Semen Cassiae

Figure 1(i) A photograph of dried ripe seed of *Cassia obtusifolia* L.

Figure 1(ii) A photograph of dried ripe seed of *Cassia tora* L.

1. NAMES

Official Name: Semen Cassiae

Chinese Name: 決明子

Chinese Phonetic Name: Juemingzi

2. SOURCE

Semen Cassiae is the dried ripe seed of *Cassia obtusifolia* L. or *Cassia tora* L. (Fabaceae). The ripe legume is collected in autumn and dried under the sun, then the seed is tapped out and foreign matter removed to obtain Semen Cassiae.

3. DESCRIPTION

***Cassia obtusifolia* L.:** Slightly rhomboid-cuboidal, short-cylindrical or tapered-cuboidal in shape, 3-7 mm in length, 1.5-4 mm in width, both ends parallel and slanting, one end truncate, the other acuminate. Externally greenish-brown to dark brown, smooth and lustrous, with a light yellowish-brown longitudinal line or band on each side. Texture hard, not easily broken; testa thin. Odour slight; taste slightly bitter [Fig. 1(i)].

***Cassia tora* L.:** Short-cylindrical to tapered-cuboidal in shape, externally usually brown, 2-6 mm in length, and 1-3 mm in width. In general, the size is somewhat smaller than that of *C. obtusifolia* L. [Fig. 1(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Testa thin. Cuticle smooth, transparent and thick. Palisade cells in 1 layer, palisade-shaped, showing 2 distinct luciferous bands. Brace or prop cells in 1 layer, nearly dumbbell-shaped, wall thick, with large intercellular spaces. Nutritive layer consists of 6-8 rows of parenchyma cells. Perisperm tissue greyish-white, translucent, rich in aleurone granules. Cotyledonous tissue is made up of 2 patches, S-shaped, with the parenchyma cells rich in cluster of calcium oxalate [Fig. 2(i) and (ii)].

Powder

Colour yellowish-brown. Palisade cells in groups, colourless or pale yellowish, arranged in 1 row, when viewed laterally narrow-rectangular in shape, 50-113 μm in length, with thick walls, the cell lumina minute, in surface view sub-polygonal. Brace cells dumbbell-shaped laterally, suborbicular or polygonal in surface view, with 2 concentric circles, 13-84 μm in diameter. Cluster of calcium oxalate numerous, scattered in the parenchyma cells, 9-44 μm in diameter [Fig. 3(i) and (ii)].

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

Standard solution

Aurantio-obtusin standard solution

Weigh 1.0 mg of aurantio-obtusin CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of *n*-hexane, isopropanol and formic acid (5:1:0.1, v/v).

Spray reagent

Dissolve 3.0 g sodium hydroxide in 100 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 20 min. Filter through a filter paper.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately aurantio-obtusin standard solution (2 μL) and the test solution (5 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 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).

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 aurantio-obtusin.

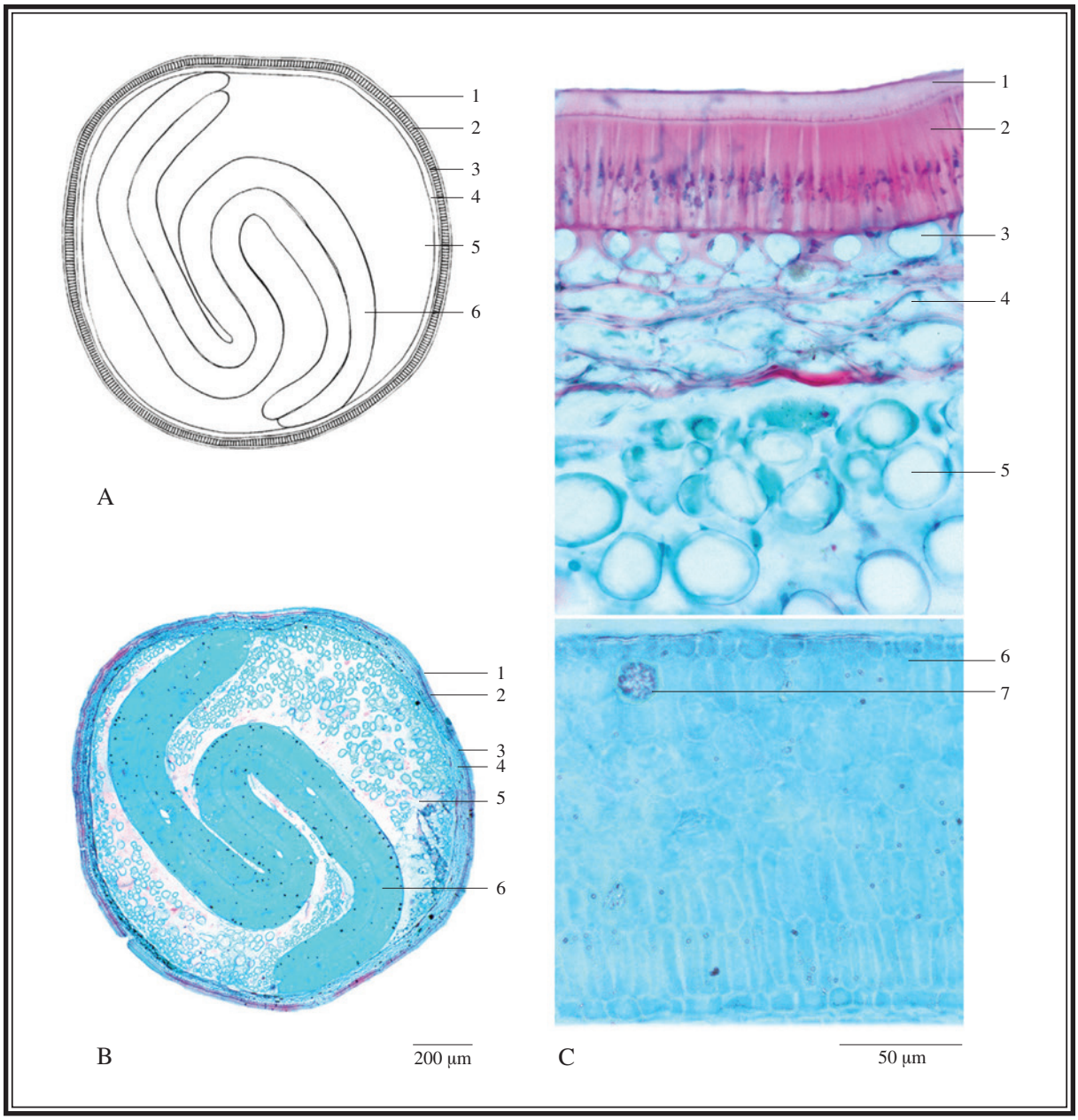

Figure 2 (i) Microscopic features of transverse section of dried ripe seed of *Cassia obtusifolia* L.

A. Sketch B-C. Section illustration

1. Cuticle 2. Palisade cells 3. Brace cells 4. Nutritive layer 5. Endosperm
6. Cotyledon 7. Cluster of calcium oxalate

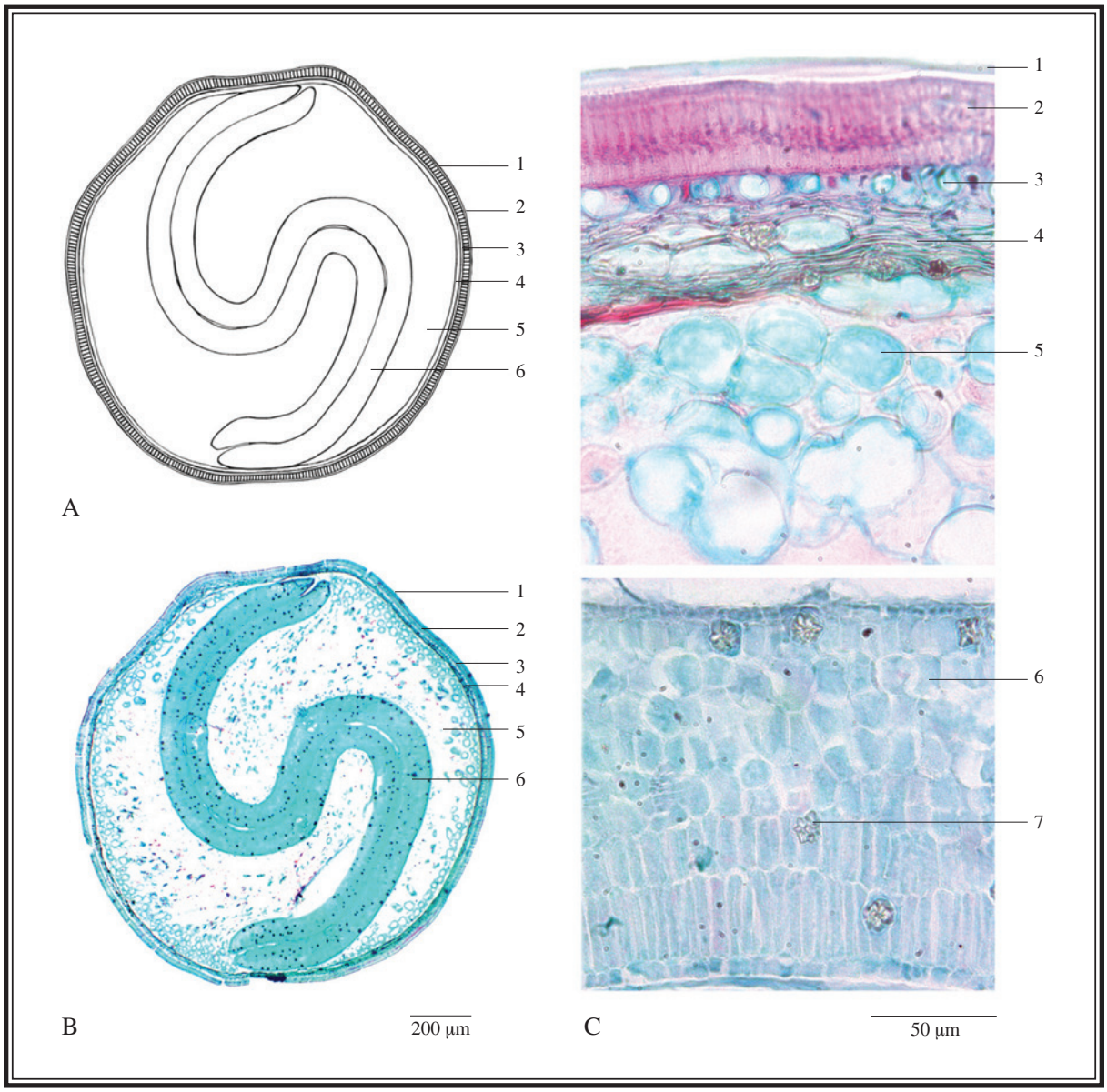

Figure 2 (ii) Microscopic features of transverse section of dried ripe seed of *Cassia tora* L.

A. Sketch B-C. Section illustration

1. Cuticle 2. Palisade cells 3. Brace cells 4. Nutritive layer 5. Endosperm
6. Cotyledon 7. Cluster of calcium oxalate

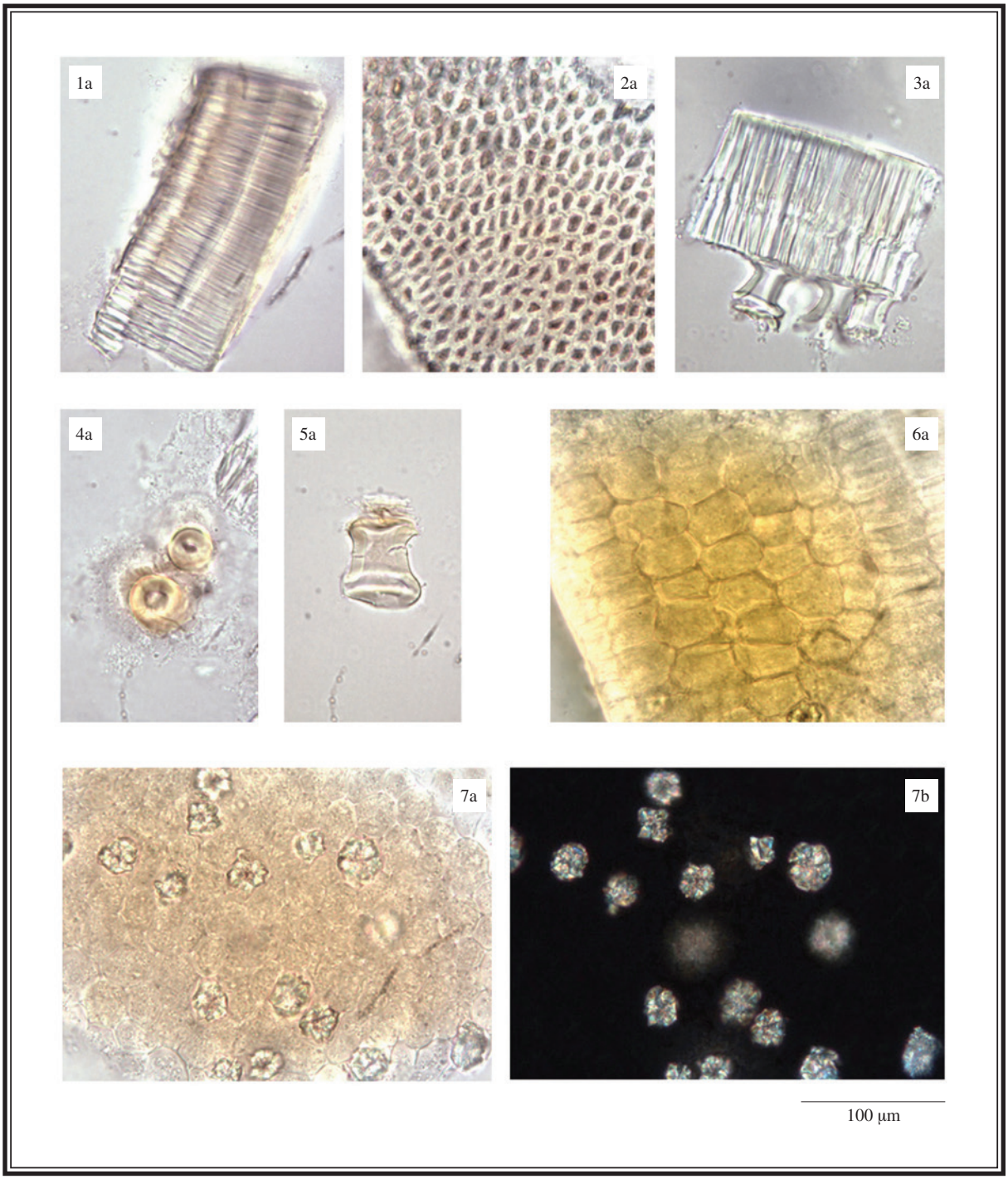

Figure 3 (i) Microscopic features of powder of dried ripe seed of *Cassia obtusifolia* L.

1. Palisade cells (lateral view) 2. Palisade cells (surface view)
3. Palisade cells and brace cells 4. Brace cells (surface view) 5. Brace cells (lateral view)
6. A fragment of cotyledon 7. Clusters of calcium oxalate

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

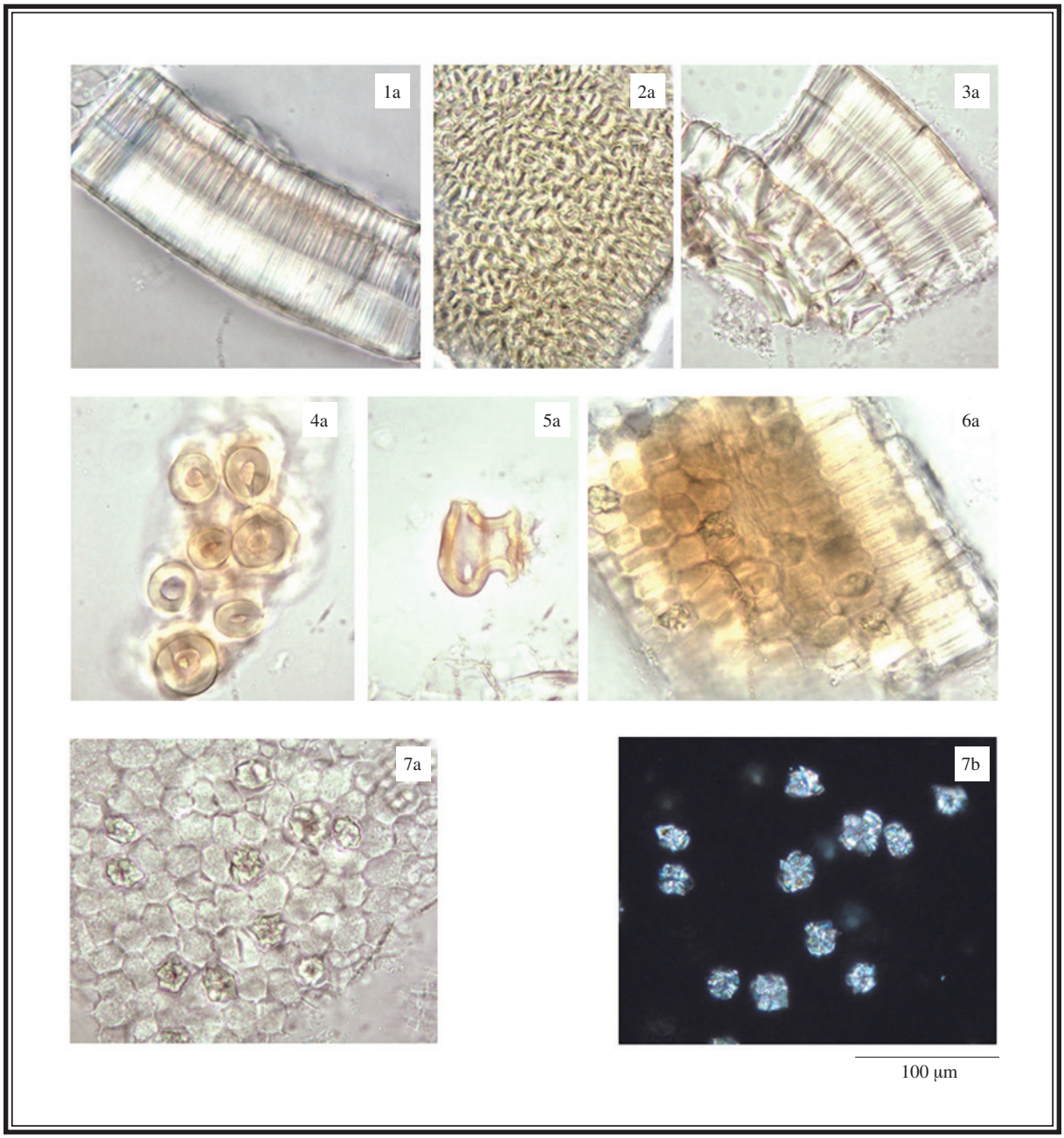

Figure 3 (ii) Microscopic features of powder of dried ripe seed of *Cassia tora* L.

1. Palisade cells (lateral view) 2. Palisade cells (surface view)
3. Palisade cells and brace cells 4. Brace cells (surface view) 5. Brace cells (lateral view)
6. A fragment of cotyledon 7. Clusters of calcium oxalate

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

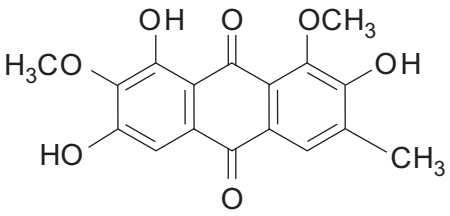

Figure 4 Chemical structure of aurantio-obtusin

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

Standard solution

Aurantio-obtusin standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of aurantio-obtusin CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (285 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)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	82→70	18→30	linear gradient
35 – 40	70→60	30→40	linear gradient
40 – 60	60→40	40→60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of aurantio-obtusin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of aurantio-obtusin should not be more than 5.0%; the RSD of the retention time of aurantio-obtusin peak should not be more than 2.0%; the column efficiency determined from aurantio-obtusin peak should not be less than 150000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 5(i) or (ii)].

Procedure

Separately inject aurantio-obtusin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of aurantio-obtusin peak in the chromatogram of aurantio-obtusin Std-FP and the retention times of the four characteristic peaks [Fig. 5(i) or (ii)] in the chromatogram of the test solution. Identify aurantio-obtusin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of aurantio-obtusin Std-FP. The retention times of aurantio-obtusin 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 Semen Cassiae extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Semen Cassiae extract

Peak No.	RRT	Acceptable Range
1	0.35	±0.03
2	0.40	±0.03
3	0.50	±0.03
4 (marker, aurantio-obtusin)	1.00	-

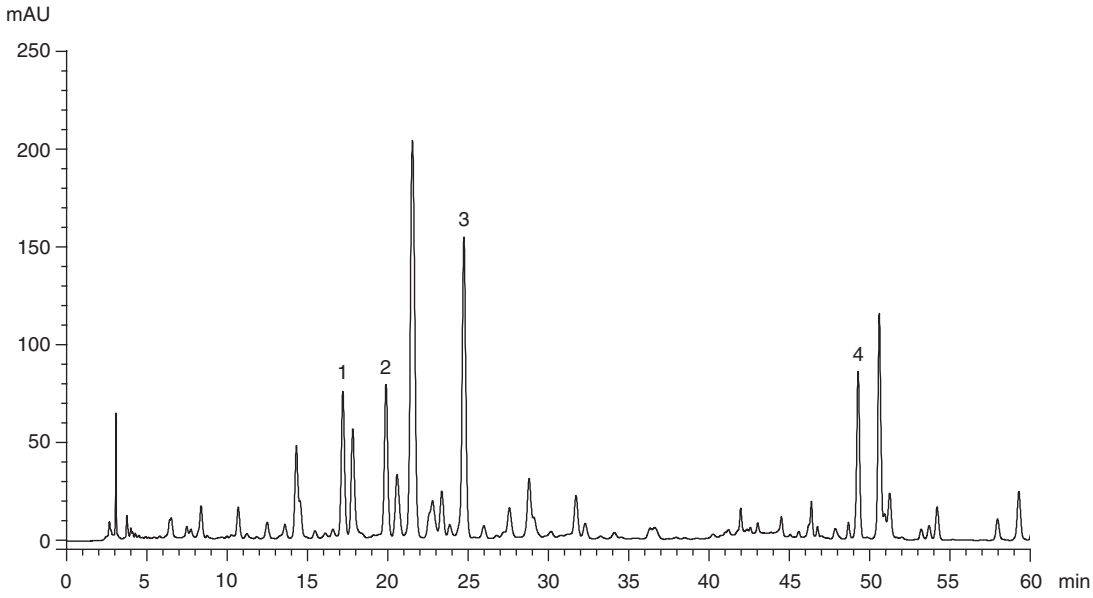

Figure 5(i) A reference fingerprint chromatogram of dried ripe seed of *Cassia obtusifolia* L. extract

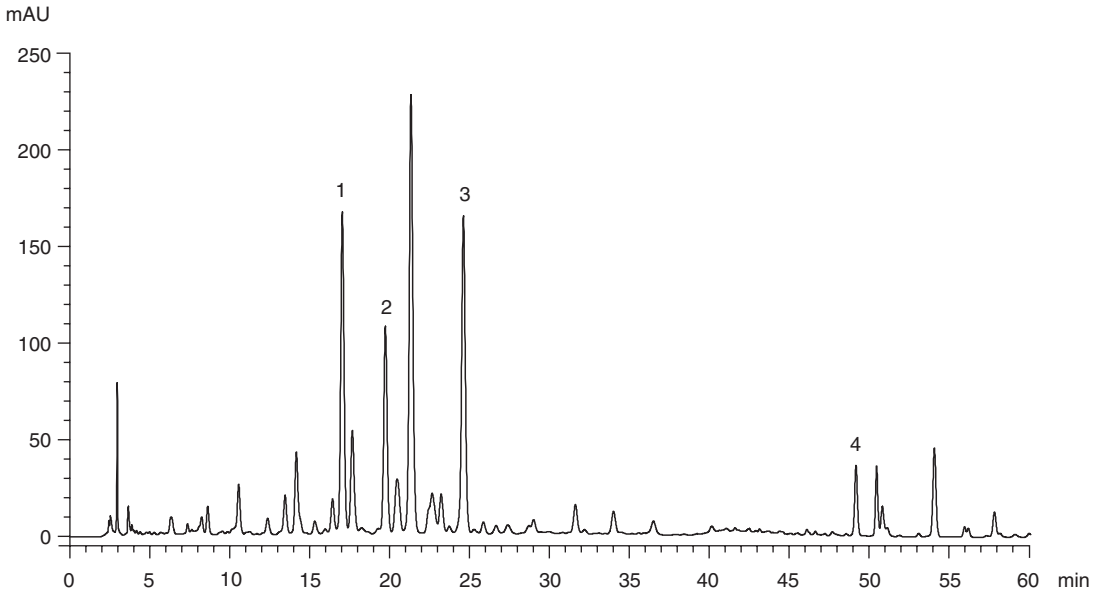

Figure 5(ii) A reference fingerprint chromatogram of dried ripe seed of *Cassia tora* L. extract

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 chromatograms [Fig. 5(i) or (ii)].

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 XV)*: 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 1.0%.

5.7 Water Content *(Appendix X)*: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 29.0%.
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

Aurantio-obtusin standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of aurantio-obtusin CRS and dissolve in 10 mL of ethanol (70%).

Aurantio-obtusin standard solution for assay, Std-AS

Measure accurately the volume of the aurantio-obtusin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 1, 4, 10, 15, 20 mg/L for aurantio-obtusin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the extracts and make up to the mark with ethanol (70%). Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (285 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. The mobile phase is a mixture of acetonitrile and 0.1% trifluoroacetic acid (33:67, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of aurantio-obtusin Std-AS (4 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of aurantio-obtusin should not be more than 5.0%; the RSD of the retention time of aurantio-obtusin peak should not be more than 2.0%; the column efficiency determined from aurantio-obtusin peak should not be less than 10000 theoretical plates.

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

Limits

The sample contains not less than 0.017% of aurantio-obtusin ($C_{17}H_{14}O_7$), calculated with reference to the dried substance.