Crotonis Fructus (unprocessed)

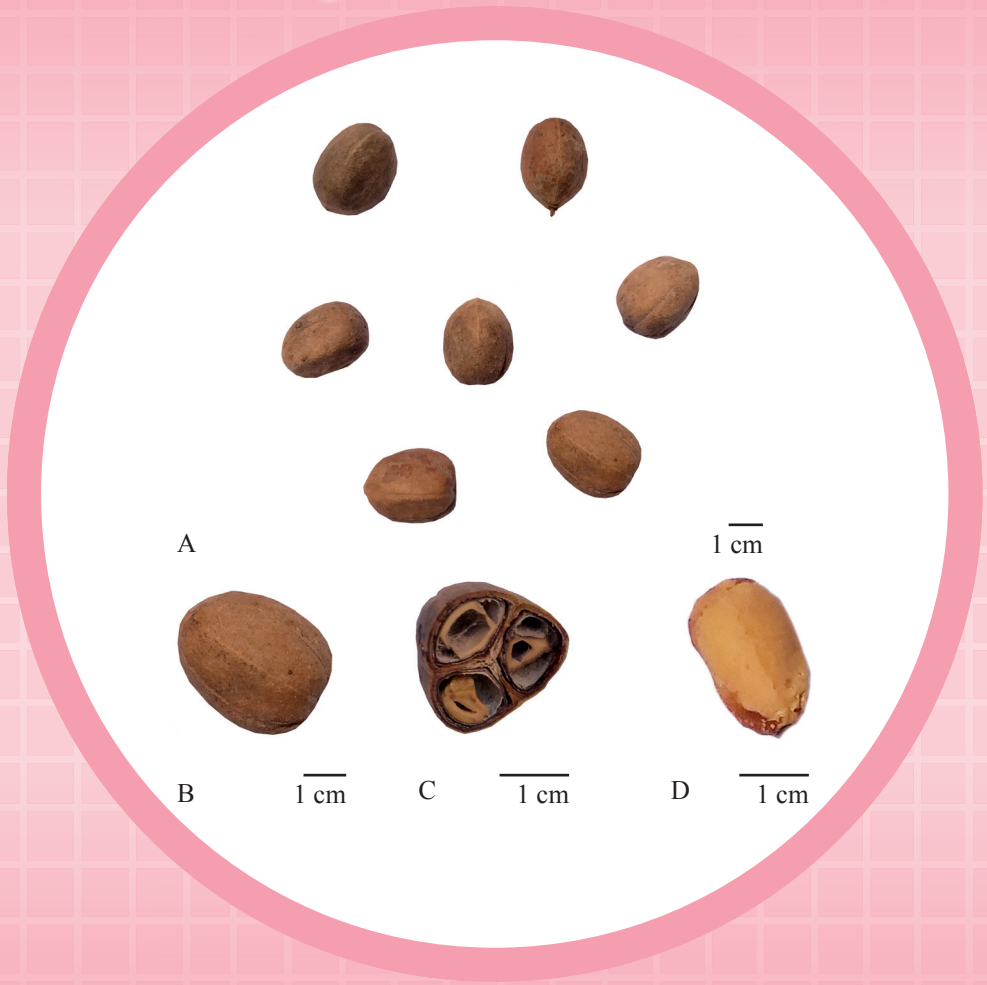

Figure 1 A photograph of Crotonis Fructus (unprocessed)

A. Crotonis Fructus (unprocessed) B. Magnified image of fruit
C. Transverse section of fruit showing seeds in 3 loculi
D. Magnified image of kernel without testa

1. NAMES

Official name: Crotonis Fructus (unprocessed)

Chinese name: 巴豆 (生)

Chinese phonetic name: Badou (Sheng)

2. SOURCE

Crotonis fructus (unprocessed) is the unprocessed dried ripe fruit of *Croton tiglium* L. (Euphorbiaceae). The fruit is collected in autumn when ripe, spread out, then dried under the sun to obtain Crotonis Fructus (unprocessed).

3. DESCRIPTION

Ellipsoidal, oblong-ovoid, oval or sub-globose, usually 3-ribbed, 1.5-3 cm long, 2 cm wide. Externally greyish to brownish, rough, with 6 longitudinal lines, radially aligned with the loculi. Apex truncate, base with fruit stalk scar. Texture hard, on opening shell, 3 loculi in shell, each containing 1 seed. Seed slightly elliptic to oblong ovate shape, 5-7 mm in diameter; externally yellowish-brown to brown. Testa thin and brittle, endotesta white, membranous; kernels yellowish-white, oily. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pericarp: Exocarp consists of 1 layer cells, flattened to irregular in shape, multi-stellate hairs occasionally found. Mesocarp consists of several layers of parenchymatous cells, scattered with stone cells, clusters of calcium oxalate in rosette aggregates and vascular bundle occasionally found; the middle part consisting of cycle band of stone cells, about 4 layers; several layers of parenchymatous cells in the inner part. Endocarp consists of 3-5 layers sclerendrymatous cells [Fig. 2(i)].

Seed: Testa cells flattened, thin, boundaries indistinct, degenerated. Endotesta membranous, consisting of 1 layer of cells. Endosperm surrounds the cotyledon, cells oblong or spherical. Clusters of calcium oxalate in rosette aggregate, scattered [Fig. 2(ii)].

Powder

Colour brownish-yellow. Mesocarp cells yellowish-brown, boundaries indistinct, shrunken. Exocarp cell reddish-brown, flattened, walls relatively thickened. Stone cells oblong, numerous, 150-232 μm in diameter. Endocarp cell walls flattened, striated and thickened, containing beaded holes. Clusters of calcium oxalate 50-175 μm in diameter, in rosette aggregate; polychromatic under polarized microscope. Fibres yellowish-brown, 25-90 μm in diameter; polychromatic under the polarized microscope. Multi-stellate hair occasionally found, star-shaped. Testa cells in groups, reddish-brown to yellowish-brown, subsquare to polygonal. Endosperm cells containing aleurone grains and oil droplets, intercellular layer distinct (Fig. 3).

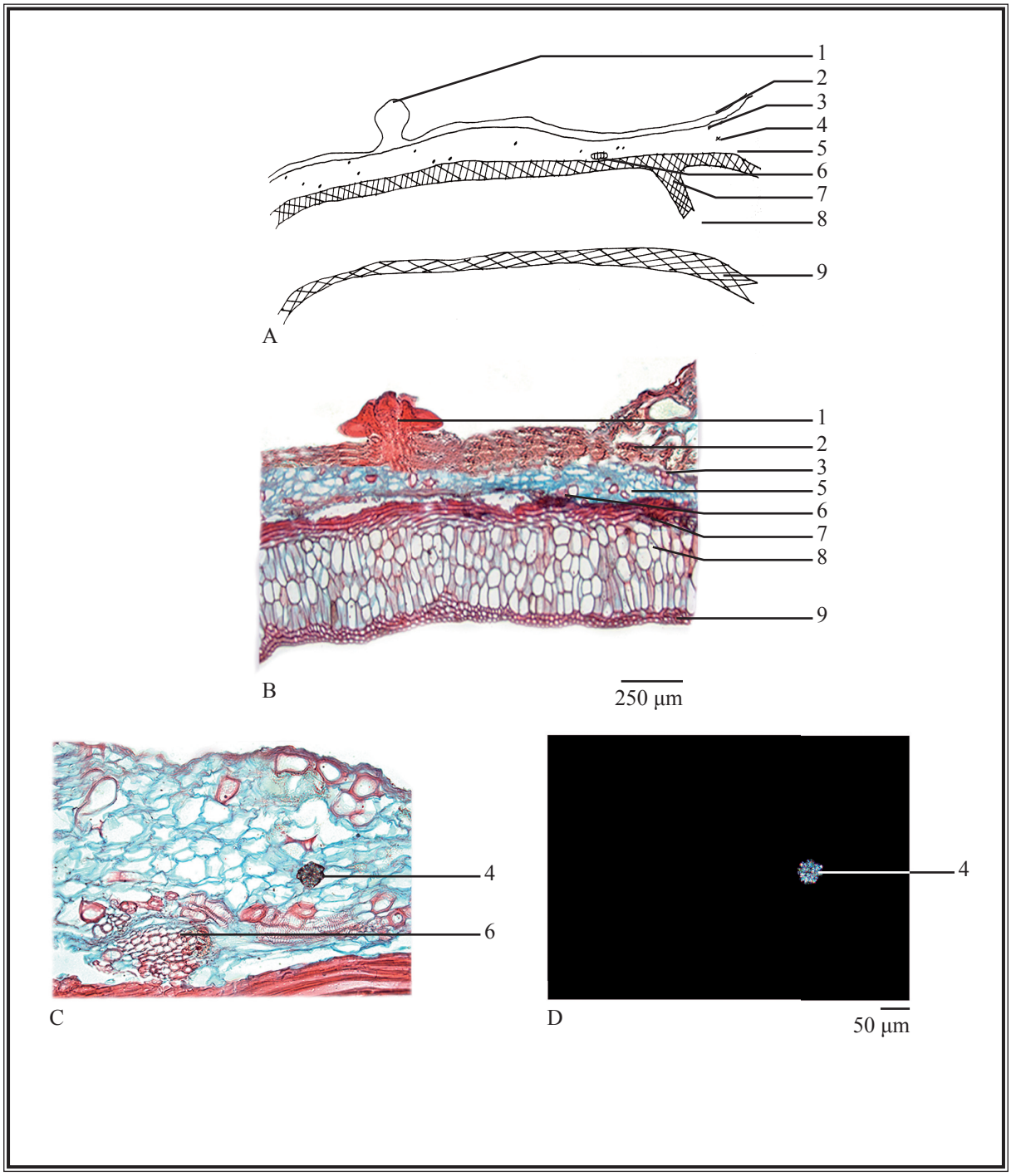

Figure 2 (i) Microscopic features of transverse section of pericarp of Crotonis Fructus (unprocessed)

A. Sketch B. Section illustration

C. Mesocarp containing cluster of calcium oxalate (under the light microscope)

D. Mesocarp containing cluster of calcium oxalate (under the polarized microscope)

1. Multi-stellate hair 2. Exocarp 3. Stone cell 4. Cluster of calcium oxalate 5. Mesocarp
6. Vascular bundle 7. Band of stone cells 8. Parenchyma 9. Endocarp

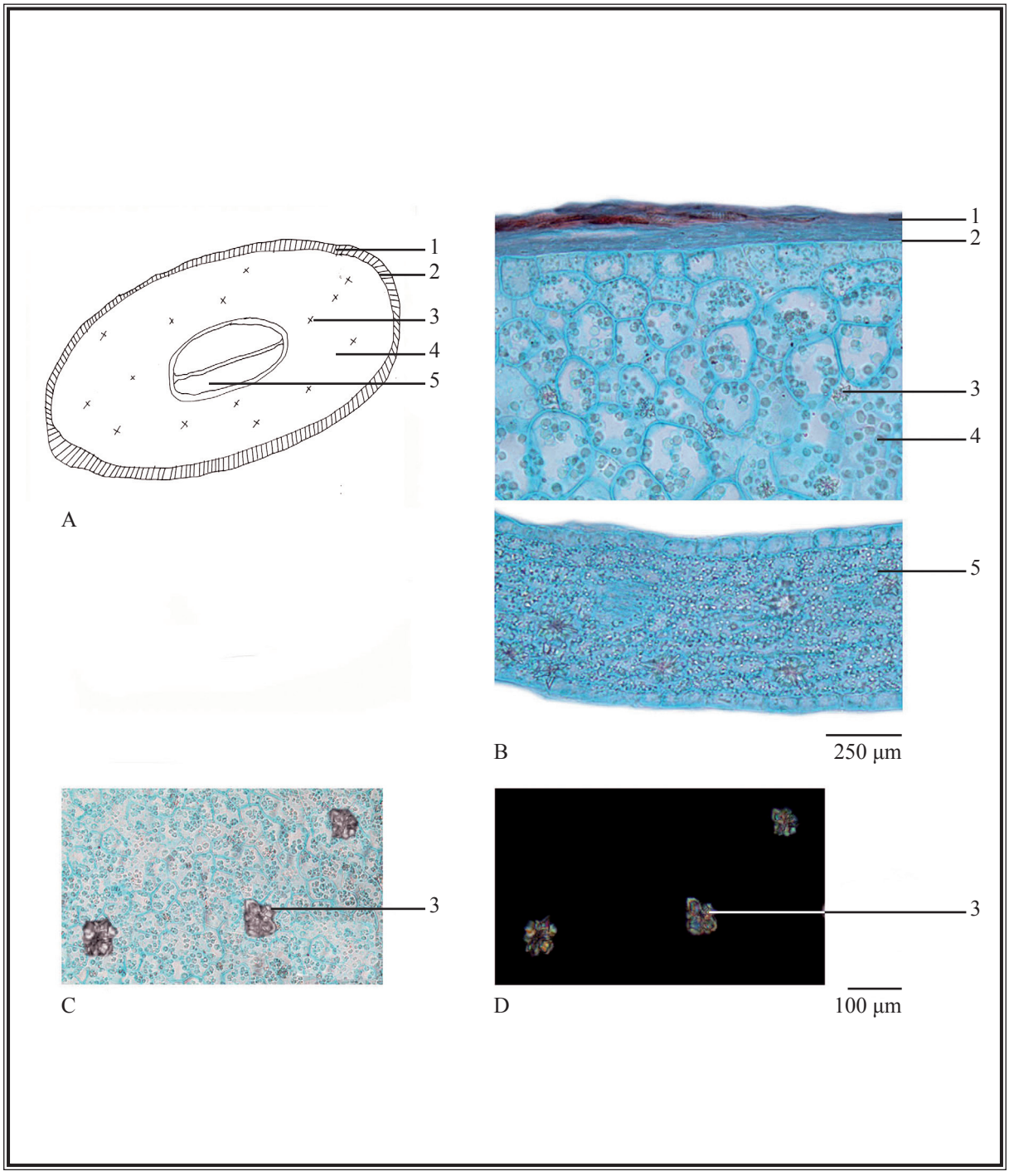

Figure 2 (ii) Microscopic features of transverse section of seed of Crotonis Fructus (unprocessed)

A. Sketch B. Section illustration

C. Clusters of calcium oxalate (under the light microscope)

D. Clusters of calcium oxalate (under the polarized microscope)

1. Testa 2. Endotesta 3. Cluster of calcium oxalate 4. Endosperm 5. Cotyledon

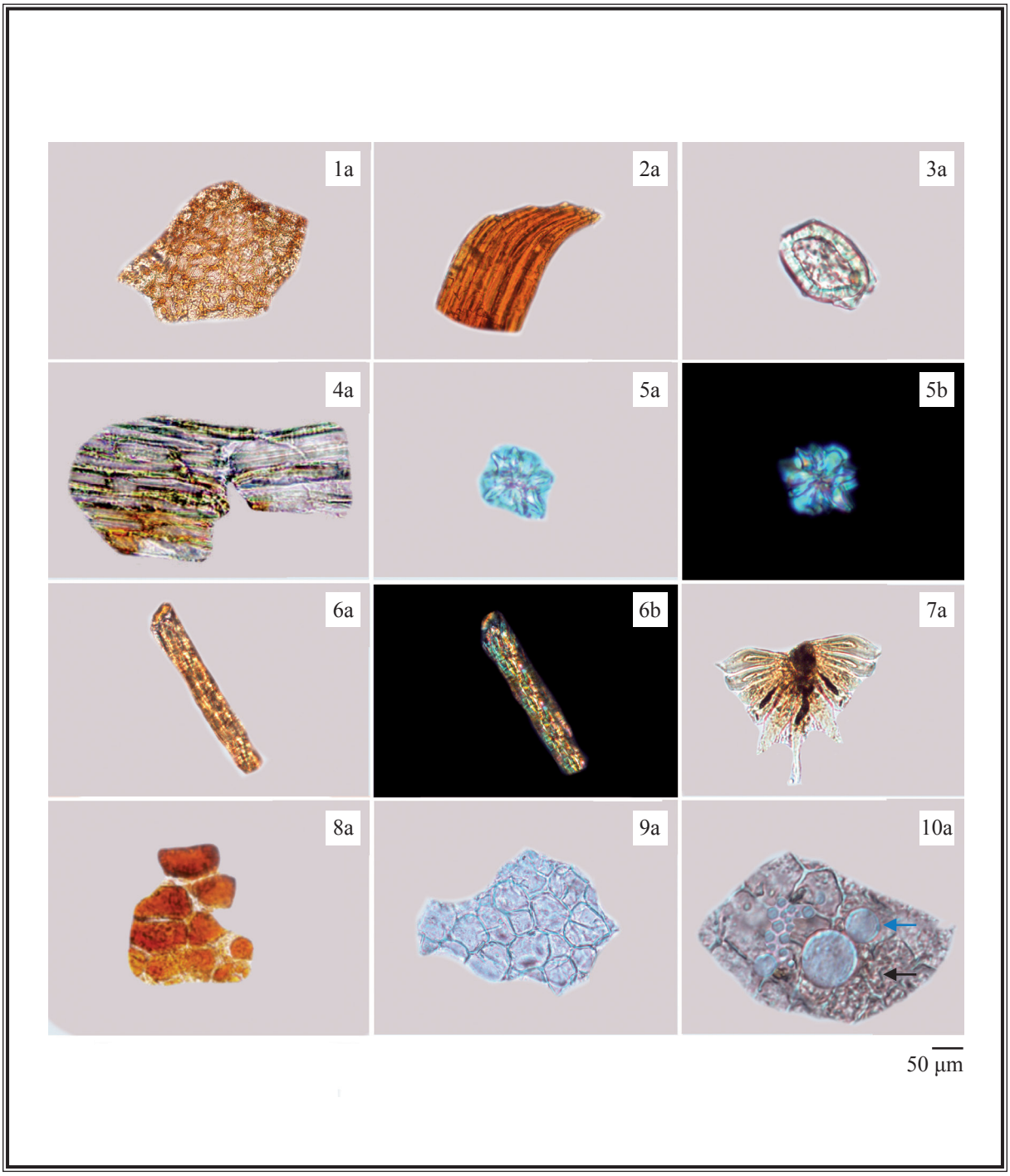

Figure 3 Microscopic features of the powder of Crotonis Fructus (unprocessed)

1. Mesocarp cells 2. Exocarp cells 3. Stone cell 4. Endocarp cells
5. Cluster of calcium oxalate 6. Fibre 7. Multi-stellate hair
8. Testa cells 9. Endosperm cells
10. Endosperm cells containing aleurone grains (→) and oil droplets (→)

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

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

Standard solution

Crotonoside standard solution

Weigh 1.2 mg of crotonoside CRS (Fig. 4) and dissolve in 1 mL of methanol (30%).

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), water, methanol and *n*-butanol (0.5: 1: 4: 4, v/v).

Staining reagent

Iodine.

Test solution

Remove the seed coat and pulverise the kernels to powder. Weigh 1.0 g of the powdered kernel sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (30%). Sonicate (200 W) the mixture for 15 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

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 crotonoside standard solution (3 μL) and the test solution (6 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 6 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Expose the plate to iodine vapour in a chamber for about 5-10 min. Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

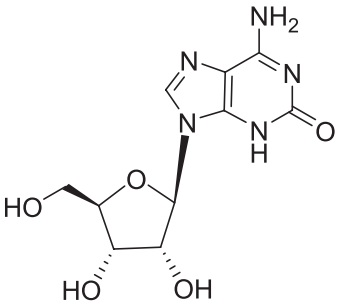

Figure 4 Chemical structure of crotonoside

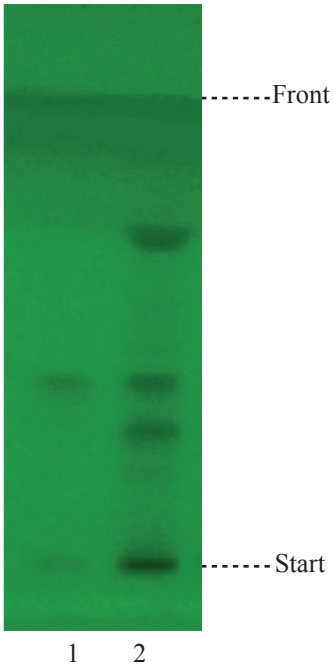

Figure 5 A reference HPTLC chromatogram of kernel of Crotonis Fructus (unprocessed) extract observed under UV light (254 nm) after staining

1. Crotonoside standard solution 2. Test solution

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 crotonoside (Fig. 5).

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

Standard solution

Crotonoside standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 5.0 mg of crotonoside CRS and dissolve in 100 mL water.

Test solution

Remove the seed coat and pulverise the kernels to powder. Weigh 0.3 g of the powdered kernel sample and place it in a 100-mL round-bottomed flask, then add 25 mL of water. Reflux the mixture for 1 h. Cool down to room temperature. Transfer the extract to a 50-mL volumetric flask. Repeat the extraction one more time. Combine the extracts and make up to the mark with water. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (215 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (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% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	100 → 95	0 → 5	linear gradient
15 – 30	95 → 65	5 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of crotonoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of crotonoside should not be more than 5.0%; the RSD of the retention time of crotonoside peak should not be more than 2.0%; the column efficiency determined from crotonoside peak should not be less than 20000 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).

Procedure

Separately inject crotonoside Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of crotonoside peak in the chromatogram of crotonoside Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify crotonoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of crotonoside Std-FP. The retention times of crotonoside 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 Crotonis Fructus (unprocessed) extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Crotonis Fructus (unprocessed) extract

Peak No.	RRT	Acceptable Range
1 (marker, crotonoside)	1.00	-
2 (magnoflorine)	2.77	± 0.05
3	2.92	± 0.05

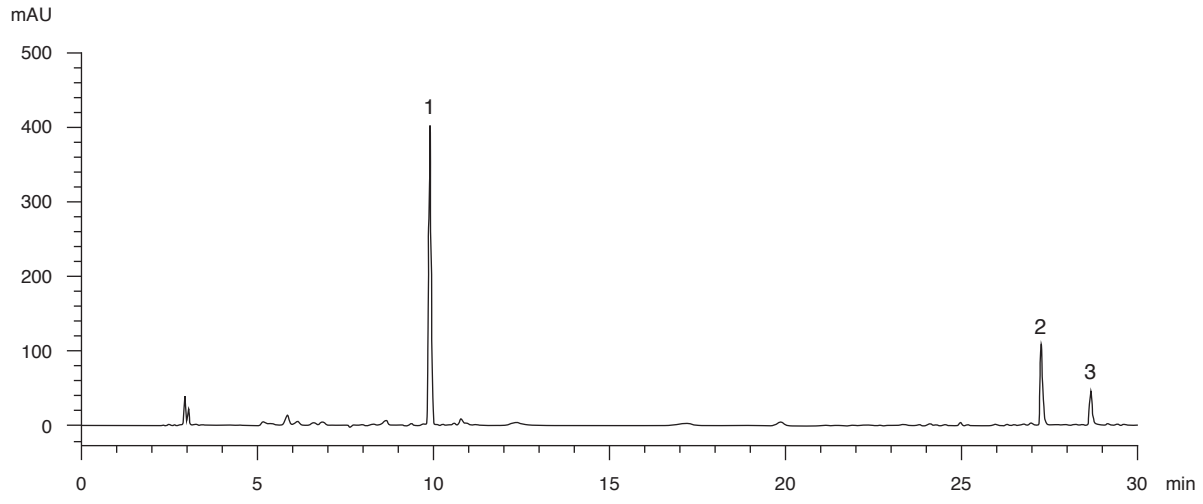

Figure 6 A reference fingerprint chromatogram of kernel of Crotonis Fructus (unprocessed) extract

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 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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 8.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 5.0 mg of crotonoside CRS and dissolve in 5 mL of water.

Crotonoside standard solution for assay, Std-AS

Measure accurately the volume of the crotonoside Std-Stock, dilute with water to produce a series of solutions of 20, 40, 50, 60, 80 mg/L for crotonoside.

Test solution

Remove the seed coat and pulverise the kernels to powder. Weigh accurately 0.3 g of the powdered kernel sample and place it in a 100-mL round-bottomed flask, then add 25 mL of water. Reflux the mixture for 1 h. Cool down to room temperature. Transfer the extract to a 50-mL volumetric flask. Repeat the extraction one more time. Combine the extracts and make up to the mark with water. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (215 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 μm particle size). 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)	Acetonitrile (%, v/v)	Elution
0 – 15	100 → 95	0 → 5	linear gradient
15 – 30	95 → 65	5 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of crotonoside Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of crotonoside should not be more than 5.0%; the RSD of the retention time of crotonoside peak should not be more than 2.0%; the column efficiency determined from crotonoside peak should not be less than 20000 theoretical plates.

The *R* value between crotonoside peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

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

Limits

The sample contains not less than 0.81% of crotonoside ($C_{10}H_{13}N_5O_5$), calculated with reference to the dried substance.

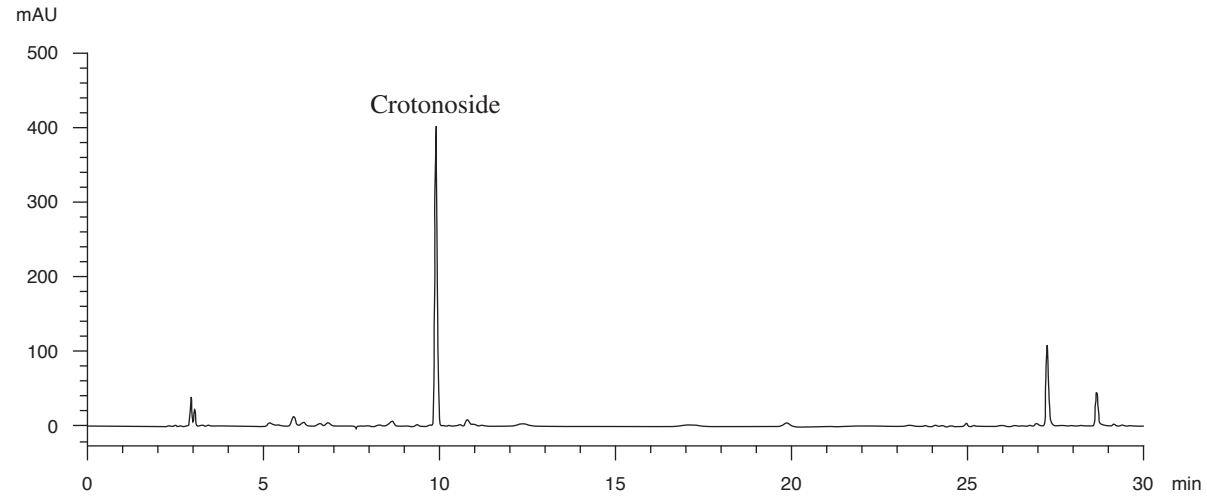

Figure 7 A reference assay chromatogram of kernel of Crotonis Fructus (unprocessed) extract

8. CAUTION

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.