Celosiae Semen

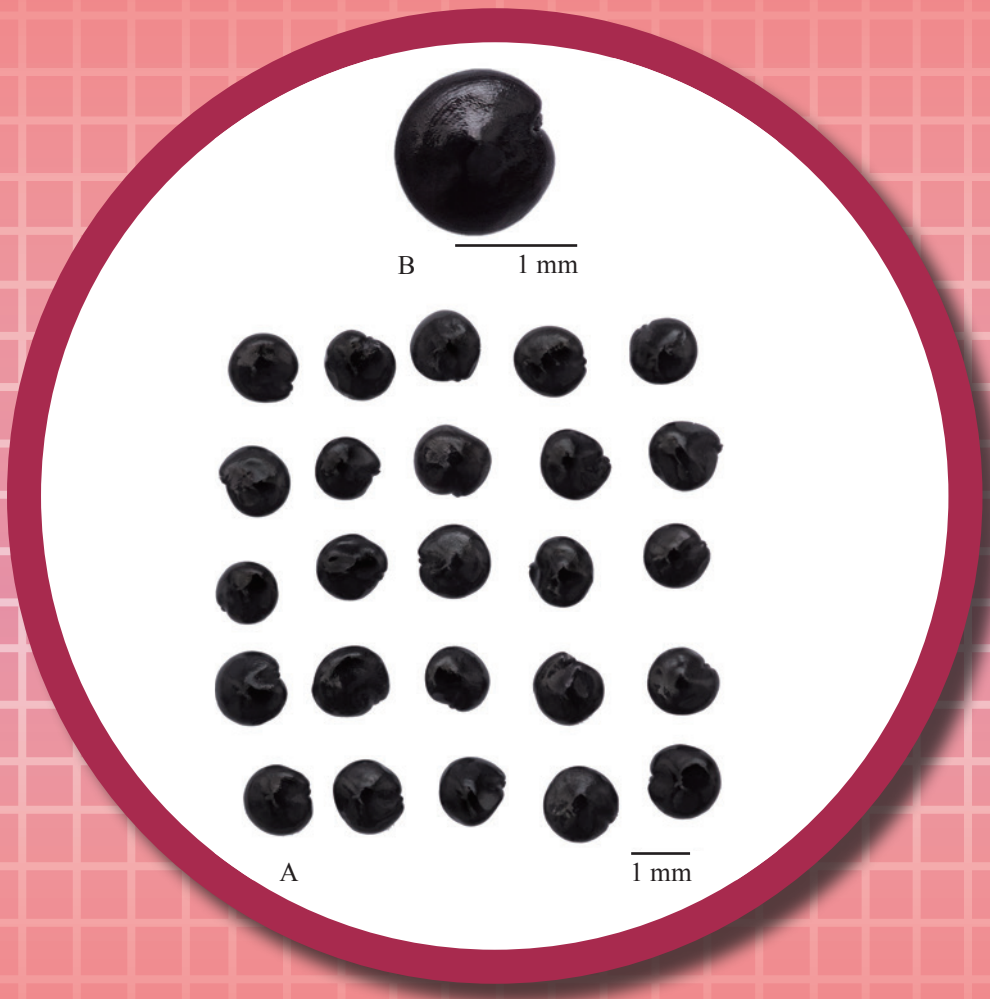

Figure 1 A photograph of Celosiae Semen

A. Celosiae Semen B. Magnified image of ripe seeds

1. NAMES

Official Name: Celosiae Semen

Chinese Name: 青葙子

Chinese Phonetic Name: Qingxiangzi

2. SOURCE

Celosiae Semen is the dried ripe seed of *Celosia argentea* L. (Amaranthaceae). The plant is cut up or the infructescence is collected in autumn when the fruit is ripe, dried under the sun, and the seeds gathered, foreign matter removed to obtain Celosiae Semen.

3. DESCRIPTION

Oblate, seldom rounded-reniform, 0.8-1.2 mm in diameter. Externally black or reddish-black, lustrous, slightly protuberant at the centre, with an oblique and small acute hilum on the slightly dented lateral side. Testa thin and fragile, polygonal reticulate striations visible when magnified. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Outer epidermal cell walls of testa dark reddish-brown, prominently thickened. Inner layer cells of testa flat, colourless or pale yellow. Endosperm cells filled with starch granules, aleurone grains and prisms of calcium oxalate. Cotyledons and radicle consist of parenchymatous cells (Fig. 2).

Powder

Colour greyish-black. Outer epidermal cells of testa dark reddish-brown, polygonal to elongated-polygonal in surface view, with polygonal, grid-shaped and thickened striations. Inner layer cells of testa colourless or pale yellow, polygonal in surface view, with dense, fine and straight striations. Prisms of calcium oxalate present in endosperm cells, diamond-shaped, square or rectangular. Endosperm cells filled with starch granules, aleurone grains and oil droplets (Fig. 3).

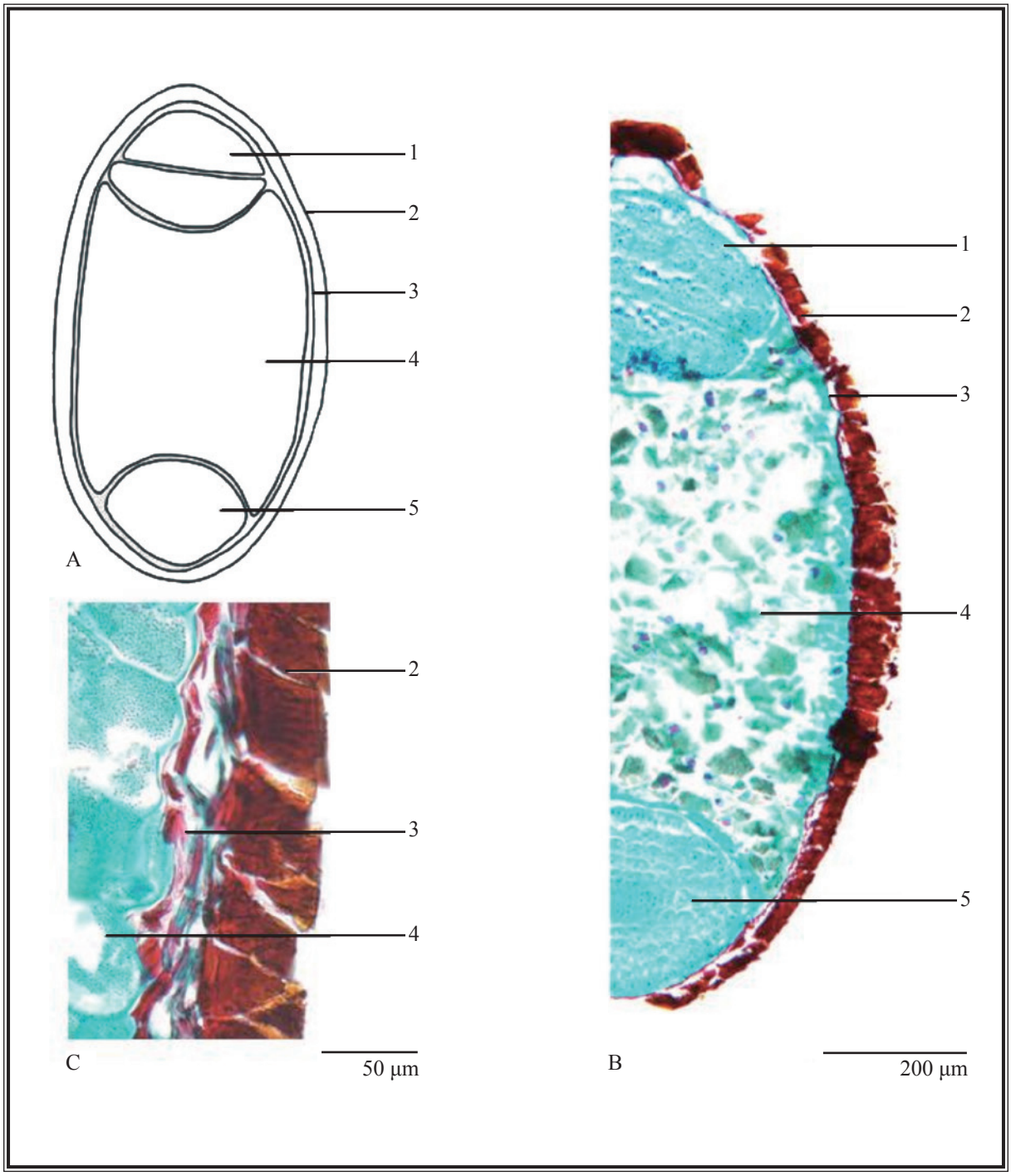

Figure 2 Microscopic features of transverse section of Celosiae Semen

A. Sketch B. Section illustration C. Section magnified

1. Cotyledon 2. Outer epidermis of testa 3. Inner layer cells of testa
4. Endosperm 5. Radicle

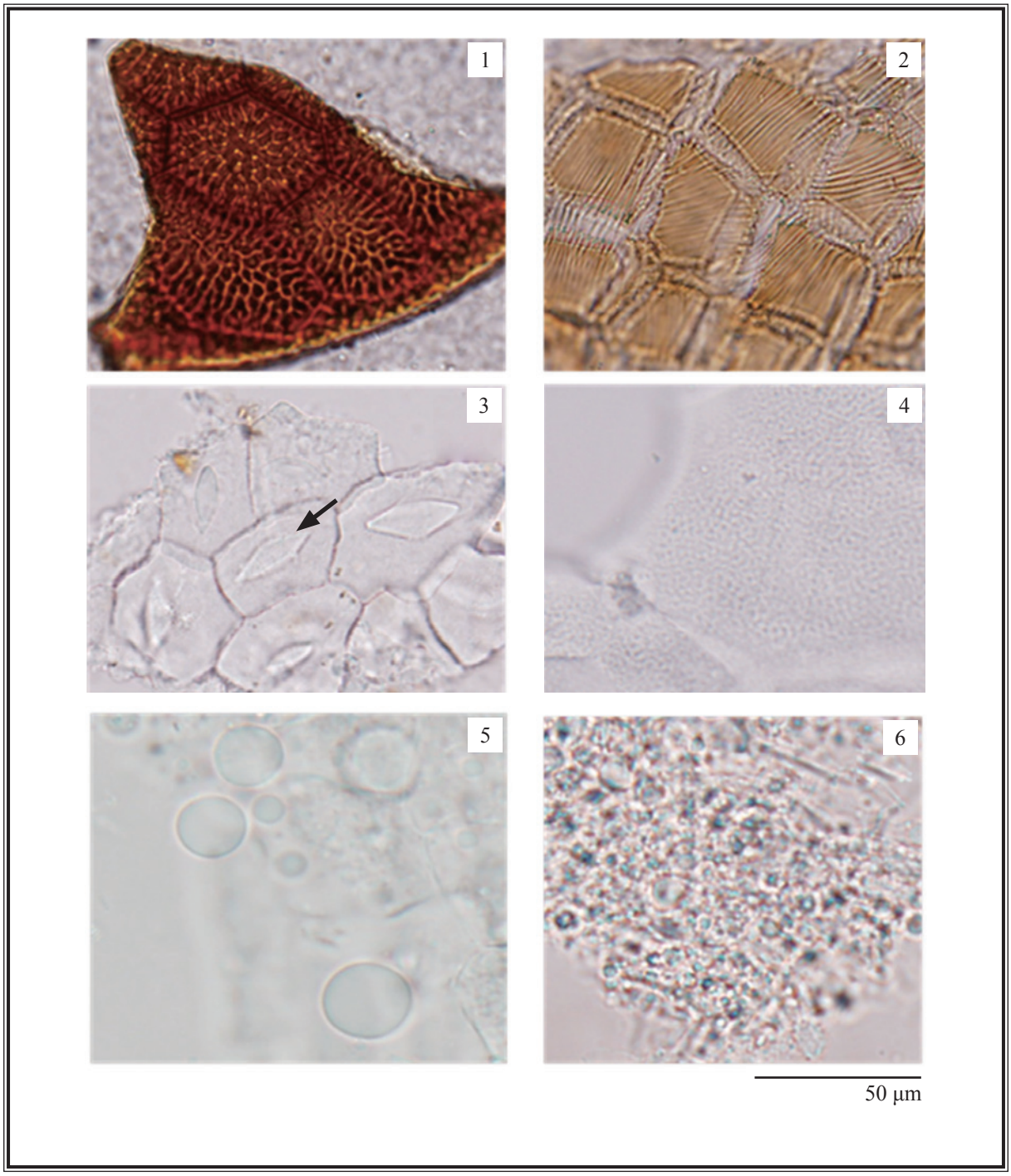

Figure 3 Microscopic features of powder of Celosiae Semen (under the light microscope)

1. Outer epidermal cells of testa 2. Inner layer cells of testa 3. Prisms of calcium oxalate (⟶)
4. Aleurone grains 5. Oil droplets 6. Starch granules

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

Standard solutions

Celosin H standard solution

Weigh 1.0 mg of celosin H CRS (Fig. 4) and dissolve in 1 mL of ethanol (50%).

Celosin I standard solution

Weigh 1.0 mg of celosin I CRS (Fig. 4) and dissolve in 1 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, methanol, water and formic acid (13:7:2:0.1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of ethanol (50%). Sonicate (250 W) the mixture for 30 min. Centrifuge at about 2280 × *g* for 10 min. Transfer the supernatant to a 150-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of water. Load the sample solution to the solid-phase extraction column containing ODS packing (12 mL, 2 g) pre-conditioned with 24 mL of methanol and 24 mL of water. Add 24 mL of water to the column. Discard the eluant. Add 24 mL of a mixture of ethanol and water (6:4, v/v) to the column. Collect the eluant and transfer to a 150-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of ethanol (50%).

Procedure

Carry out the method by using a HPTLC silica gel plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately celosin H standard solution (1 μL), celosin I standard solution (1 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 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 105ºC until the spots or bands become visible (about 15 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

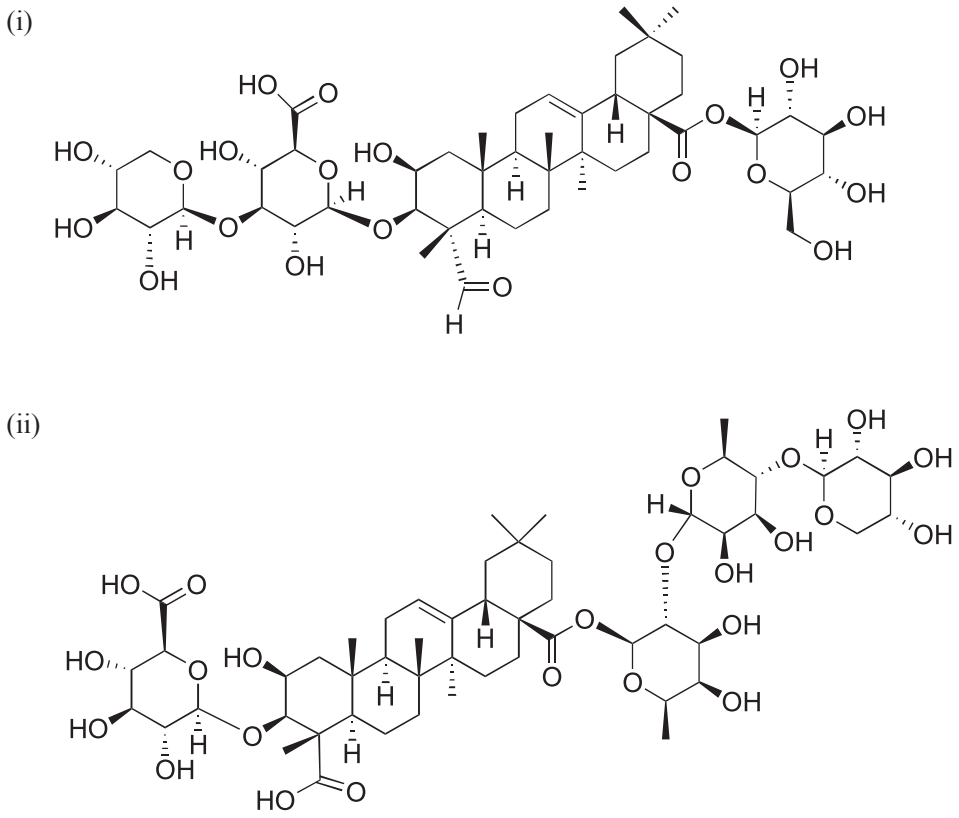

Figure 4 Chemical structures of (i) celosin H and (ii) celosin I

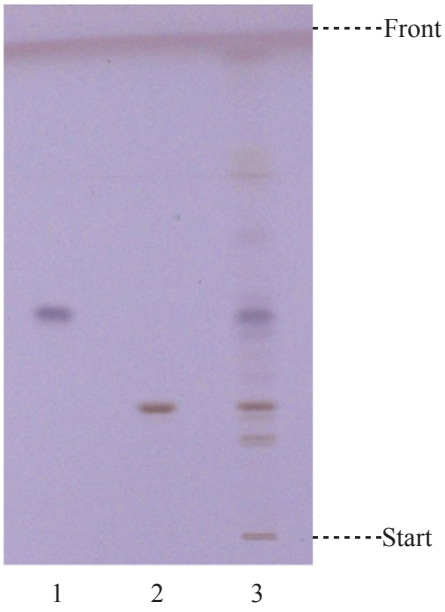

Figure 5 A reference HPTLC chromatogram of Celosiae Semen extract observed under visible light after staining

1. Celosin H standard solution 2. Celosin I standard solution 3. Test solution

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 celosin H and celosin I (Fig. 5).

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

Standard solutions

Celosin H standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of celosin H CRS and dissolve in 10 mL of ethanol (50%).

Celosin I standard solution for fingerprinting, Std-FP (120 mg/L)

Weigh 1.2 mg of celosin I CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 50 mL of ethanol (50%). Sonicate (250 W) the mixture for 30 min. Centrifuge at about 2280 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction with 30 mL of ethanol (50%). Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the solution to a 10-mL volumetric flask and make up to the mark with ethanol (50%). Filter through a 0.20-µm polyester filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 110ºC; nebulizer gas (N_2) flow rate: 2.5 L/min] and a column (4.6 × 150 mm) packed with ODS bonded silica gel (3 µm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.6 mL/min. The mobile phase is a mixture of 0.1% formic acid and acetonitrile with 0.1% formic acid (70:30, v/v). The elution time is about 45 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of celosin H Std-FP and celosin I Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of celosin H and celosin I should not be more than 5.0%; the RSD of the retention times of celosin H and celosin I peaks should not be more than 2.0%; the column efficiencies determined from celosin H and celosin I peaks should not be less than 25000 theoretical plates.

The *R* value between peak 3 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. 6).

Procedure

Separately inject celosin H Std-FP, celosin I Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of celosin H and celosin I peaks in the chromatograms of celosin H Std-FP, celosin I Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify celosin H and celosin I peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of celosin H Std-FP and celosin I Std-FP. The retention times of celosin H and celosin I 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 Celosiae Semen extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the five characteristic peaks of Celosiae Semen extract

Peak No.	RRT	Acceptable Range
1	0.55	± 0.03
2	0.65	± 0.03
3 (marker, celosin H)	1.00	-
4	1.24	± 0.03
5 (celosin I)	1.49	± 0.03

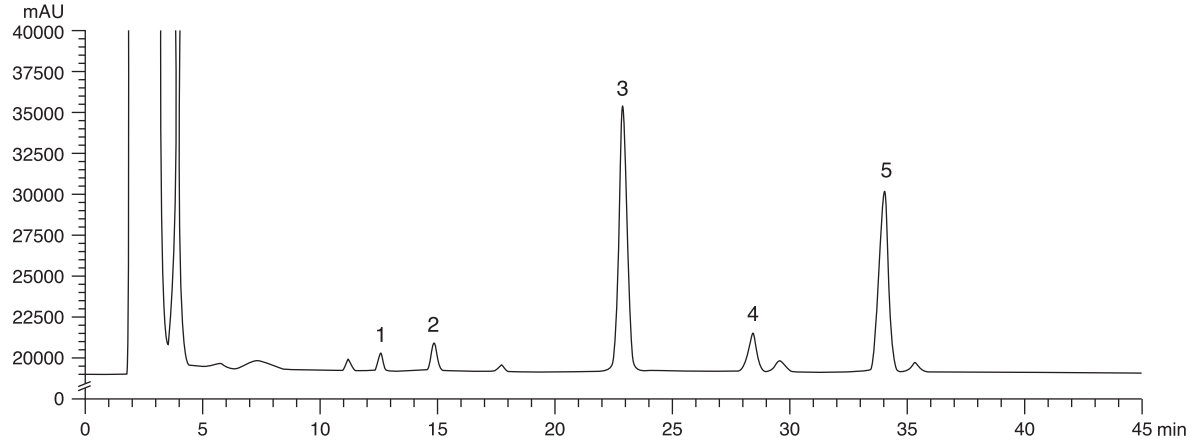

Figure 6 A reference fingerprint chromatogram of Celosiae Semen extract

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. 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 XVI)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.
Acid-insoluble ash: not more than 2.0%.

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 7.0%.
Ethanol-soluble extractives (cold extraction method): not less than 3.0%.

7. ASSAY

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

Standard solution

Mixed celosin H and celosin I standard stock solution, Std-Stock (1000 mg/L for celosin H and 1200 mg/L for celosin I)

Weigh accurately 10.0 mg of celosin H CRS and 12.0 mg of celosin I CRS, and dissolve in 10 mL of ethanol (50%).

Mixed celosin H and celosin I standard solution for assay, Std-AS

Measure accurately the volume of the mixed celosin H and celosin I Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 30, 50, 100, 320, 540 mg/L for celosin H and 35, 60, 120, 360, 600 mg/L for celosin I.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 50 mL of ethanol (50%). Sonicate (250 W) the mixture for 30 min. Centrifuge at about 2280 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction with 30 mL of ethanol (50%). Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the solution to a 10-mL volumetric flask and make up to the mark with ethanol (50%). Filter through a 0.20-μm polyester filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 110ºC; nebulizer gas (N_2) flow rate: 2.5 L/min] and a column (4.6 × 150 mm) packed with ODS bonded silica gel (3 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.6 mL/min. The mobile phase is a mixture of 0.1% formic acid and acetonitrile with 0.1% formic acid (70:30, v/v). The elution time is about 45 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed celosin H and celosin I Std-AS (100 mg/L for celosin H and 120 mg/L for celosin I). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of celosin H and celosin I should not be more than 5.0%; the RSD of the retention times of celosin H and celosin I peaks should not be more than 2.0%; the column efficiencies determined from celosin H and celosin I peaks should not be less than 25000 theoretical plates.

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

Limits

The sample contains not less than 0.23% of the total content of celosin H ($C_{47}H_{72}O_{20}$) and celosin I ($C_{53}H_{82}O_{24}$), calculated with reference to the dried substance.