Chebulae Fructus

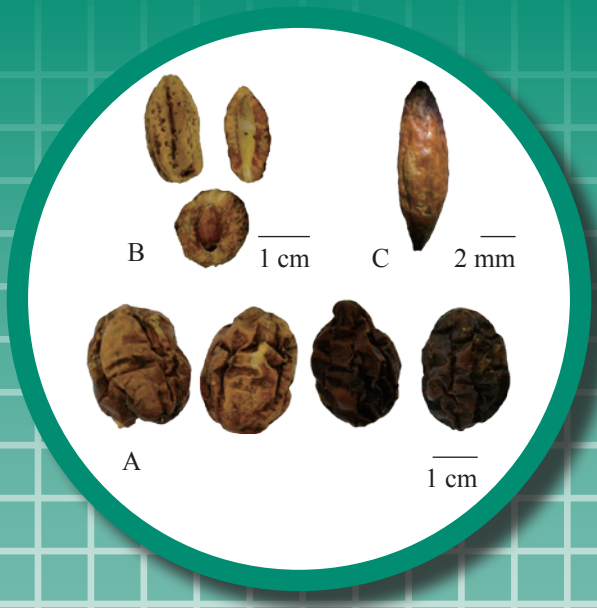

Figure 1 (i) A photograph of the dried ripe fruit of *Terminalia chebula* Retz.

A. Fruits B. Kernels C. Magnified seed

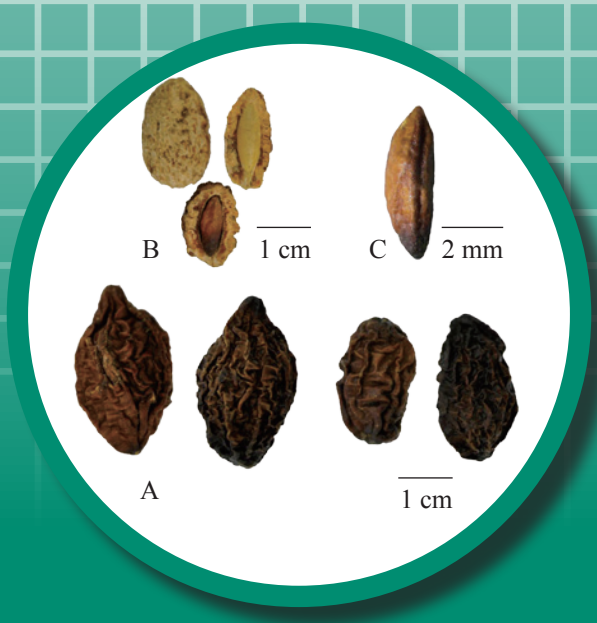

Figure 1 (ii) A photograph of the dried ripe fruit of *Terminalia chebula* Retz. var. *tomentella* Kurt.

A. Fruits B. Kernels C. Magnified seed

1. NAMES

Official Name: Chebulae Fructus

Chinese Name: 訶子

Chinese Phonetic Name: Hezi

2. SOURCE

Chebulae Fructus is the dried ripe fruit of *Terminalia chebula* Retz. or *Terminalia chebula* Retz. var. *tomentella* Kurt. (Combretaceae). The fruit is collected in autumn and winter when ripe, foreign matter removed, then dried under the sun to obtain Chebulae Fructus.

3. DESCRIPTION

***Terminalia chebula* Retz.:** Ellipsoid to ovoid, 2.2-3.8 cm long and 14-23 mm in diameter. Externally yellowish-brown to brown, slightly lustrous, marked with 5-6 longitudinal ribs and irregular wrinkles, the base with a rounded fruit stalk scar. Texture hard and compact. Sarcocarp yellowish-brown to dark yellowish-brown; kernels 1.4-2.5 cm long, 9-16 mm in diameter, pale yellow, rough and hard. Seeds narrowly fusiform, testa yellowish-brown. Odour slight; taste sour, astringent, and followed by sweet [Fig. 1 (i)].

***Terminalia chebula* Retz. var. *tomentella* Kurt.:** Ellipsoid to ovoid, 2.3-3.5 cm long and 13-19 mm in diameter. Externally yellowish-brown to dark brown, with extremely shrunk wrinkles. Sarcocarp yellowish-brown to brown, kernels 1.6-3.2 cm long, 11-15 mm in diameter [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pericarp:

***Terminalia chebula* Retz.:** Exocarp consists of 2-8 layers of irregular square collenchymatous cells, arranged closely, covered with cuticle. Mesocarp consists of several layers of big parenchymatous cells, arranged loosely with slightly thickened wall, 10-40 μm long radically, 50-140 μm long tangentially, containing brown resin mass. Sclerenchyma consists of 3-5 layers of rope-shaped sclerenchymatous cells, tangentially telescoped, with numerous radically ramifications. Mesocarp parenchyma consists of several layers of parenchymatous cells, with vessels and small vascular bundles among. Vessels arranged in group closely, with ramification. Clusters of calcium oxalate scattered in sclerenchymatous cells and parenchymatous cells [Fig. 2 (i)].

***Terminalia chebula* Retz. var. *tomentella* Kurt.:** Exocarp consists of 2-5 layers of collenchymatous cells, relatively flat rectangular, arranged closely and covered with cuticle. Rope-shaped sclerenchymatous cells 3-9 layers [Fig. 2 (ii)].

Powder

***Terminalia chebula* Retz.:** Colour yellowish-brown. Exocarp epidermal cells colourless to pale yellow, polygonal in top view, walls slightly thickened, some contain brown masses. Sclerenchymatous cells colourless to yellow, fibre-like or varying in shape, 50-250 μm long, some contain brown masses and a few contain clusters of calcium oxalate. Stone cells pale yellow or bright yellow, some branched, walls 2-6 μm thick. Vessels mainly reticulate, spiral vessels sometimes visible, in bundles, 10-30 μm in diameter; bright white under the polarized microscope. Clusters of calcium oxalate scattered or arranged as strip-shaped in cells, 10-40 μm in diameter; polychromatic under the polarized microscope [Fig. 3 (i)].

***Terminalia chebula* Retz. var. *tomentella* Kurt.:** Sclerenchymatous cells arrange in crisscross pattern or in other shapes, 80-230 μm long. Stone cells 3-8 μm in diameter. Spiral vessels 10-25 μm in diameter. Clusters of calcium oxalate 10-50 μm in diameter [Fig. 3 (ii)].

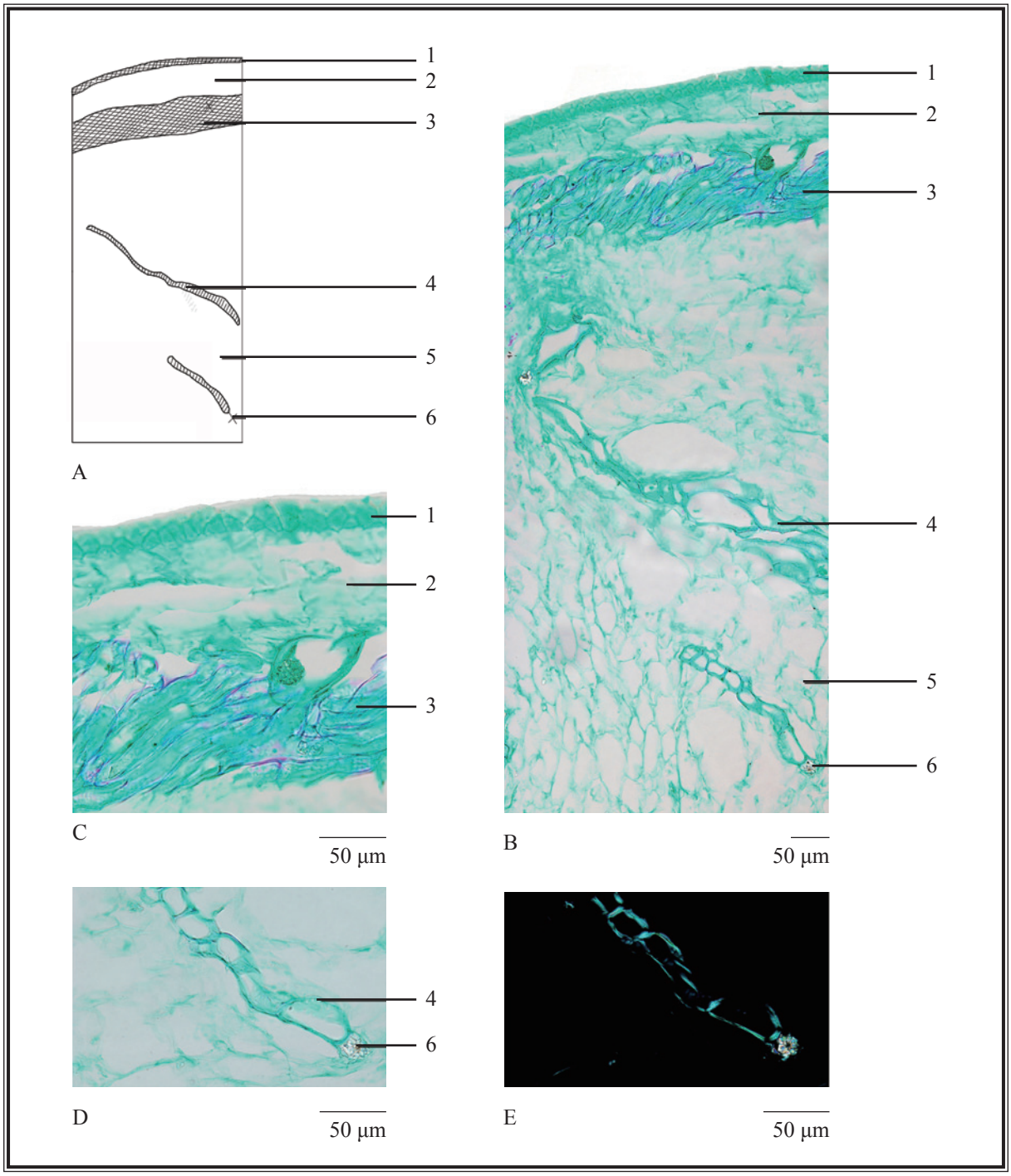

Figure 2 (i) Microscopic features of transverse section of dried pericarp of *Terminalia chebula* Retz.

A. Sketch B. Section illustration C. Pericarp magnified
D. Clusters of calcium oxalate
E. Clusters of calcium oxalate (under the polarized microscope)

1. Exocarp 2. Mesocarp 3. Sclerenchyma 4. Vessels
5. Mesocarp parenchyma 6. Cluster of calcium oxalate

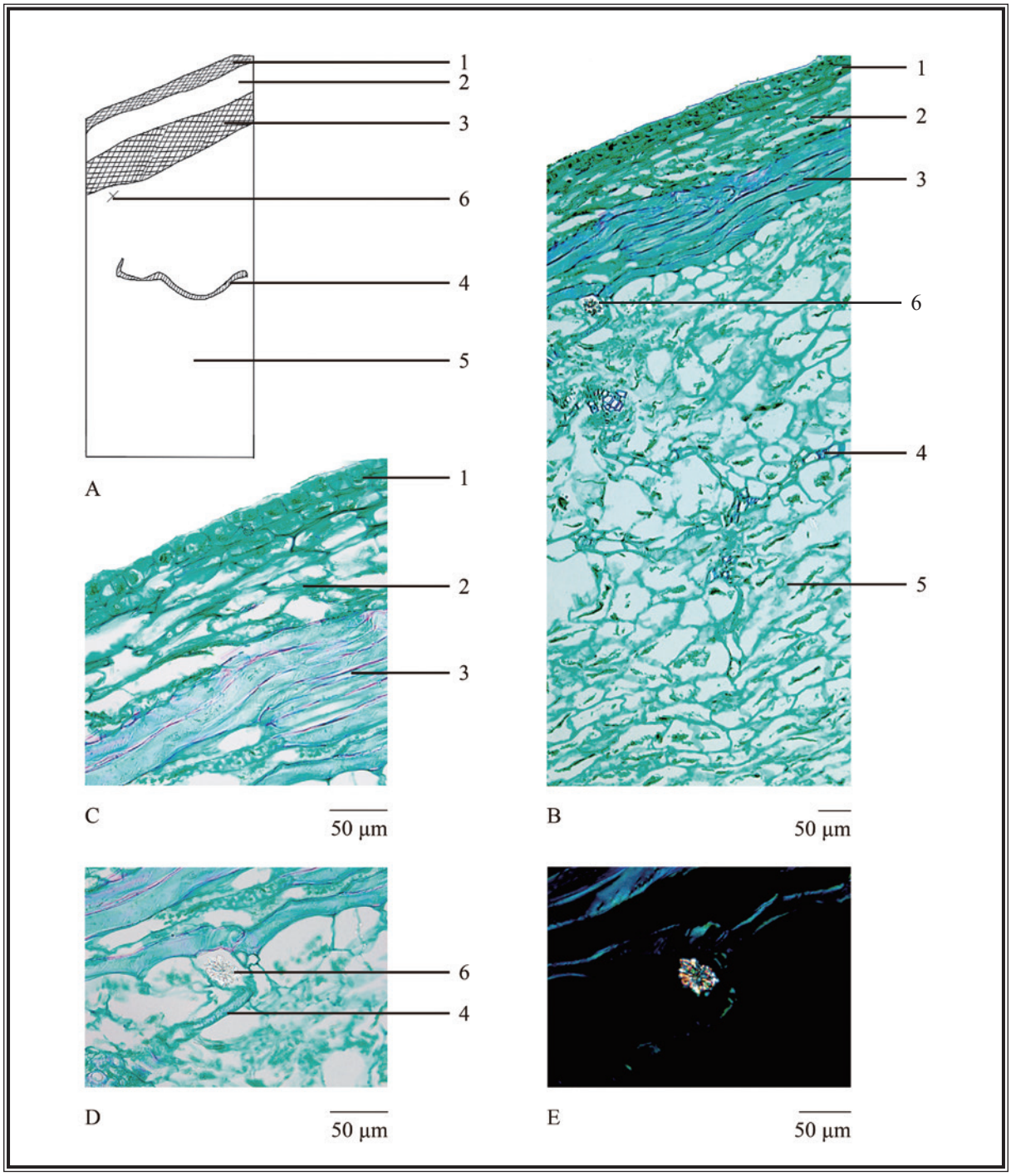

Figure 2 (ii) Microscopic features of transverse section of dried pericarp of *Terminalia chebula* Retz. var. *tomentella* Kurt.

A. Sketch B. Section illustration C. Pericarp magnified D. Clusters of calcium oxalate
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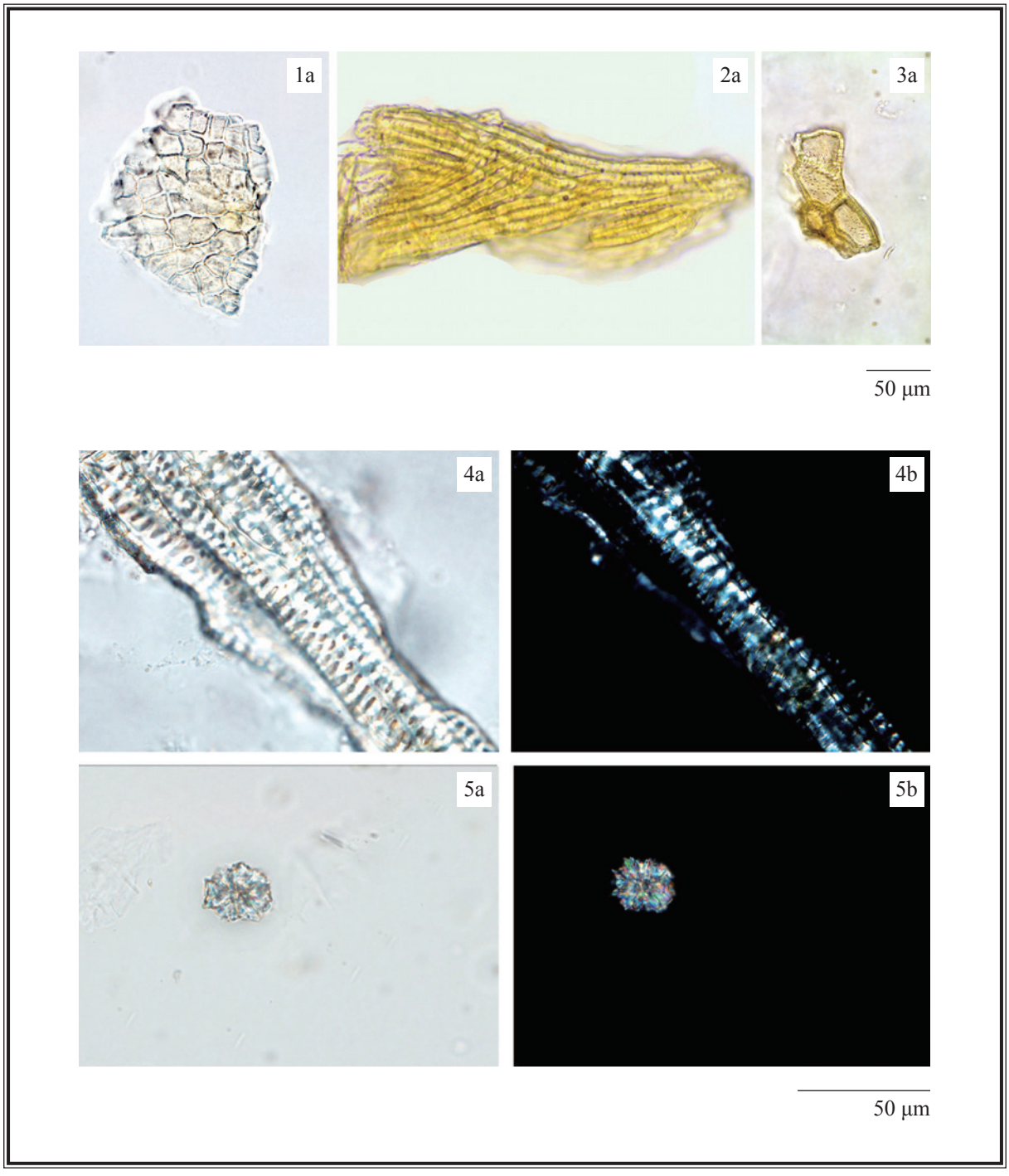

Figure 3 (i) Microscopic features of powder of dried pericarp of *Terminalia chebula* Retz.

1. Exocarp epidermal cells 2. Sclerenchymatous cells 3. Stone cells
4. Vessels 5. Cluster of calcium oxalate

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

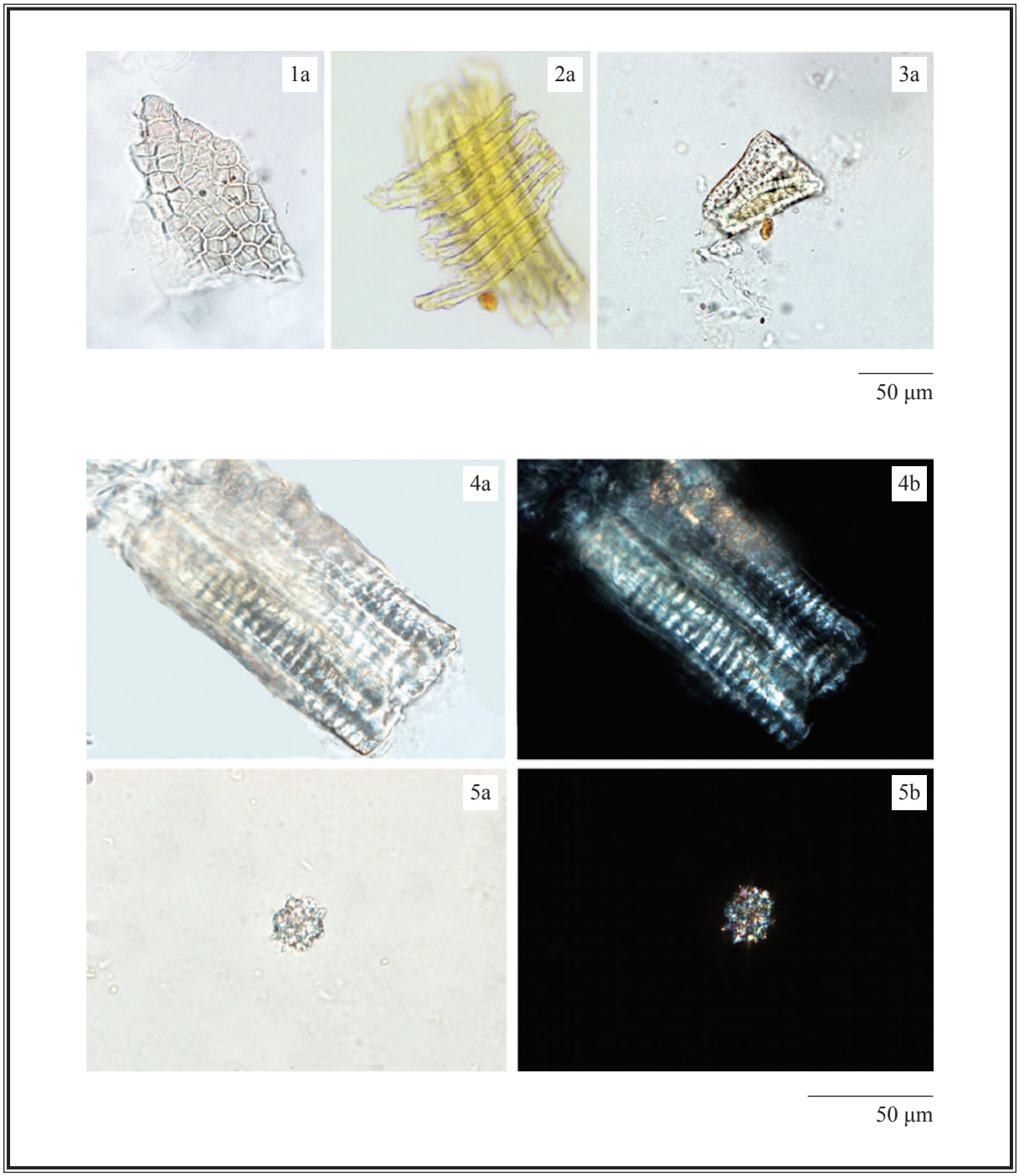

Figure 3 (ii) Microscopic features of powder of dried pericarp of *Terminalia chebula* Retz. var. *tomentella* Kurt.

1. Exocarp epidermal cells 2. Sclerenchymatous cells 3. Stone cells
4. Vessels 5. Cluster of calcium oxalate

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

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

Standard solution

Gallic acid standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane and glacial acetic acid (4:3:3, v/v).

Spray reagent

Weigh 1 g of ferric trichloride and dissolve in 100 mL of ethanol.

Test solution

Kernels of Chebulae Fructus are removed before grinding and powdering. Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. 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 and a freshly prepared developing solvent system as described above. Apply separately gallic acid standard solution and the test solution (2 μL each) 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 dry in air. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

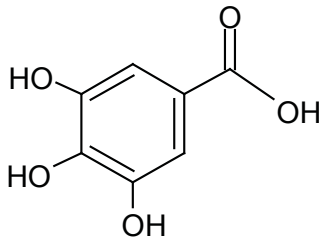

Figure 4 Chemical structure of gallic acid

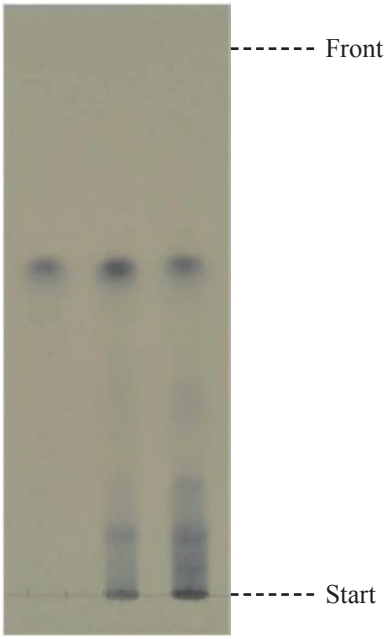

Figure 5 A reference HPTLC chromatogram of Chebulae Fructus extract observed under visible light after staining

1. Gallic acid standard solution
2. Test solution of
(i) dried ripe fruit of *Terminalia chebula* Retz.
(ii) dried ripe fruit of *Terminalia chebula* Retz. var. *tomentella* Kurt.

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 gallic acid (Fig. 5).

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

Standard solution

Gallic acid standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of gallic acid CRS and dissolve in 10 mL of methanol.

Test solution

Kernels of Chebulae Fructus are removed before grinding and powdering. Weigh 0.1 g of the powdered sample and place it in a 250-mL conical flask, then add 100 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-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 (275 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The internal diameter of inlet column tubing is about 0.5 mm. 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% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	100	0	isocratic
10 – 70	100 → 30	0 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of gallic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of gallic acid should not be more than 5.0%; the RSD of the retention time of gallic acid peak should not be more than 2.0%; the column efficiency determined from gallic acid peak should not be less than 40000 theoretical plates.

The *R* value between peak 1 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 gallic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of gallic acid peak in the chromatogram of gallic acid Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify gallic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of gallic acid Std-FP. The retention times of gallic acid 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 Chebulae Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Chebulae Fructus extract

Peak No.	RRT	Acceptable Range
1 (marker, gallic acid)	1.00	-
2	2.19	± 0.09
3	2.56	± 0.09
4	2.61	± 0.10

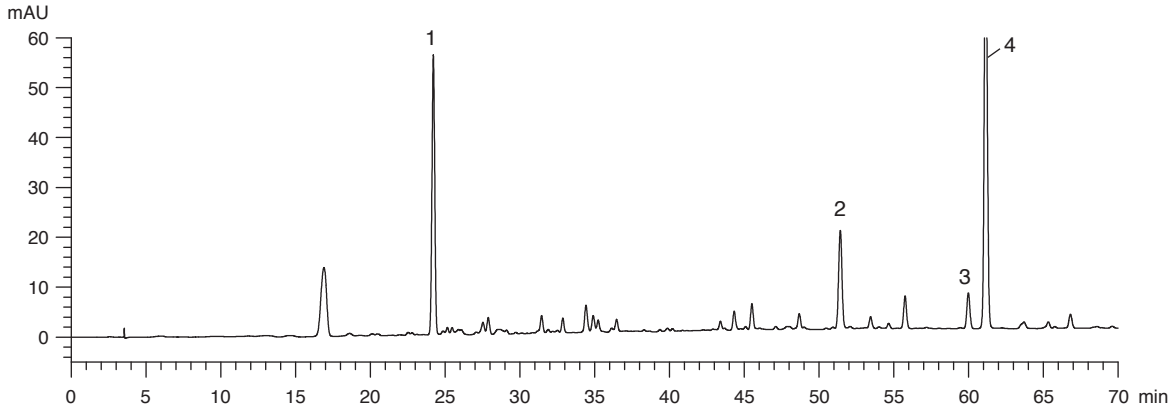

Figure 6 (i) A reference fingerprint chromatogram of dried ripe fruit of *Terminalia chebula* Retz. extract

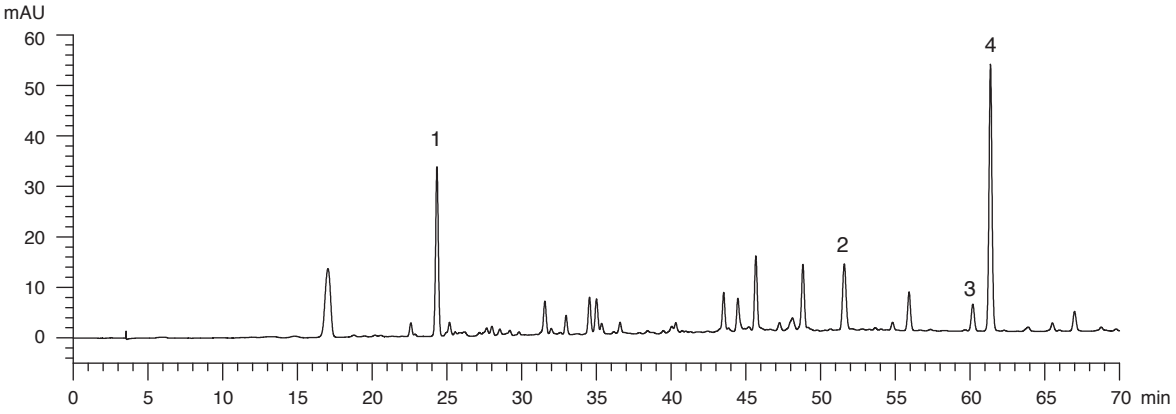

Figure 6 (ii) A reference fingerprint chromatogram of dried ripe fruit of *Terminalia chebula* Retz. var. *tomentella* Kurt. extract

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. 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 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 5.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 44.0%.
Ethanol-soluble extractives (cold extraction method): not less than 46.0%.

7. ASSAY

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

Standard solution

Gallic acid standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 5.0 mg of gallic acid CRS and dissolve in 50 mL of methanol.

Gallic acid standard solution for assay, Std-AS

Measure accurately the volume of the gallic acid Std-Stock, dilute with methanol to produce a series of solutions of 5, 10, 30, 50, 100 mg/L for gallic acid.

Test solution

Kernels of Chebulae Fructus are removed before grinding and powdering. Weigh accurately 0.1 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for two 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 (275 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The internal diameter of inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	100	0	isocratic
10 – 30	100 → 75	0 → 25	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of gallic acid Std-AS (30 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of gallic acid should not be more than 5.0%; the RSD of the retention time of gallic acid peak should not be more than 2.0%; the column efficiency determined from gallic acid peak should not be less than 40000 theoretical plates.

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

Limits

The sample contains not less than 1.2% of gallic acid ($C_7H_6O_5$), calculated with reference to the dried substance.