4) and dissolve in 1 mL of methanol.

Imperatorin standard solution

Weigh 1.0 mg of imperatorin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Osthole standard solution

Weigh 2.0 mg of osthole CRS (Fig. 4) and dissolve in 1 mL of methanol.

Xanthotoxin standard solution

Weigh 1.0 mg of xanthotoxin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of methanol, water and glacial acetic acid (70∶30∶5, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 5 mL of methanol. Sonicate (220 W) the mixture for 10 min. Filter the mixture.

Procedure

Carry out the method by using a TLC RP-18 F_{254} (2-10 μm) plate and a freshly prepared developing solvent system as described above. Apply separately bergapten standard solution (2 μL), imperatorin standard solution (2 μL), osthole standard solution (4 μL), xanthotoxin standard solution (2 μL) and the test solution (2 μL) to the plate. Develop over a path of about 8 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).

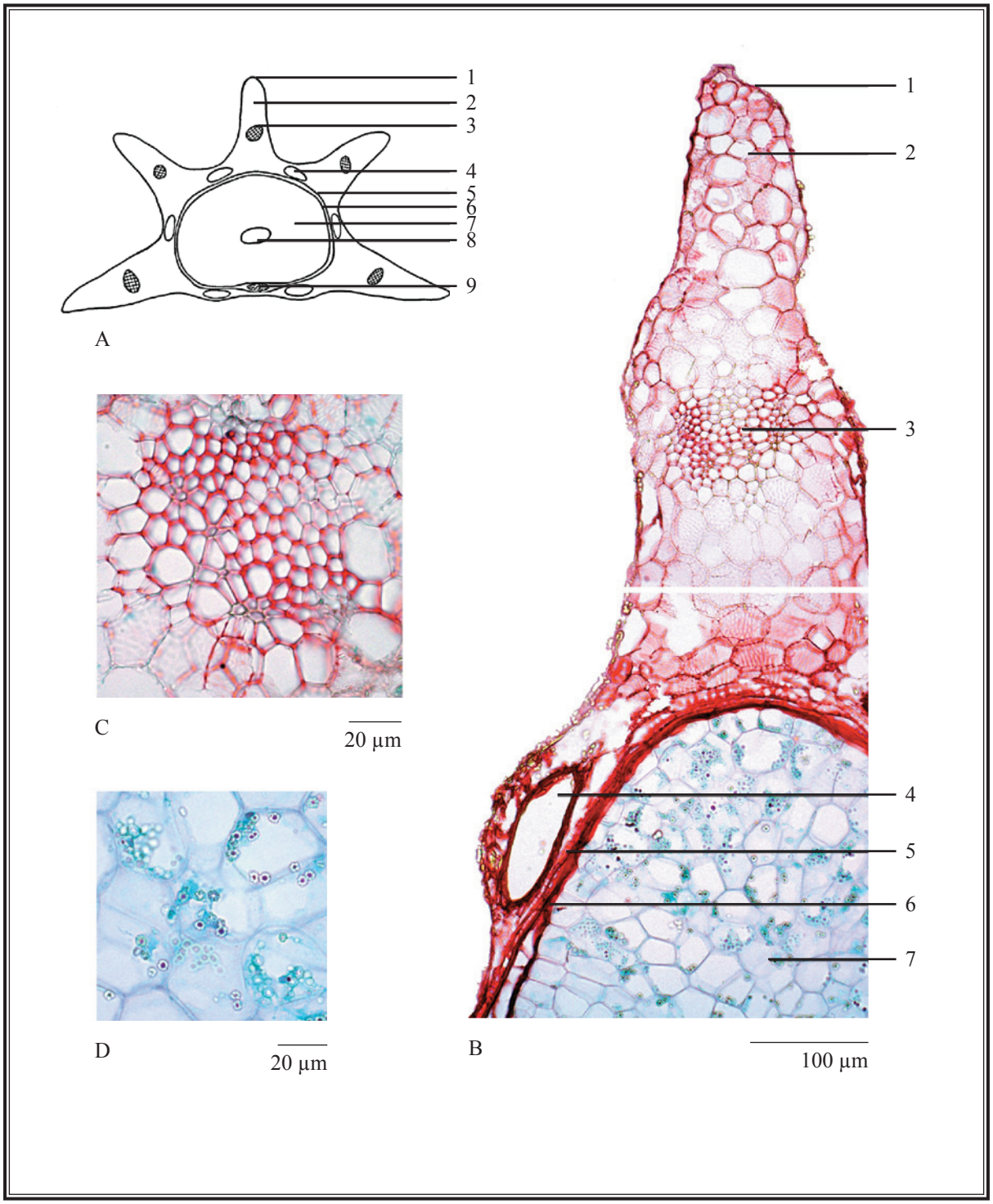


Figure 2 Microscopic features of transverse section of Cnidii Fructus

A. Sketch B. Section illustration C. Vascular bundle D. Endosperm

1. Exocarp 2. Mesocarp 3. Vascular bundles 4. Vitta 5. Endocarp 6. Testa
7. Endosperm 8. Embryo 9. Raphe vascular bundle

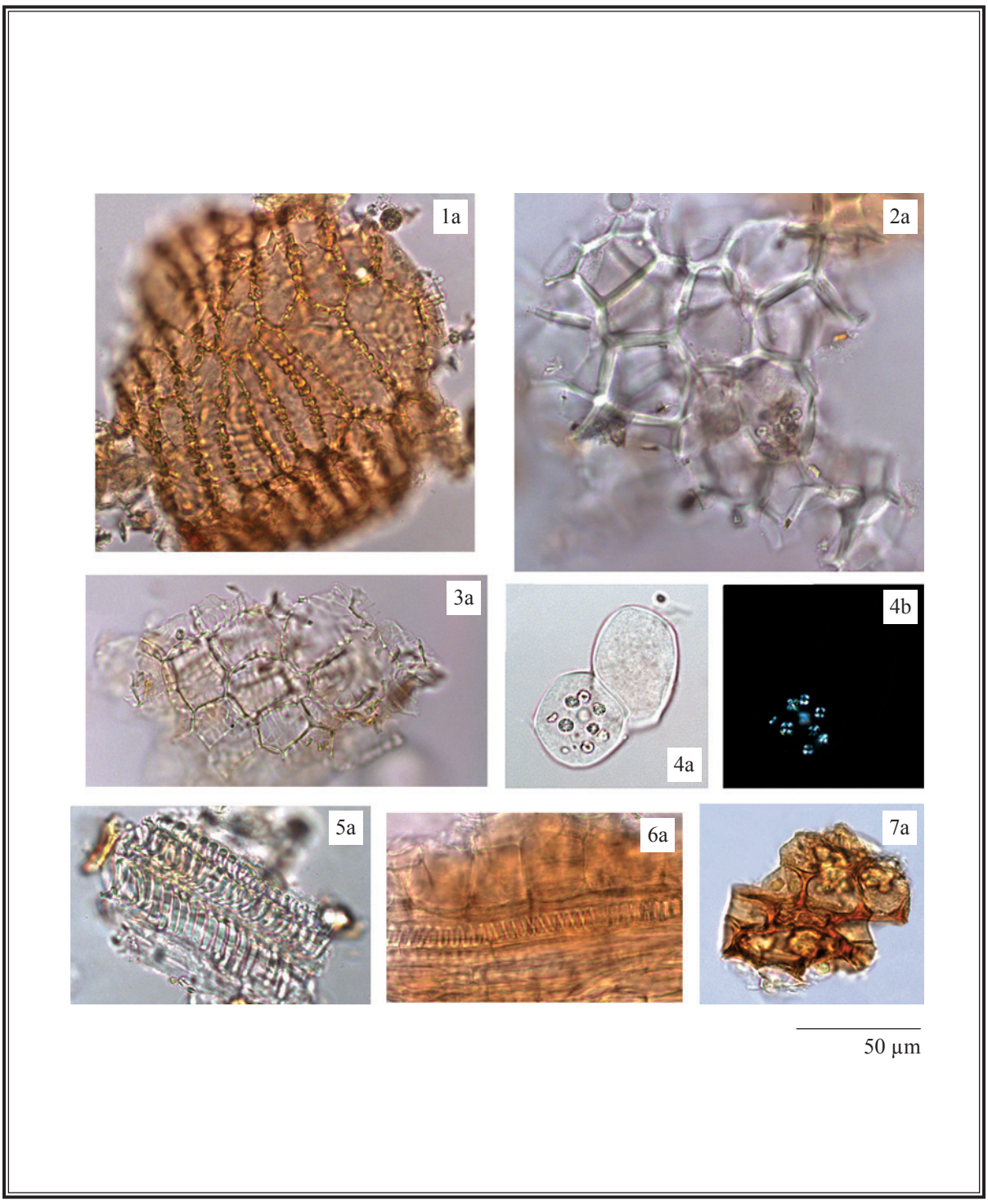


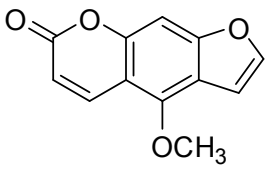
Figure 3 Microscopic features of powder of Cnidii Fructus

1. Inlaid (endocarp) cells 2. Endosperm cells 3. Reticulated cells
4. Endosperm cells (containing aleurone granules and clusters of calcium oxalate)
5, 6. Spiral vessels 7. Vitta

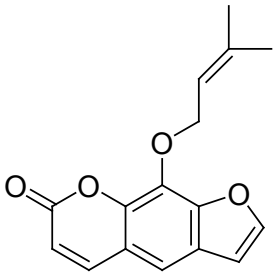
a. Features under the light microscope b. Features under the polarized microscope

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 bergapten, imperatorin, osthole and xanthotoxin.

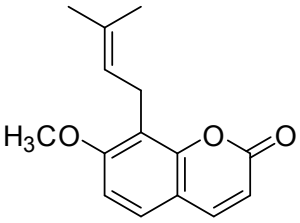
(i)



(ii)



(iii)



(iv)

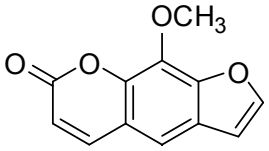


Figure 4 Chemical structures of (i) bergapten (ii) imperatorin (iii) osthole and (iv) xanthotoxin

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

Standard solutions

Bergapten standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of bergapten CRS and dissolve in 10 mL of methanol.

Imperatorin standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of imperatorin CRS and dissolve in 10 mL of methanol.

Osthole standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of osthole CRS and dissolve in 10 mL of methanol.

Xanthotoxin standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of xanthotoxin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. 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 (310 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)	Methanol (%, v/v)	Water (%, v/v)	Elution
0 – 40	40 → 60	60 → 40	linear gradient
40 – 60	60 → 80	40 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of bergapten Std-FP, imperatorin Std-FP, osthole Std-FP and xanthotoxin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bergapten, imperatorin, osthole and xanthotoxin should not be more than 5.0%; the RSD of the retention times of bergapten, imperatorin, osthole and xanthotoxin peaks should not be more than 2.0%; the column efficiencies determined from bergapten, imperatorin, osthole and xanthotoxin peaks should not be less than 30000, 95000, 150000 and 20000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; the *R* value between peak 4 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. The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1 (xanthotoxin)	0.49	± 0.03
2	0.63	± 0.03
3 (bergapten)	0.68	± 0.03
4 (marker, imperatorin)	1.00	-
5 (osthole)	1.11	± 0.03

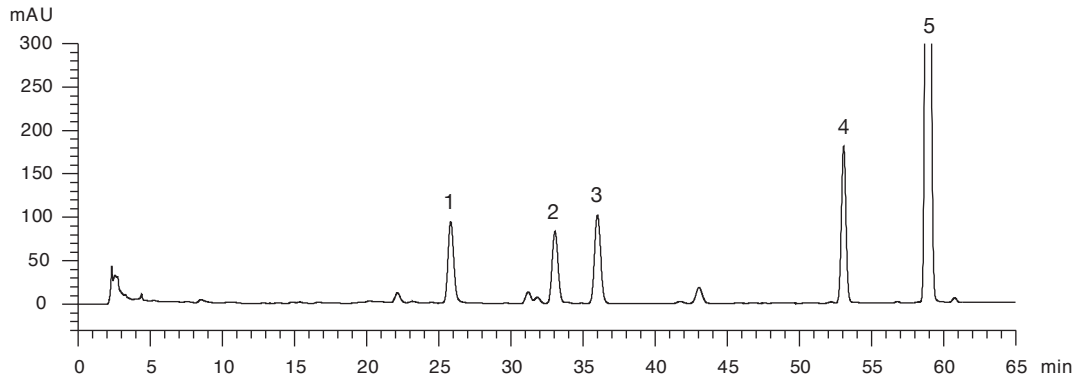


Figure 5 A reference fingerprint chromatogram of Cnidii Fructus extract

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 XVIII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 13.0%.
Acid-insoluble ash: not more than 6.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

Mixed bergapten, imperatorin, osthole and xanthotoxin standard stock solution, *Std-Stock* (5 mg/L for bergapten, 25 mg/L for imperatorin, 50 mg/L for osthole and 5 mg/L for xanthotoxin)

Weigh accurately 0.25 mg of bergapten CRS, 1.25 mg of imperatorin CRS, 2.5 mg of osthole CRS and 0.25 mg of xanthotoxin CRS and dissolve in 50 mL of methanol.

Mixed bergapten, imperatorin, osthole and xanthotoxin standard solution for assay, Std-AS

Measure accurately the volume of the mixed bergapten, imperatorin, osthole and xanthotoxin Std-Stock, dilute with methanol to produce a series of solutions of 0.25, 0.5, 1.0, 2.5, 5.0 mg/L for bergapten, 1.25, 2.5, 5.0, 12.5, 25 mg/L for imperatorin, 2.5, 5.0, 10, 25, 50 mg/L for osthole and 0.25, 0.5, 1.0, 2.5, 5.0 mg/L for xanthotoxin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (220 W) the mixture for 30 min. Centrifuge at about 3200 × *g* for 5 min. Filter the supernatant to a 100-mL volumetric flask. Repeat the extraction for four more times. Combine the filtrates 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 (250 nm for bergapten, imperatorin and xanthotoxin; 322 nm for osthole) 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	70 → 10	30 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed bergapten, imperatorin, osthole and xanthotoxin Std-AS (1 mg/L for bergapten, 5 mg/L for imperatorin, 10 mg/L for osthole and 1 mg/L for xanthotoxin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bergapten, imperatorin, osthole and xanthotoxin should not be more than

5.0%; the RSD of the retention times of bergapten, imperatorin, osthole and xanthotoxin peaks should not be more than 2.0%; the column efficiencies determined from bergapten, imperatorin, osthole and xanthotoxin peaks should not be less than 20000, 75000, 80000 and 25000 theoretical plates respectively.

The *R* value between bergapten peak and the closest peak; the *R* value between imperatorin peak and the closest peak; the *R* value between osthole peak and the closest peak; and the *R* value between xanthotoxin 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 bergapten, imperatorin, osthole and xanthotoxin Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of bergapten, imperatorin, osthole and xanthotoxin against the corresponding concentrations of the mixed bergapten, imperatorin, osthole and xanthotoxin Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify bergapten, imperatorin, osthole and xanthotoxin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed bergapten, imperatorin, osthole and xanthotoxin Std-AS. The retention times of bergapten, imperatorin, osthole and xanthotoxin 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 bergapten, imperatorin, osthole and xanthotoxin in the test solution, and calculate the percentage contents of bergapten, imperatorin, osthole and xanthotoxin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.13% of the total content of bergapten ($C_{12}H_8O_4$) and xanthotoxin ($C_{12}H_8O_4$); not less than 0.42% of imperatorin ($C_{16}H_{14}O_4$); and not less than 1.4% of osthole ($C_{15}H_{16}O_3$), calculated with reference to the dried substance.