Trichosanthis Semen

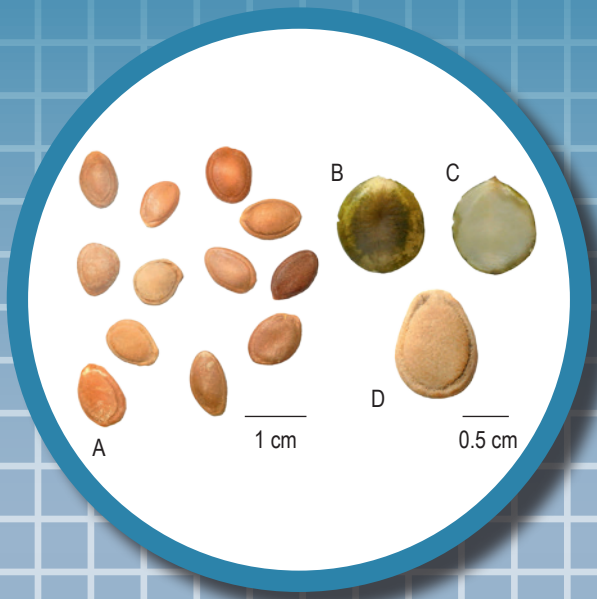

Figure 1 (i) A photograph of seed of *Trichosanthes kirilowii* Maxim.

A. Trichosanthis Semen B. Outer surface of cotyledon C. Inner surface of cotyledon
D. Magnified seed

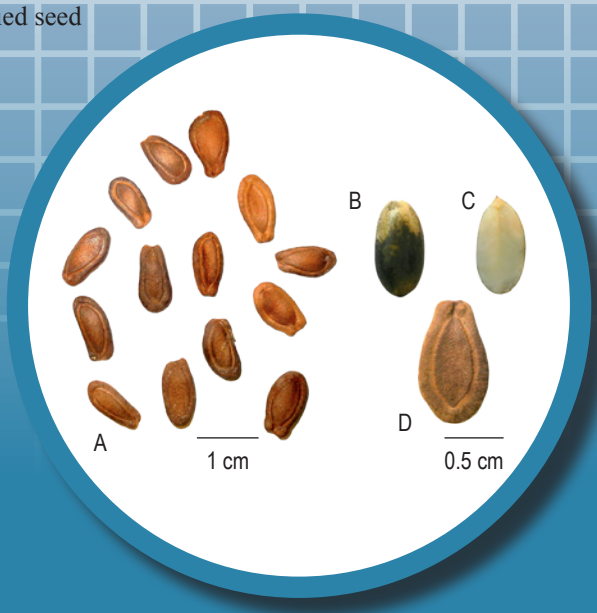

Figure 1 (ii) A photograph of seed of *Trichosanthes rosthornii* Harms

A. Trichosanthis Semen B. Outer surface of cotyledon C. Inner surface of cotyledon
D. Magnified seed

1. NAMES

Official Name: Trichosanthis Semen

Chinese Name: 瓜蔞子

Chinese Phonetic Name: Gualouzi

2. SOURCE

Trichosanthis Semen is the dried ripe seed of *Trichosanthes kirilowii* Maxim. or *Trichosanthes rosthornii* Harms (Cucurbitaceae). The ripe fruit is collected in autumn and dissected. The seed is gathered, washed clean, then dried under the sun to obtain Trichosanthis Semen.

3. DESCRIPTION

***Trichosanthes kirilowii* Maxim.:** Flattish-ellipsoidal, 1-1.8 cm long, 8-12 mm wide, 2-3.5 mm thick. Externally yellowish-green to brown, smooth, with a circle of furrow along the edge. Apex relatively acute, with a hilum, base rounded-obtuse or relatively narrow. Testa hard; tegmen membranous, greyish-green; cotyledons 2, yellowish-white, oily. Odour slight; taste weak [Fig. 1 (i)].

***Trichosanthes rosthornii* Harms:** Flattish-ellipsoidal to elliptic-rectangular, 1.4-1.9 cm long, 6-10 mm wide, 1.5-3 mm thick. Externally brown, the circular furrow around the edge prominent and somewhat closer to the central part. Apex even and truncated [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Testa consists of 1 layer of epidermal cells, the cells subrectangular, tangentially elongated, externally covered with cuticle. Sclerenchyma beneath the epidermis, consisting of 6-14 layers of lignified cells, the cells of the 1-3 layers on the outside relatively small, long-rounded or subrounded, elongated tangentially; the cells of the outer to inner 3-6 layers varied in size, arranged irregularly and densely, with relatively small lumen; the innermost 1-3 layers of the

stone cells subsquare or polygonal, with prominently thickened wall, arranged densely. Inside the stone cell layer, the lacunar parenchyma situated, consisting of 1-3 layers of cells relatively small at the outside, elongated-rounded, subrounded or elliptical, those increased in size or dilapidated towards inner side, with gradually thickened wall, forming sclerenchyma near the joint of cotyledon in the two ends of seed. Vascular bundle located in the lacunar parenchyma of the two ends of the seed, with the vessels relatively small. Endosperm consists of several layers of flatten cells. Parenchyma of cotyledon occupied the most part of seed, filled with oil droplets and aleurone grains [Fig. 2 (i) and (ii)].

Powder

Colour dark reddish-brown or pale brown. Stone cells relatively small, scattered singly or in groups, brown, pale yellowish-brown or greyish-green, irregularly squared or rounded, edges indistinct, wall sinuous or branched, some with striations, pits relatively sparse or with indistinct pit canals at one side, lumen occasionally containing dark brown substances. Sclerenchymatous cells mostly scattered singly or in groups, some attached to epidermal cells, brownish-yellow, orange or greyish-green, irregularly rectangular, elongated-rounded or subtriangular 22-79 μm in diameter, 42-108 μm long, wall 3-16 μm thick, sinuous, often with several branches or protuberance, tips obtuse. Epidermal cells of testa subpolygonal or irregular in surface view, periclinal wall slightly curved or straight, with cuticled striations. Parenchymatous cells of cotyledons polygonal, subpolygonal or subrounded, filled with oil droplets and aleurone grains. Fragments of endosperm seldom found, yellowish-green, polygonal or subpolygonal, containing aleurone grains [Fig. 3 (i) and (ii)].

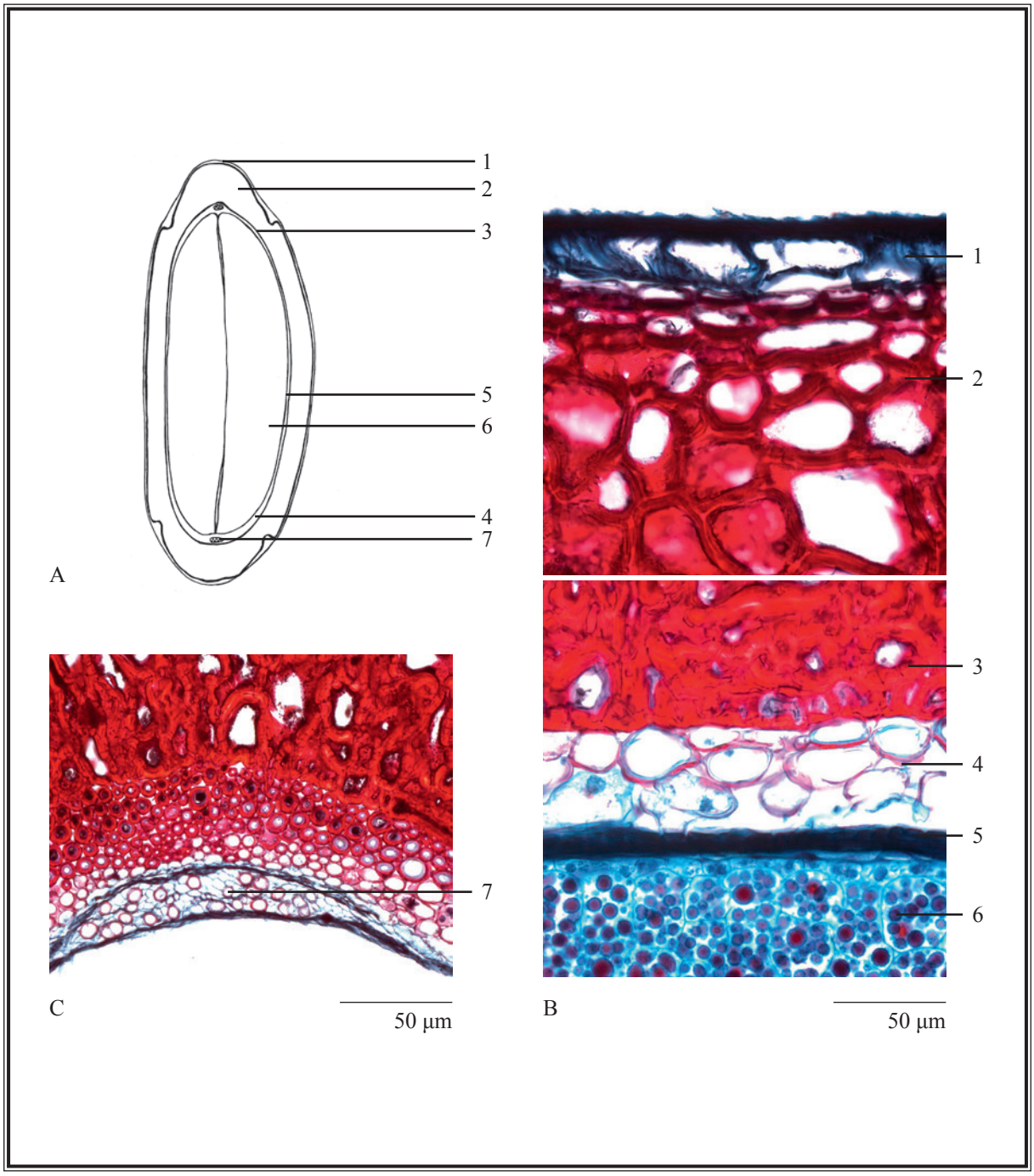

Figure 2 (i) Microscopic features of transverse section of dried ripe seed of *Trichosanthes kirilowii* Maxim.

A. Sketch B. Section illustration C. Vascular bundle

1. Epidermis of testa 2. Sclerenchyma 3. Stone cells 4. Lacunar parenchyma
5. Endosperm 6. Cotyledon 7. Vascular bundle

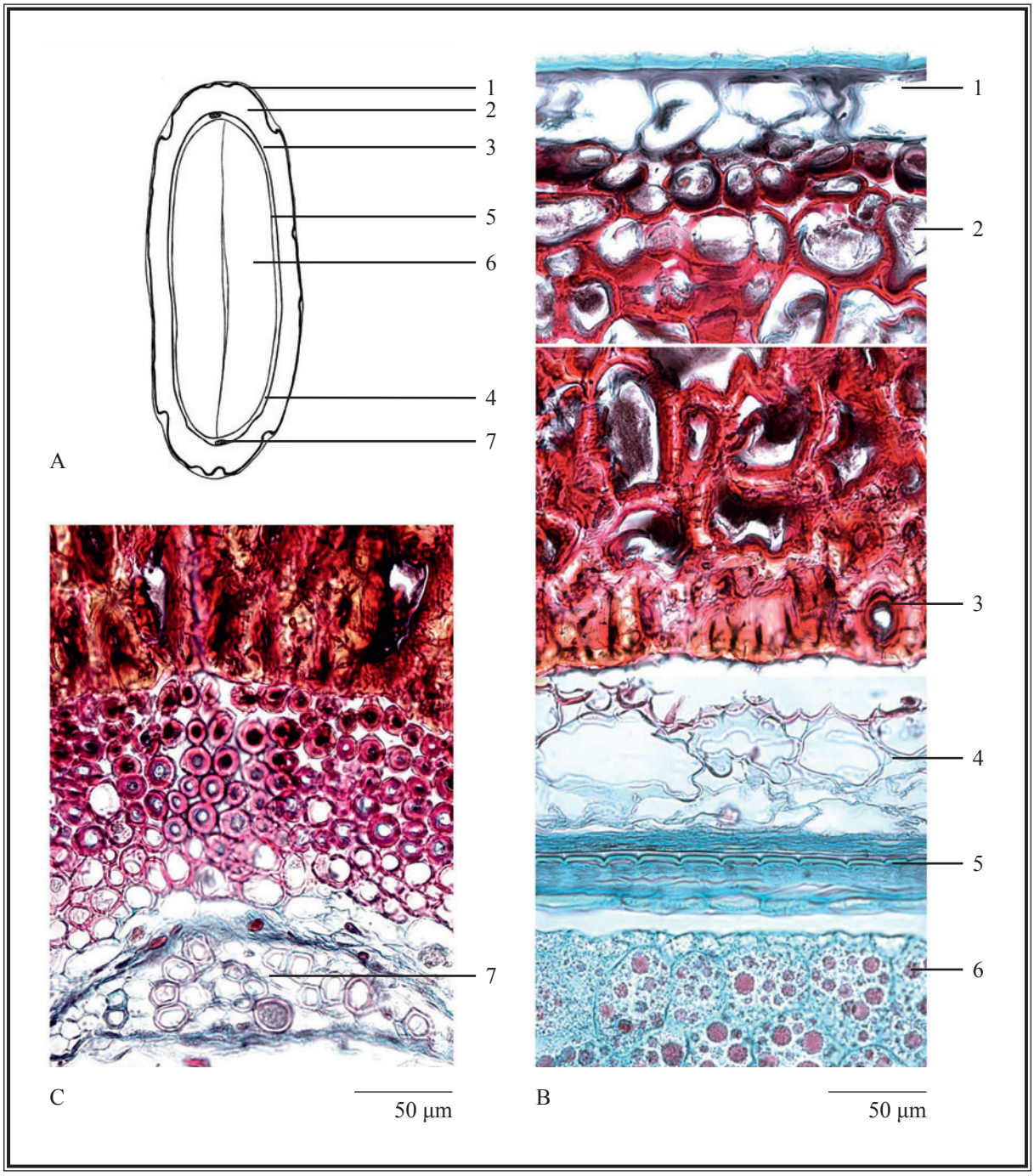

Figure 2 (ii) Microscopic features of transverse section of dried ripe seed of *Trichosanthes rosthornii* Harms

A. Sketch B. Section illustration C. Vascular bundle

1. Epidermis of testa 2. Sclerenchyma 3. Stone cells 4. Lacunar parenchyma
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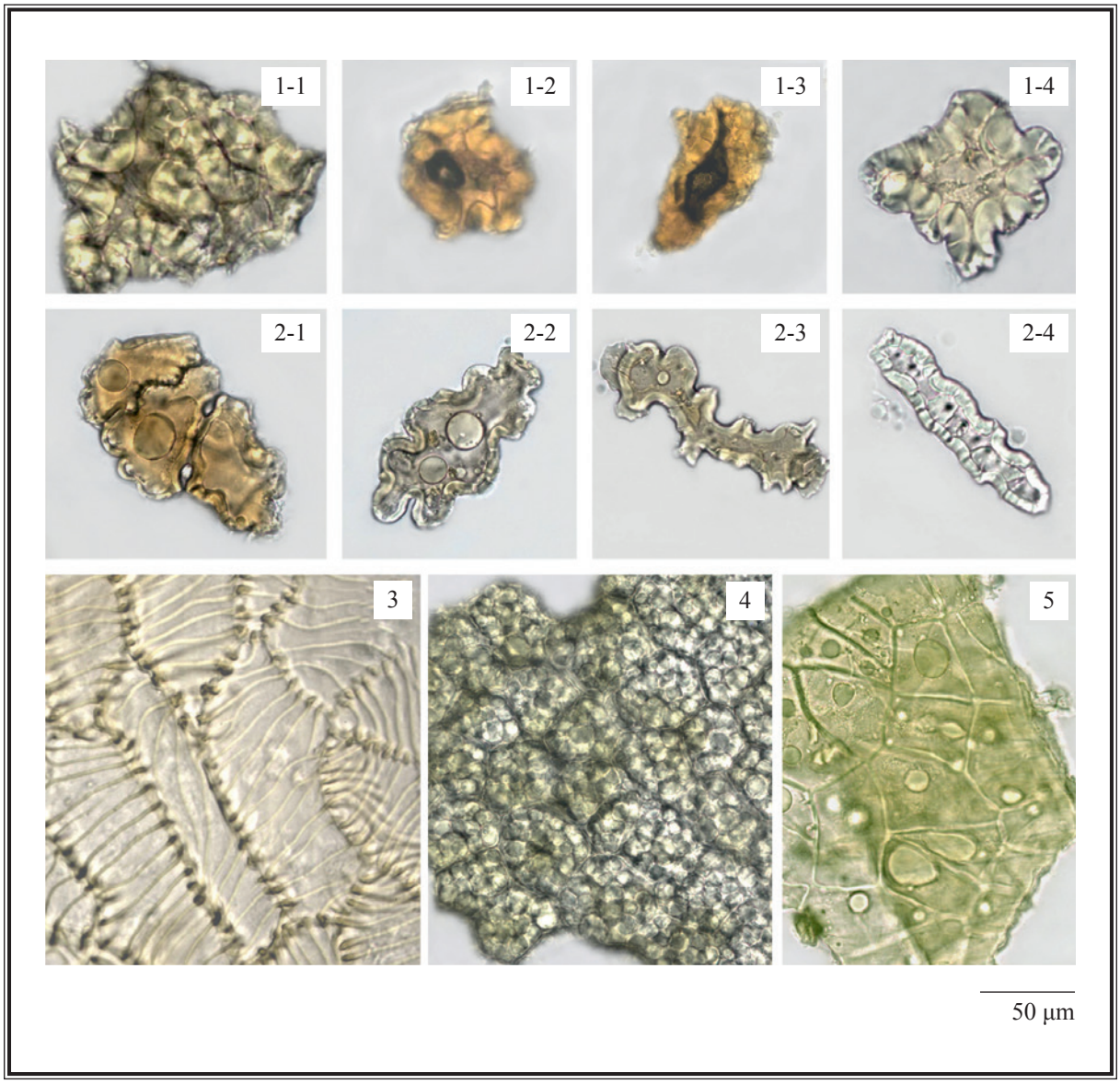

Figure 3 (i) Microscopic features of powder of dried ripe seed of *Trichosanthes kirilowii* Maxim. (under the light microscope)

1. Stone cells 2. Sclerenchymatous cells 3. Epidermal cells of testa
4. Cotyledon cells 5. Endosperm cells

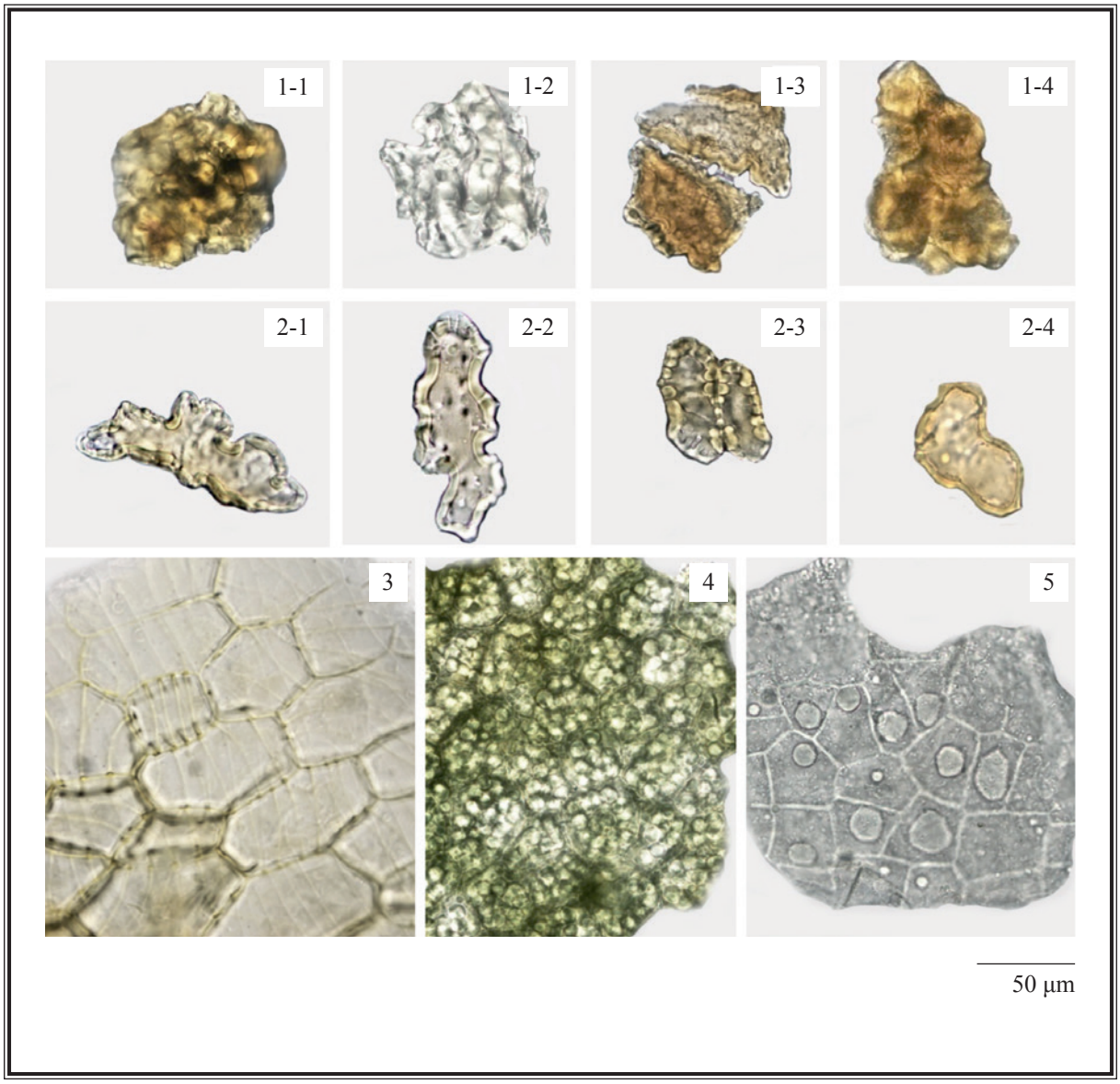

Figure 3 (ii) Microscopic features of powder of dried ripe seed of *Trichosanthes rosthornii* Harms (under the light microscope)

1. Stone cells 2. Sclerenchymatous cells 3. Epidermal cells of testa
4. Cotyledon cells 5. Endosperm cells

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

Standard solution

3, 29-Dibenzoyl-karounitriol standard solution

Weigh 1.0 mg of 3, 29-dibenzoyl-karounitriol CRS (Fig. 4) and dissolve in 10 mL of petroleum ether (60-80˚C).

Developing solvent system

Prepare a mixture of cyclohexane and ethyl acetate (3:1, v/v).

Spray reagent

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

Test solution

Weigh 1.0 g of the freshly powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol. Sonicate (100 W) the mixture for 20 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of ethyl acetate. Transfer the solution to a separating funnel. Extract with a mixture of 16 mL of ammonium hydroxide solution (25%, v/v) and 24 mL of water. Transfer the upper layer 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 cyclohexane.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately 3, 29-dibenzoyl-karounitriol standard solution (5 µL) and the test solution (10 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. Spray the plate evenly with the spray reagent and heat at about 105˚C until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. 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 3, 29-dibenzoyl-karounitriol.

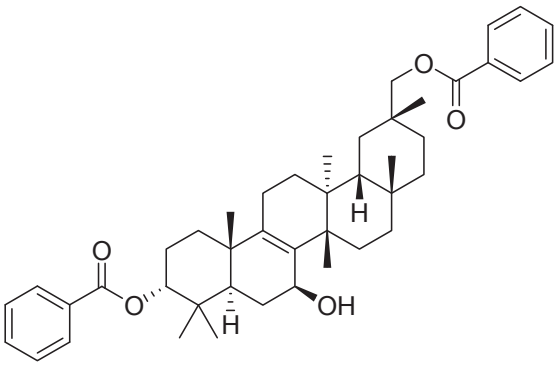

Figure 4 Chemical structure of 3, 29-dibenzoyl-karounitriol

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

Standard solution

3, 29-Dibenzoyl-karounitriol standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of 3, 29-dibenzoyl-karounitriol CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 1.0 g of the freshly powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (230 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)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 25	30 → 100	70 → 0	linear gradient
25 – 60	100	0	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of 3, 29-dibenzoyl-karounitriol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of 3, 29-dibenzoyl-karounitriol should not be more than 5.0%; the RSD of the retention time of 3, 29-dibenzoyl-karounitriol peak should not be more than 2.0%; the column efficiency determined from 3, 29-dibenzoyl-karounitriol peak should not be less than 90000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 5 (i) or (ii)].

Procedure

Separately inject 3, 29-dibenzoyl-karounitriol Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of 3, 29-dibenzoyl-karounitriol peak in the chromatogram of 3, 29-dibenzoyl-karounitriol Std-FP and the retention times of the four characteristic peaks [Fig. 5 (i) or (ii)] in the chromatogram of the test solution. Identify 3, 29-dibenzoyl-karounitriol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 3, 29-dibenzoyl-karounitriol Std-FP. The retention times of 3, 29-dibenzoyl-karounitriol 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 Trichosanthis Semen extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.54	± 0.03
2 (marker, 3, 29-dibenzoyl-karounitriol)	1.00	-
3	1.14	± 0.03
4	1.18	± 0.05

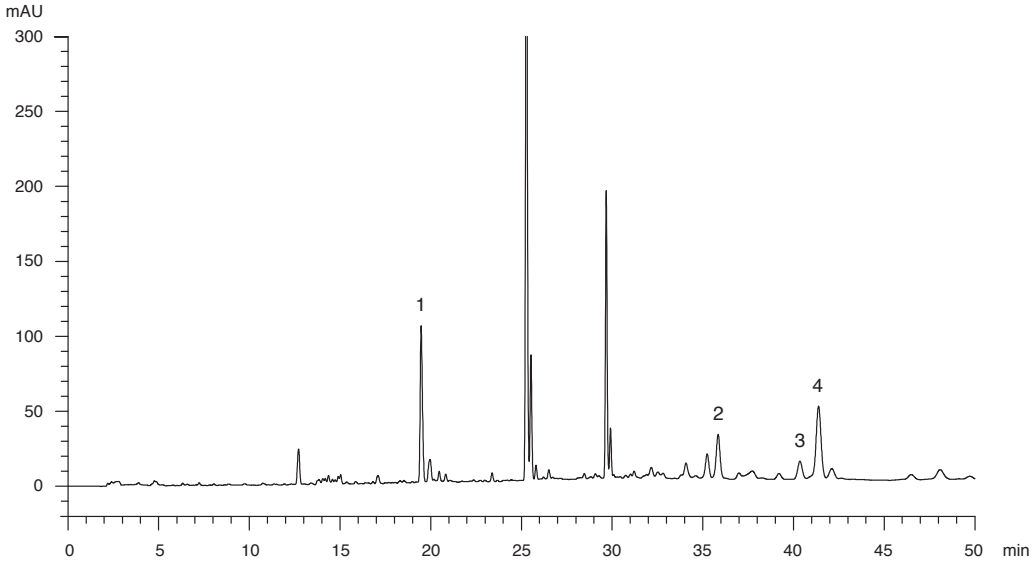

Figure 5 (i) A reference fingerprint chromatogram of dried ripe seed of *Trichosanthes kirilowii* Maxim. extract

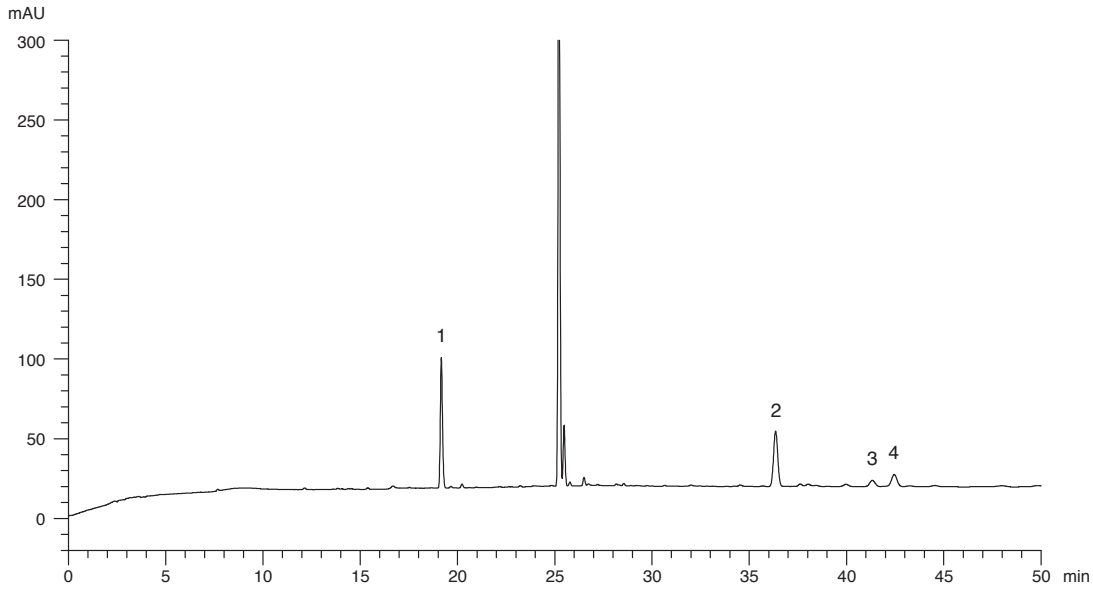

Figure 5 (ii) A reference fingerprint chromatogram of dried ripe seed of *Trichosanthes rosthornii* Harms extract

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 respective 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 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 3.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 3.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

3, 29-Dibenzoyl-karounitriol standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 2.5 mg of 3, 29-dibenzoyl-karounitriol CRS and dissolve in 5 mL of ethanol.

3, 29-Dibenzoyl-karounitriol standard solution for assay, Std-AS

Measure accurately the volume of the 3, 29-dibenzoyl-karounitriol Std-Stock, dilute with ethanol to produce a series of solutions of 25, 50, 75, 100, 125 mg/L for 3, 29-dibenzoyl-karounitriol.

Test solution

Weigh accurately 1.0 g of the freshly powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Combine the supernatants and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with ethanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (230 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 methanol and water (96:4, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of 3, 29-dibenzoyl-karounitriol Std-AS (75 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 3, 29-dibenzoyl-karounitriol should not be more than 5.0%; the RSD of the retention time of 3, 29-dibenzoyl-karounitriol peak should not be more than 2.0%; the column efficiency determined from 3, 29-dibenzoyl-karounitriol peak should not be less than 7000 theoretical plates.

The *R* value between 3, 29-dibenzoyl-karounitriol 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 3, 29-dibenzoyl-karounitriol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of 3, 29-dibenzoyl-karounitriol against the corresponding concentrations of 3, 29-dibenzoyl-karounitriol 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 3, 29-dibenzoyl-karounitriol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 3, 29-dibenzoyl-karounitriol Std-AS. The retention times of 3, 29-dibenzoyl-karounitriol peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of 3, 29-dibenzoyl-karounitriol in the test solution, and calculate the percentage content of 3, 29-dibenzoyl-karounitriol in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.080% of 3, 29-dibenzoyl-karounitriol ($C_{44}H_{58}O_5$), calculated with reference to the dried substance.