Amomi Fructus Rotundus

Figure 1 A photograph of Amomi Fructus Rotundus

A. Amomi Fructus Rotundus

B. Transverse section of fruit and magnified image of seeds

1. NAMES

Official Name: Amomi Fructus Rotundus

Chinese Name: 豆蔻

Chinese Phonetic Name: Doukou

2. SOURCE

Amomi Fructus Rotundus is the dried ripe fruit of *Amomum compactum* Soland ex Maton (Zingiberaceae). The fruit-spike is collected mostly in July and August when the fruit is nearly ripe and turns yellow but indehiscent, persistent perianth and fruit stalk removed, then dried under the sun to obtain Amomi Fructus Rotundus**.**

3. DESCRIPTION

Subspherical, 8-15 mm in diameter. Externally yellowish-white to yellowish-brown, sometimes slightly showing purplish-brown, with 3 relatively deep longitudinal furrows, apex with a prominent stylopodium, base with a fruit stalk scar, both ends bearing pale brown pubescence. Texture of pericarp fragile and light in weight, easily broken longitudinally, divided into 3 loculi, each containing 4-10 seeds. Seeds irregularly polyhedral, dorsal surface slightly protuberant, 3-4 mm in diameter, externally greyish-brown or dark brown, with wrinkles, covered with whitish membranous aril. Odour aromatic; taste pungent and cool, slightly camphor-like (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Longitudinal section of seed

Aril sometimes remnant, consisting of serveral layers of slender parenchymatous cells, some containing crystal sand of calcium oxalate. Epidermis of testa consists of 1 layer of cells, cells radially elongated, covered with cuticle. Hypodermis of testa consists 1-2 layers of cells, containing yellowish-brown or reddish-brown contents (before stained). Oil cells layer consists of 1 layer of subrectangular cells, 40-75 μm in diameter, with thin walls. Pigment layer consists several layers of cells, containing dark reddish-brown contents (before stained). Endotesta consists 1 layer of cells, reddish-brown or yellowish-brown (before stained), 10-35 μm in

diameter, with extremely thick inner and side walls, lumen located on the upper part of the cells, containing silica bodies. Perisperm cells filled with small starch granules, some containing prisms of calcium oxalate. Endosperm and parenchymatous cells of embryo contain aleurone granules and oil droplets (before stained). Hilum position located on cupped side of the seed, cells contain reddish-brown contents (before stained) (Fig. 2).

Transverse section of pericarp

Exocarp consists of 1 layer of flattened rectangular parenchymatous cells, 25-60 μm long, about 8 μm wide. Parenchymatous cells of mesocarp subrounded to oblong. Collateral vascular bundles located on the inner side of mesocarp; crescent-shaped fibre bundles located on the outer side of vascular bundles. 1-4 layers of stone cells arranged in an interrupted band, located in between vascular bundles, stone cells subrounded to subsquare, with distinct pits. Parenchymatous cells scattered with prisms of calcium oxalate. Endocarp consists 1 layer of rectangular parenchymatous cells, mostly shrunken (Fig. 3).

Powder

Colour pale brown or greyish-brown. Stone cells varied in shape, 35-150 μm in diameter, pits distinct, lumens relatively large. Endotesta cells yellowish-brown or reddish-brown, polygonal in surface view, 10-35 μm in diameter, containing silica bodies; 1 layer of palisade-like cells in lateral view, with extremely thick inner and side walls, lumens located on the upper part of the cells, containing silica bodies. Epidermal cells of testa pale yellow, long stripe-shaped in surface view, walls slightly thickened, usually arranged vertically with yellowish-brown hypodermal cells. Perisperm cells subrectangular, containing small starch masses, usually with prisms of calcium oxalate; polychromatic under the polarized microscope. Oil cells nearly colourless to pale yellow, subsquare, 40-75 μm in diameter. Prisms of calcium oxalate scattered, 2-20 μm in diameter; bright white or polychromatic under the polarized microscope. Aril cells with thin walls, the wall slightly curved, some cells contain crystal sand of calcium oxalate. Fibres mostly broken, 15-25 μm in diameter, lumens distinct (Fig. 4).

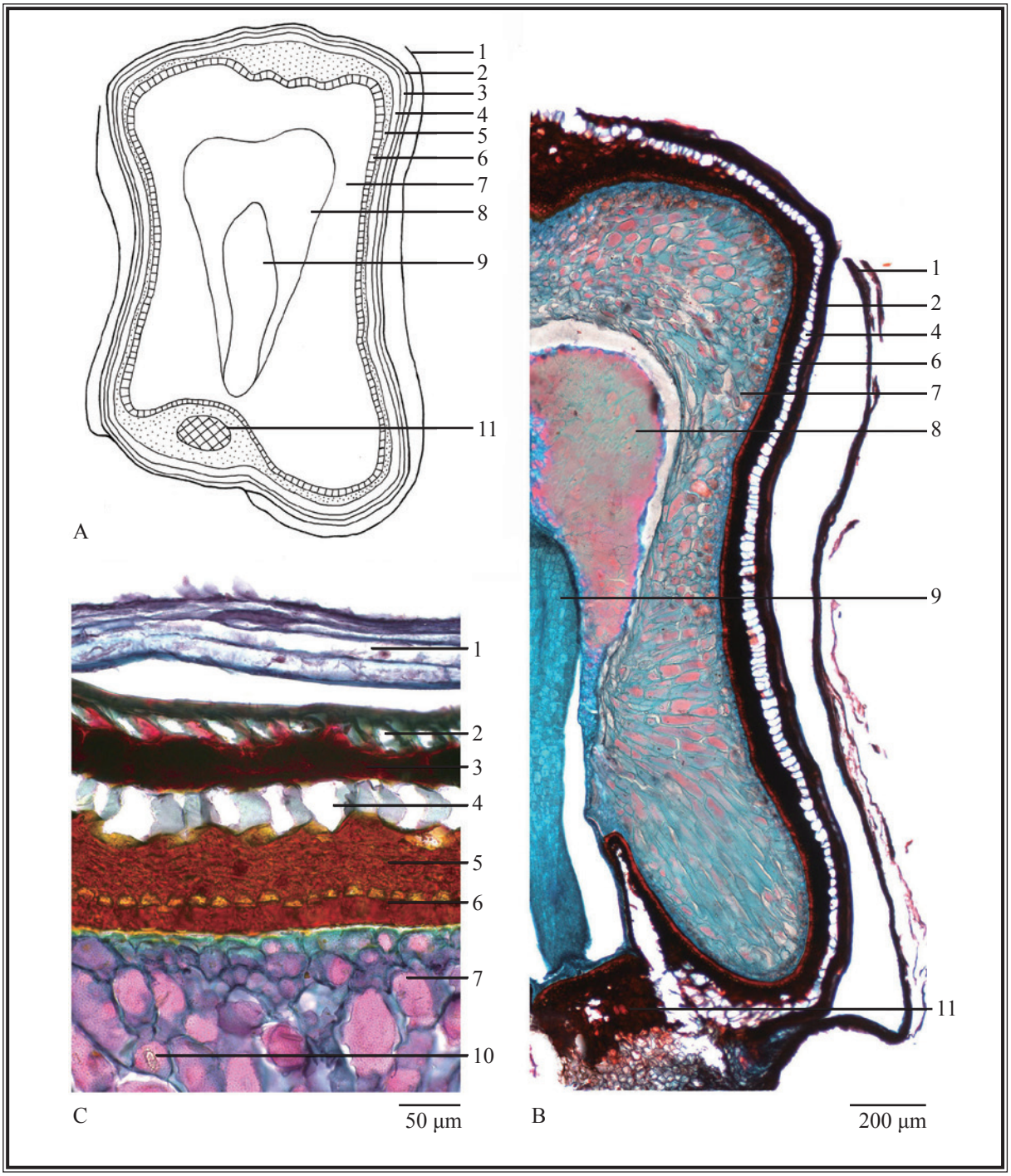

Figure 2 Microscopic features of longitudinal section of seed of Amomi Fructus Rotundus

A. Sketch B. Section illustration C. Section magnified

1. Aril 2. Epidermis of testa 3. Hypodermis of testa 4. Oil cells layer
5. Pigment layer 6. Endotesta 7. Perisperm 8. Endosperm 9. Embryo
10. Prism of calcium oxalate 11. Hilum

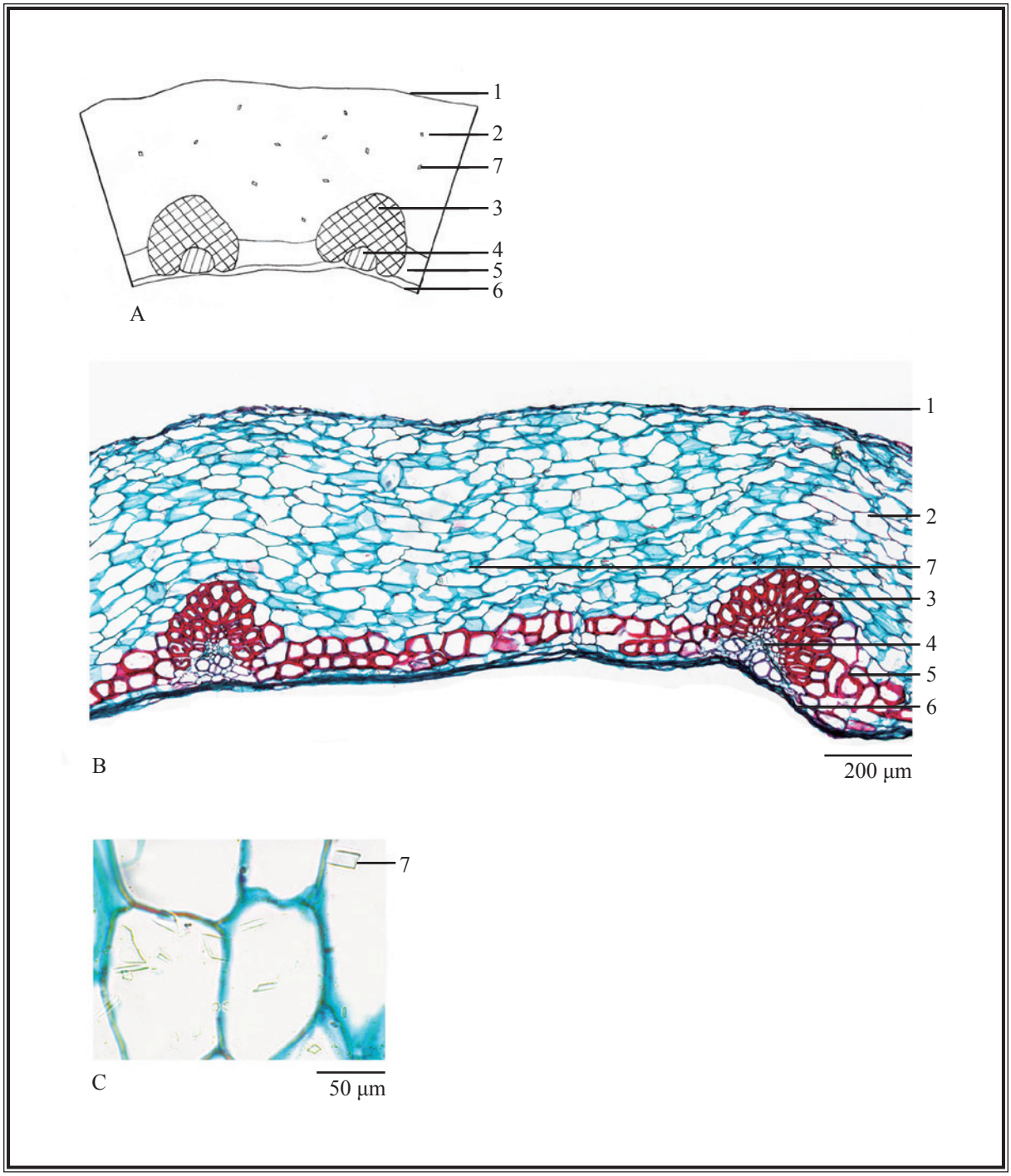

Figure 3 Microscopic features of transverse section of pericarp of Amomi Fructus Rotundus

A. Sketch B. Section illustration C. Prisms of calcium oxalate in mesocarp

1. Exocarp 2. Mesocarp 3. Fibre bundle 4. Vascular bundle
5. Stone cell 6. Endocarp 7. Prism of calcium oxalate

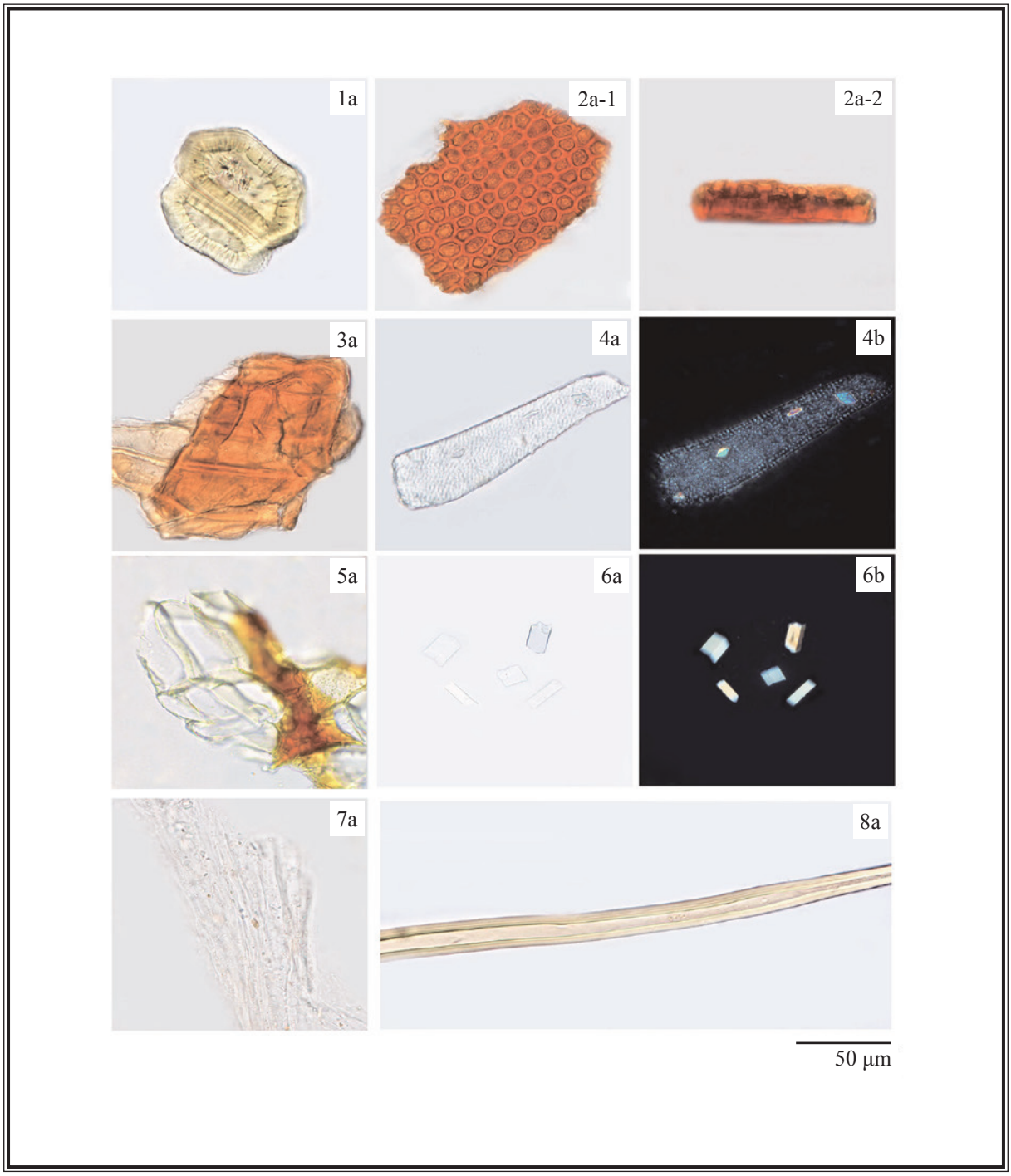

Figure 4 Microscopic features of powder of Amomi Fructus Rotundus

1. Stone cells 2. Endotesta cells with silica bodies (2-1 in surface view, 2-2 in lateral view)
3. Epidermal cells of testa 4. Perisperm cells 5. Oil cells
6. Prisms of calcium oxalate 7. Aril cells 8. Fibre

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

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

Standard solution

1,8-Cineole standard solution

Weigh 5.0 mg of 1,8-cineole CRS (Fig. 5) and dissolve in 5 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of cyclohexane and ethyl acetate (19:1, v/v).

Spray reagent

Weigh 5 g of vanillin and dissolve in 100 mL of sulphuric acid.

Test solution

Kieselguhr is added to the sample before powdering in the ratio of 1:2. Weigh 1.5 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of ethyl acetate. Sonicate (320 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at about 40ºC at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of ethyl acetate.

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 1,8-cineole standard solution (3 μL) and the test solution (2 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 visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

Figure 5 Chemical structure of 1,8-cineole

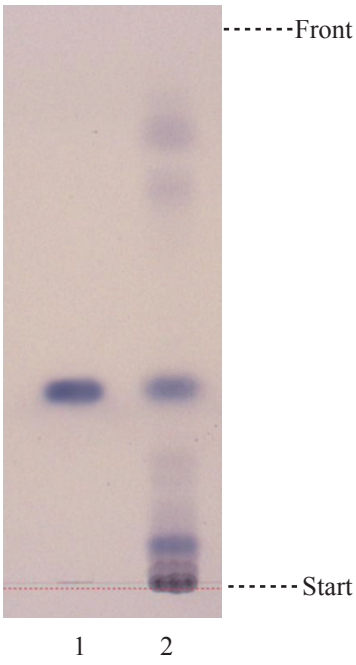

Figure 6 A reference HPTLC chromatogram of Amomi Fructus Rotundus extract observed under visible light after staining

1. 1,8-Cineole 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 1,8-cineole (Fig. 6).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

1,8-Cineole standard solution for fingerprinting, Std-FP (1000 mg/L)

Weigh 10.0 mg of 1,8-cineole CRS and dissolve in 10 mL of *n*-hexane.

Test solution

Kieselguhr is added to the sample before powdering in the ratio of 1:2. Weigh 1.5 g of the powdered sample and place it in a 500-mL round-bottomed flask, then add 200 mL of water. Connect the round-bottomed flask to a volatile oil determination tube, then add 20 mL of water and 3 mL of *n*-hexane. Connect to the condenser and heat the flask gently until boiling for 2 h. Cool down and allow to stand until two layers can be separated. Transfer the *n*-hexane layer to a 25-mL volumetric flask. Transfer the aqueous layer to a 100-mL separating funnel. Rinse the volatile oil determination tube with 5 mL of *n*-hexane. Transfer the solution to the separating funnel and extract with aqueous layer. Collect the *n*-hexane extract and filter through the funnel containing 1 g of anhydrous sodium sulphate. Transfer the filtrate to a 25-mL volumetric flask. Extract the aqueous layer with 5 mL of *n*-hexane. Collect the *n*-hexane extract and filter through the funnel containing 1 g of anhydrous sodium sulphate. Transfer the filtrate to a 25-mL volumetric flask. Wash the filter funnel with 5 mL of *n*-hexane. Combine the *n*-hexane extracts and make up to the mark with *n*-hexane. Filter through a 0.45-μm nylon filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-1701, 0.32 mm × 30 m) of which the internal wall is covered with (14%-cyanopropyl-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 210ºC. The detector temperature is at 230ºC. The split injection mode at a ratio of 20:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 3	40	–
3 – 39	40 → 220	5

System suitability requirements

Perform at least five replicate injections, each using 1 μL of 1,8-cineole Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of 1,8-cineole should not be more than 5.0%; the RSD of the retention time of 1,8-cineole peak should not be more than 2.0%; the column efficiency determined from 1,8-cineole peak should not be less than 100000 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. 7).

Procedure

Separately inject 1,8-cineole Std-FP and the test solution (1 µL each) into the GC system and record the chromatograms. Measure the retention time of 1,8-cineole peak in the chromatogram of 1,8-cineole Std-FP and the retention times of the five characteristic peaks (Fig. 7) in the chromatogram of the test solution. Identify 1,8-cineole peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 1,8-cineole Std-FP. The retention times of 1,8-cineole 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 five characteristic peaks of Amomi Fructus Rotundus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Amomi Fructus Rotundus extract

Peak No.	RRT	Acceptable Range
1 (α-pinene)	0.74	± 0.03
2	0.89	± 0.04
3 (limonene)	0.96	± 0.03
4 (marker, 1,8-cineole)	1.00	-
5 (terpinen-4-ol)	1.42	± 0.03

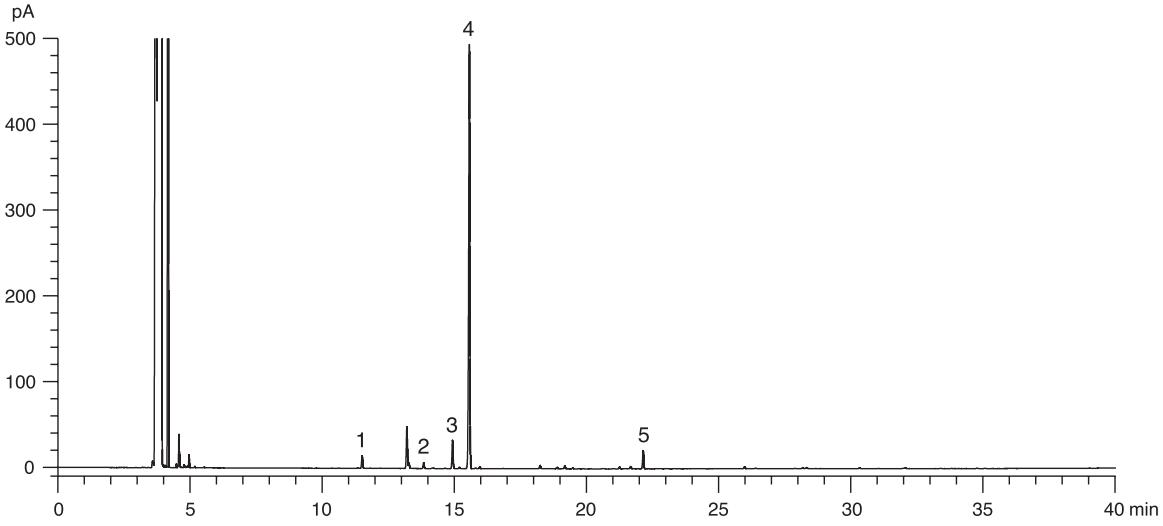

Figure 7 A reference GC fingerprint chromatogram of Amomi Fructus Rotundus extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference GC fingerprint chromatogram (Fig. 7).

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 11.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 12.0%.
Ethanol-soluble extractives (hot extraction method): not less than 5.0%.

7. ASSAY

7.1 Assay of 1,8-Cineole

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

Standard solution

1,8-Cineole standard stock solution, Std-Stock (5000 mg/L)

Weigh accurately 50.0 mg of 1,8-cineole CRS and dissolve in 10 mL of *n*-hexane.

1,8-Cineole standard solution for assay, Std-AS

Measure accurately the volume of the 1,8-cineole Std-Stock, dilute with *n*-hexane to produce a series of solutions of 200, 500, 1000, 2000, 2500 mg/L for 1,8-cineole.

Test solution

Kieselguhr is added to the sample before powdering in the ratio of 1:2. Weigh accurately 1.5 g of the powdered sample and place it in a 500-mL round-bottomed flask, then add 200 mL of water. Connect the round-bottomed flask to a volatile oil determination tube, then add 20 mL of water and 3 mL of *n*-hexane. Connect to the condenser and heat the flask gently until boiling for 2 h. Cool down and allow to stand until two layers can be separated. Transfer the *n*-hexane layer to a 25-mL volumetric flask. Transfer the aqueous layer to a 100-mL separating funnel. Rinse the volatile oil determination tube with 5 mL of *n*-hexane. Transfer the solution to the separating funnel and extract with aqueous layer. Collect the *n*-hexane extract and filter through the funnel containing 1 g of anhydrous sodium sulphate. Transfer the filtrate to a 25-mL volumetric flask. Extract the aqueous layer with 5 mL of n-hexane. Collect the *n*-hexane extract and filter through the funnel containing 1 g of anhydrous sodium sulphate. Transfer the filtrate to a 25-mL volumetric flask. Wash the filter funnel with 5 mL of *n*-hexane. Combine the *n*-hexane extracts and make up to the mark with *n*-hexane. Filter through a 0.45-μm nylon filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-1701, 0.32 mm × 30 m) of which the internal wall is covered with (14%-cyanopropyl-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 210ºC. The detector temperature is at 230ºC. The split injection mode at a ratio of 20:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 3	40	-
3 – 39	40 → 220	5

System suitability requirements

Perform at least five replicate injections, each using 1 μL of 1,8-cineole Std-AS (1000 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 1,8-cineole should not be more than 5.0%; the RSD of the retention time of 1,8-cineole peak should not be more than 2.0%; the column efficiency determined from 1,8-cineole peak should not be less than 100000 theoretical plates.

The *R* value between 1,8-cineole 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 1,8-cineole Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of 1,8-cineole against the corresponding concentrations of 1,8-cineole Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify 1,8-cineole peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 1,8-cineole Std-AS. The retention times of 1,8-cineole 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 1,8-cineole in the test solution, and calculate the percentage content of 1,8-cineole in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 3.0% of 1,8-cineole ($C_{10}H_{18}O$), calculated with reference to the dried substance.

7.2 Assay of Volatile Oil

Weigh accurately 30 g of the powdered sample and place it in a 1000-mL round-bottomed flask. Add 500 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method A).

Limits

The sample contains not less than 4.0% (v/w) of volatile oil.