Trichosanthis Radix

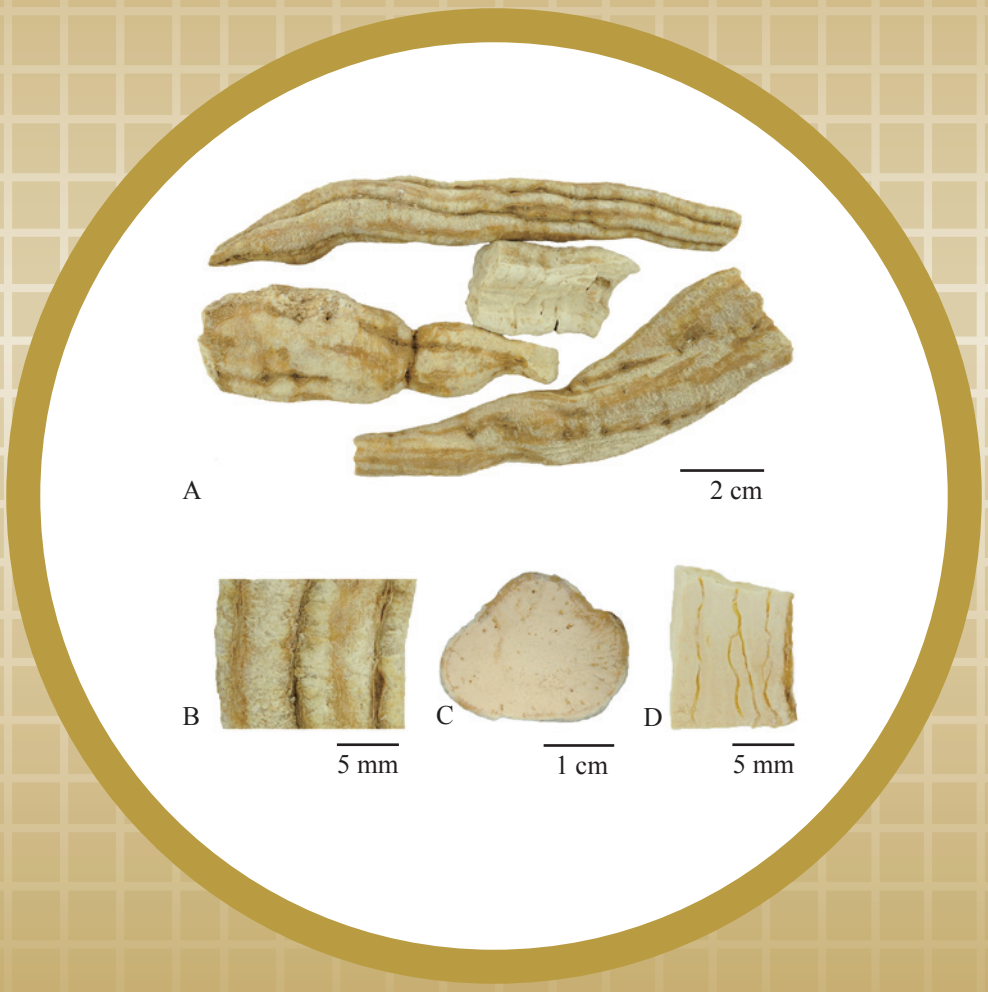

Figure 1 (i) A photograph of dried root of *Trichosanthes kirilowii* Maxim.

A. Roots B. Magnified image of outer appearance

C. Magnified image of transverse section

D. Magnified image of longitudinal section

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

A. Roots B. Magnified image of outer appearance

C. Magnified image of transverse section

D. Magnified image of longitudinal section

1. NAMES

Official Name: Trichosanthis Radix

Chinese Name: 天花粉

Chinese Phonetic Name: Tianhuafen

2. SOURCE

Trichosanthis Radix is the dried root of *Trichosanthes kirilowii* Maxim. or *Trichosanthes rosthornii* Harms (Cucurbitaceae). The root is collected in autumn and winter, washed clean, cork of *Trichosanthes kirilowii* Maxim. mostly peeled, cork of *Trichosanths rosthornii* Harms mostly unpeeled, then cut into segments or sliced longitudinally to obtain Trichosanthis Radix.

3. DESCRIPTION

***Trichosanthes kirilowii* Maxim.:** Irregularly cylindrical, spindle-shaped, or in pieces, 5-24 cm long, 5-39 mm in diameter. Externally yellowish-white to pale brownish-yellow, with longitudinal wrinkles, rootlet scars, and a slightly impressed transverse lenticel. Remnants of yellowish-brown outer bark scattered. Texture compact, fracture white to yellowish-white, starchy, a radially arranged yellow xylem visible in transverse section, striated yellow xylem visible in longitudinal section. Odour slight; taste slightly bitter [Fig. 1 (i)].

***Trichosanthes rosthornii* Harms:** Irregularly cylindrical, spindle-shaped, or in pieces, 4-21 cm long, 9-49 mm in diameter. Externally pale greyish-yellow to brownish-yellow, with reticulate wrinkles. Remnants of brownish-yellow outer bark visible [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

***Trichosanthes kirilowii* Maxim.:** Cork completely broken or peeled off, 2-12 layers of cork cells visible occasionally. Stone cells occasionally found, irregular in shape, several cells in groups, arranged in an interrupted ring. Fibres located close to stone cells, mainly in groups. Phloem narrow. Cambium indistinct. Xylem broad, vessels singly scattered or in groups, 22-432 μm in diameter, surrounded by xylem fibres, radially arranged starting near the cambium or irregularly. Starch granules numerous, scattered in parenchymatous cells [Fig. 2 (i)].

***Trichosanthes rosthornii* Harms:** Lower layer of cork visible, consisting of 2-10 layers of cork cells. Stone cells numerous, irregular in shape, several cells in groups, arranged in an interrupted ring. Vessels 18-473 μm in diameter, surrounded by xylem fibres, radially arranged starting near the cambium or irregularly [Fig. 2 (ii)].

Powder

***Trichosanthes kirilowii* Maxim.:** Colour yellowish-white. Starch granules numerous, simple starch granules relatively less abundant, subspherical, globose, subglobose or helmeted, 3-36 μm in diameter, the hilum pointed, shortly cleft or forming a V shape, with striations faintly visible in the small granules, but clear and sharp in the large ones; compound starch granules more abundant, composed of 2-14 units, frequently composed of one large unit and several small ones; black and cruciate-shaped under the polarized microscope. Stone cells greenish-yellow, singly scattered or occasionally in groups, the cells rectangular, elliptic, subsquare, polygonal or fusiform, 11-79 μm in diameter, but may be as long as 131 μm, densely fine-pitted, walls 4-14 μm in thickness. Bordered-pitted vessel large, mostly broken, densely and clearly pitted, tightly arranged, two types: one type with the pits hexagonal, pentagonal or square; the other type with the pits oblong to oval. Fibres 5-56 μm in diameter, two types: one type tracheid-like fibres and broken; the other type frequently single, straight and thick walled; polychromatic under the polarized microscope. Cork cell polygonal, tightly packed [Fig. 3 (i)].

***Trichosanthes rosthornii* Harms:** Starch granules 3-45 μm in diameter; compound granules composed of 2-15 units. Stone cells relatively abundant and easily observed, singly scattered or in groups, the cells rectangular, elliptic, subsquare, polygonal or fusiform, 14-107 μm in diameter, but may be as long as 192 μm, densely fine-pitted, walls 3-21 μm in thickness. Fibres 6-59 μm in diameter [Fig. 3 (ii)].

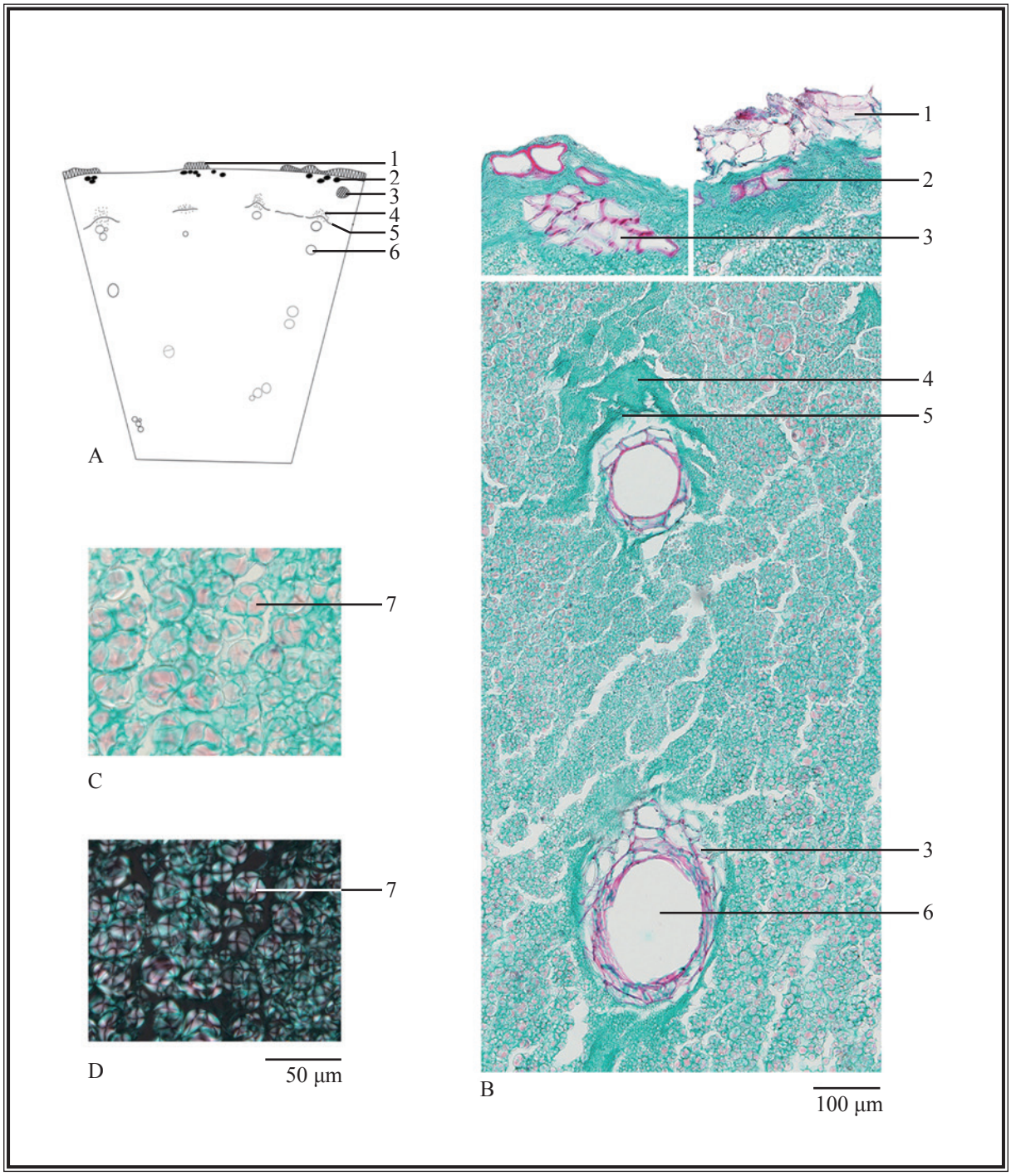

Figure 2 (i) Microscopic features of transverse section of dried root of *Trichosanthes kirilowii* Maxim.

A. Sketch B. Section illustration C. Starch granules (under the light microscope)
D. Starch granules (under the polarized microscope)

1. Cork 2. Stone cell 3. Fibre 4. Phloem 5. Cambium 6. Xylem 7. Starch granule

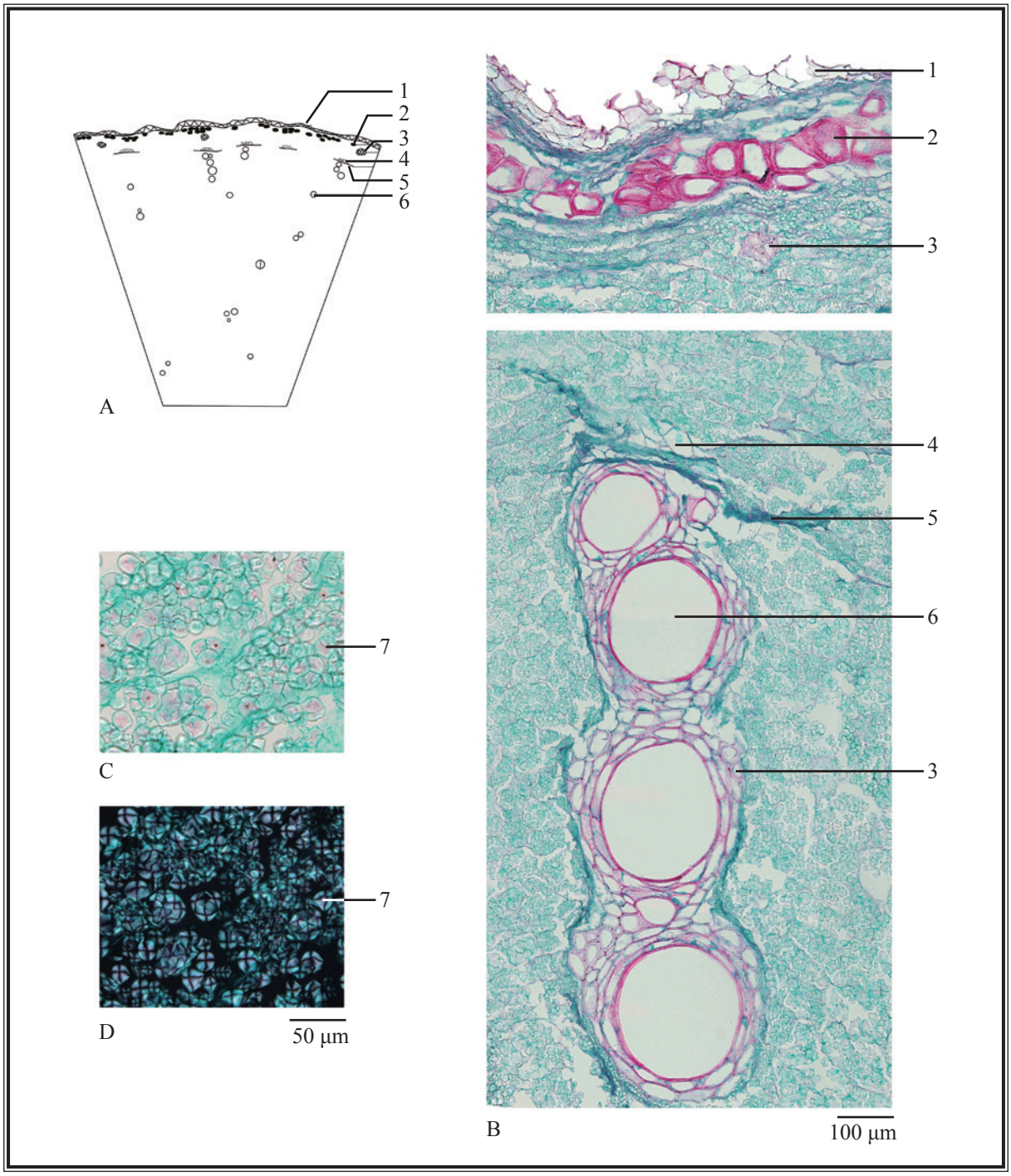

Figure 2 (ii) Microscopic features of transverse section of dried root of *Trichosanthes rosthornii* Harms

A. Sketch B. Section illustration C. Starch granules (under the light microscope)
D. Starch granules (under the polarized microscope)

1. Cork 2. Stone cell 3. Fibre 4. Phloem 5. Cambium 6. Xylem 7. Starch granule

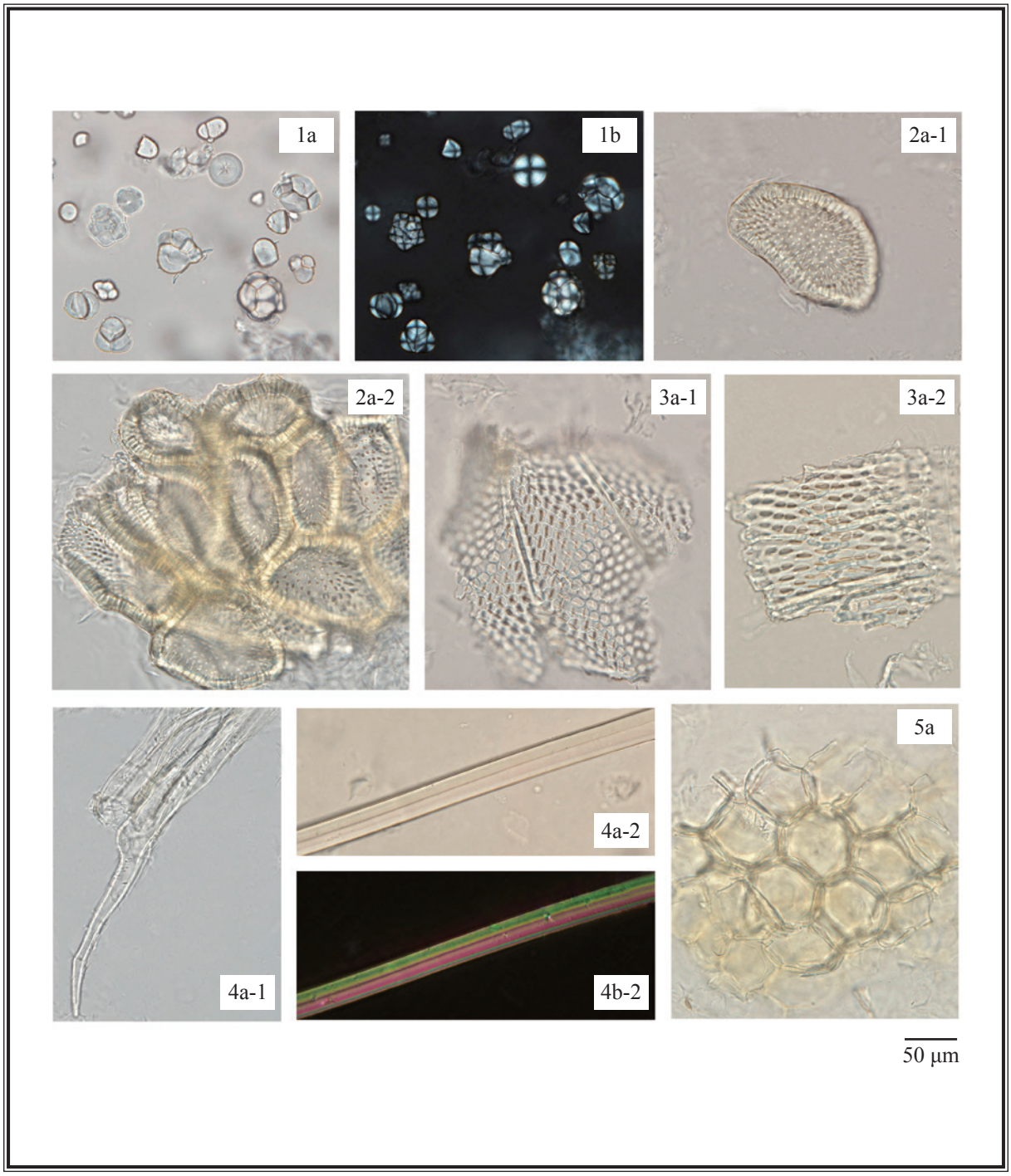

Figure 3 (i) Microscopic features of powder of dried root of *Trichosanthes kirilowii* Maxim.

1. Starch granules 2. Stone cells (2-1 single stone cell, 2-2 cluster of stone cells)
3. Bordered-pitted vessels (3-1 bordered pits polygonal, 3-2 bordered pits oblong)
4. Fibres (4-1 tracheid-like fibres, 4-2 straight fibre with a thick cell wall) 5. Cork cells

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

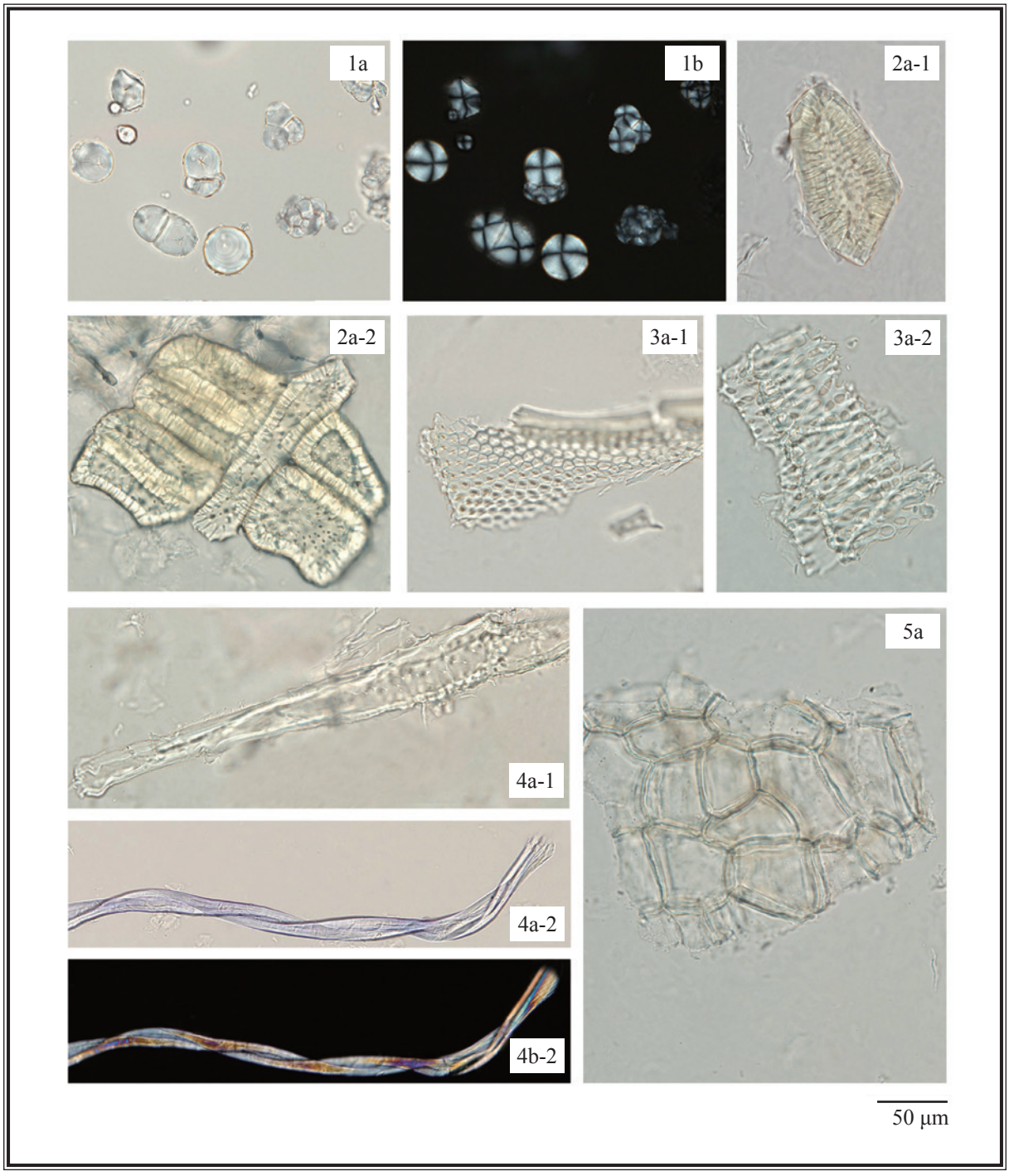

Figure 3 (ii) Microscopic features of powder of dried root of *Trichosanthes rosthornii* Harms

1. Starch granules 2. Stone cells (2-1 single cell, 2-2 cluster of stone cells)
3. Bordered-pitted vessels (3-1 bordered pits polygonal, 3-2 bordered pits oblong)
4. Fibres (4-1 tracheid-like fibre, 4-2 curved fibre with a thick cell wall) 5. Cork cells

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

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

Standard solution

L-Citrulline standard solution

Weigh 1.0 mg of L-citrulline CRS (Fig. 4) and dissolve in 1 mL of methanol (50%).

Developing solvent system

Prepare a mixture of *n*-butanol, ethanol, glacial acetic acid and water (3:2:2:2, v/v).

Spray reagent

Weigh 1 g of ninhydrin and dissolve in 50 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 methanol (50%). Sonicate (220 W) the mixture for 30 min. Filter the mixture.

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 L-citrulline 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 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 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

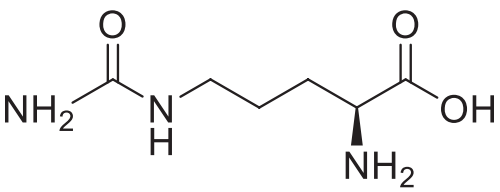

Figure 4 Chemical structure of L-citrulline

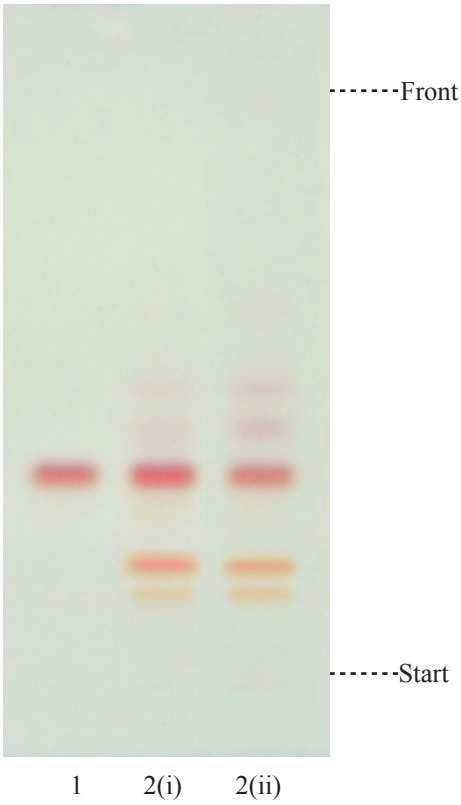

Figure 5 A reference HPTLC chromatogram of Trichosanthis Radix extract observed under visible light after staining

1. L-Citrulline standard solution
2. Test solution of
(i) dried root of *Trichosanthes kirilowii* Maxim.
(ii) dried root of *Trichosanthes rosthornii* Harms

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 L-citrulline (Fig. 5).

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

Standard solution

L-Citrulline standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of L-citrulline CRS and dissolve in 10 mL of water.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 150-mL conical flask, then add 90 mL of methanol (50%). Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for three times each with 3 mL of methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 115°C; nebulizer gas (N_2) flow rate: 3.0 L/min] and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.7 mL/min. The mobile phase is a mixture of 0.3% trifluoroacetic acid and 0.15% heptafluorobutyric acid in water. The elution time is about 20 min.

System suitability requirements

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

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 6 (i) or (ii)].

Procedure

Separately inject L-citrulline Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of L-citrulline peak in the chromatogram of L-citrulline Std-FP and the retention times of the three characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify L-citrulline peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of L-citrulline Std-FP. The retention times of L-citrulline 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 three characteristic peaks of Trichosanthis Radix extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the three characteristic peaks of Trichosanthis Radix extract

Peak No.	RRT	Acceptable Range
1	0.47	± 0.03
2	0.66	± 0.03
3 (marker, L-citrulline)	1.00	-

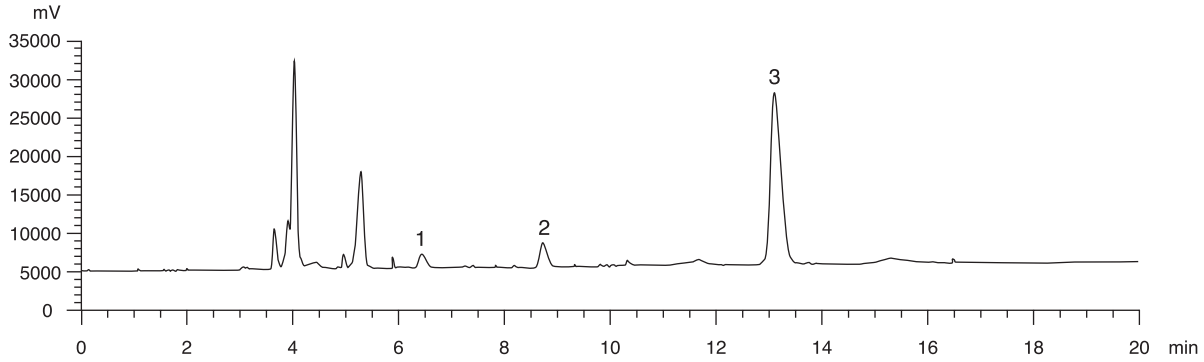

Figure 6 (i) A reference fingerprint chromatogram of dried root of *Trichosanthes kirilowii* Maxim. extract

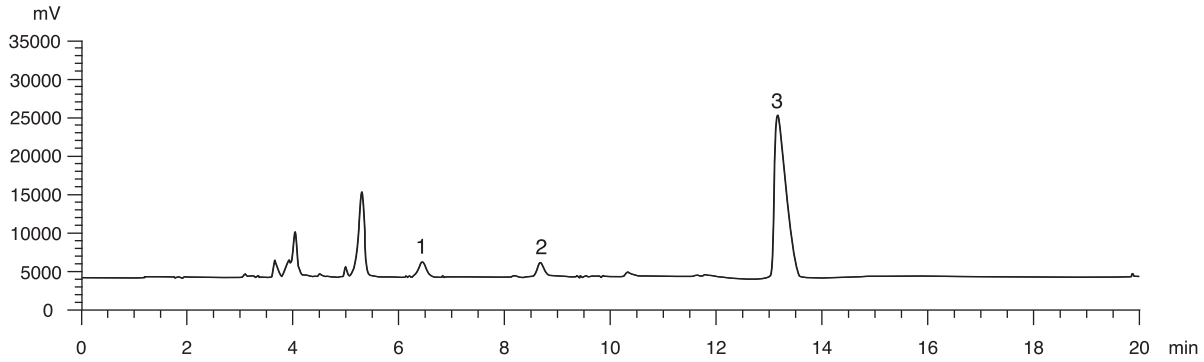

Figure 6 (ii) A reference fingerprint chromatogram of dried root of *Trichosanthes rosthornii* Harms extract

For positive identification, the sample must give the above three characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference fingerprint chromatograms [Fig. 6 (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 XVI)*: not more than 400 mg/kg.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
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

L-Citrulline standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of L-citrulline CRS and dissolve in 5 mL of water.

L-Citrulline standard solution for assay, Std-AS

Measure accurately the volume of the L-citrulline Std-Stock, dilute with water to produce a series of solutions of 10, 20, 50, 80, 300 mg/L for L-citrulline.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 150-mL conical flask, then add 90 mL of methanol (50%). Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for three times each with 3 mL of methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 115°C; nebulizer gas (N_2) flow rate: 3.0 L/min] and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.7 mL/min. The mobile phase is a mixture of 0.3% trifluoroacetic acid and 0.15% heptafluorobutyric acid in water. The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of L-citrulline Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of L-citrulline should not be more than 5.0%; the RSD of the retention time of L-citrulline peak should not be more than 2.0%; the column efficiency determined from L-citrulline peak should not be less than 10000 theoretical plates.

The *R* value between L-citrulline peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 7 (i) or (ii)].

Calibration curve

Inject a series of L-citrulline Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of L-citrulline against the natural logarithm of the corresponding concentrations of L-citrulline 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 L-citrulline peak [Fig. 7 (i) or (ii)] in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of L-citrulline Std-AS. The retention times of L-citrulline 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 L-citrulline in the test solution, and calculate the percentage content of L-citrulline in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.73% of L-citrulline ($C_6H_{13}N_3O_3$), calculated with reference to the dried substance.

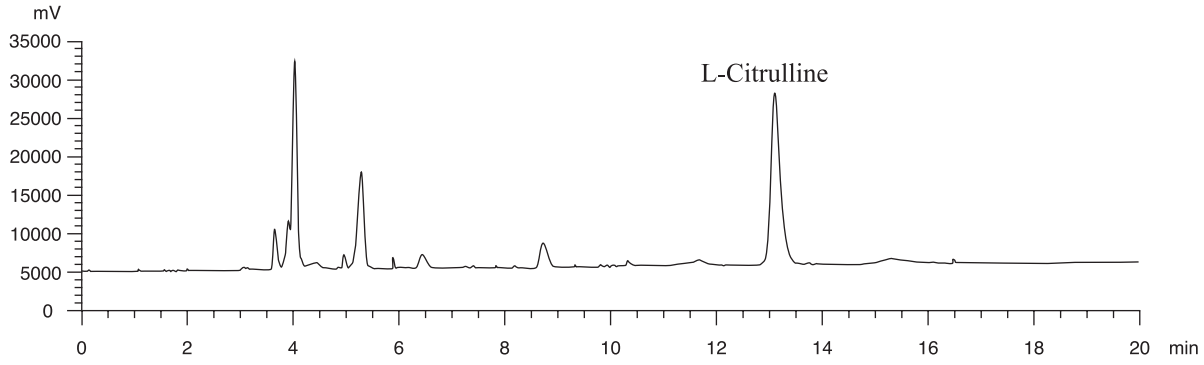

Figure 7 (i) A reference assay chromatogram of dried root of *Trichosanthes kirilowii* Maxim. extract

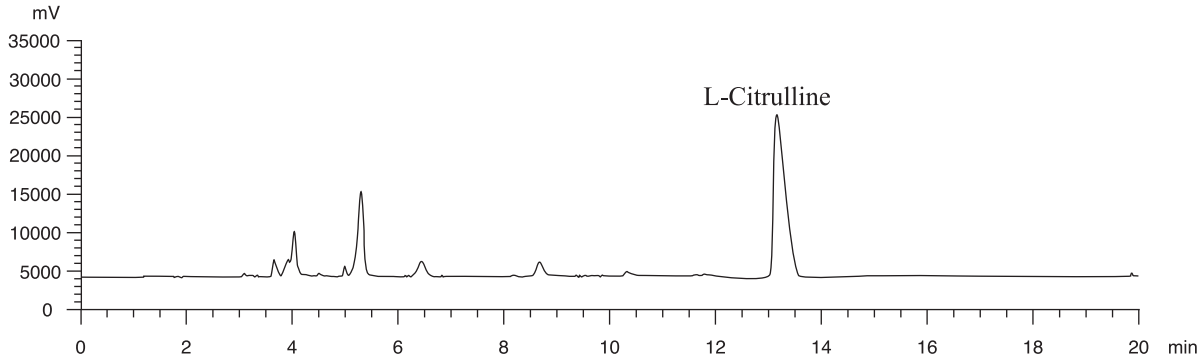

Figure 7 (ii) A reference assay chromatogram of dried root of *Trichosanthes rosthornii* Harms extract