Amomi Fructus

Figure 1 (i) A photograph of dried ripe fruit of *Amomum villosum* Lour.

A. Fruits B. Outer surface of pericarp
C. Inner surface of pericarp D. Seed mass
E. Magnified image of seeds

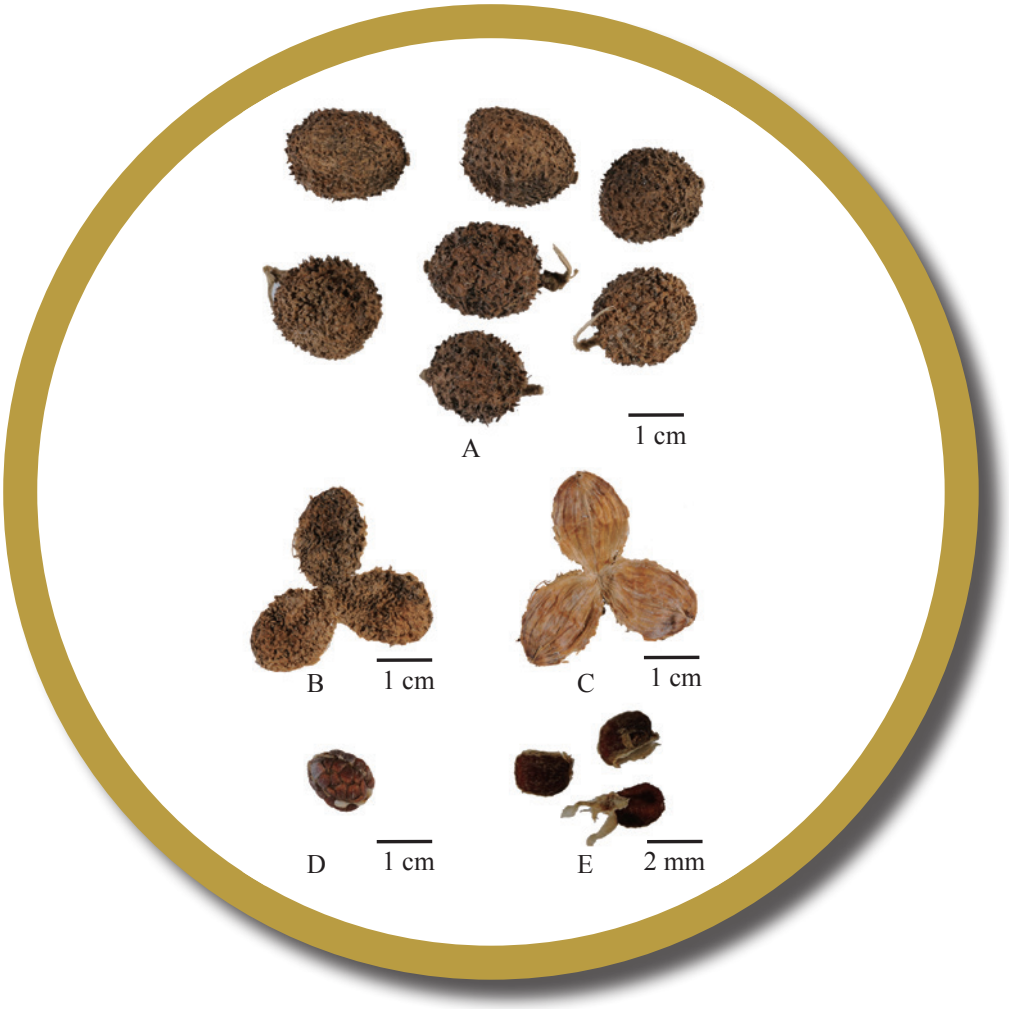


Figure 1 (ii) A photograph of dried ripe fruit of *Amomum villosum* Lour. var. *xanthioides* T.L. Wu et Senjen

A. Fruits B. Outer surface of pericarp C. Inner surface of pericarp
D. Seed mass E. Magnified image of seeds

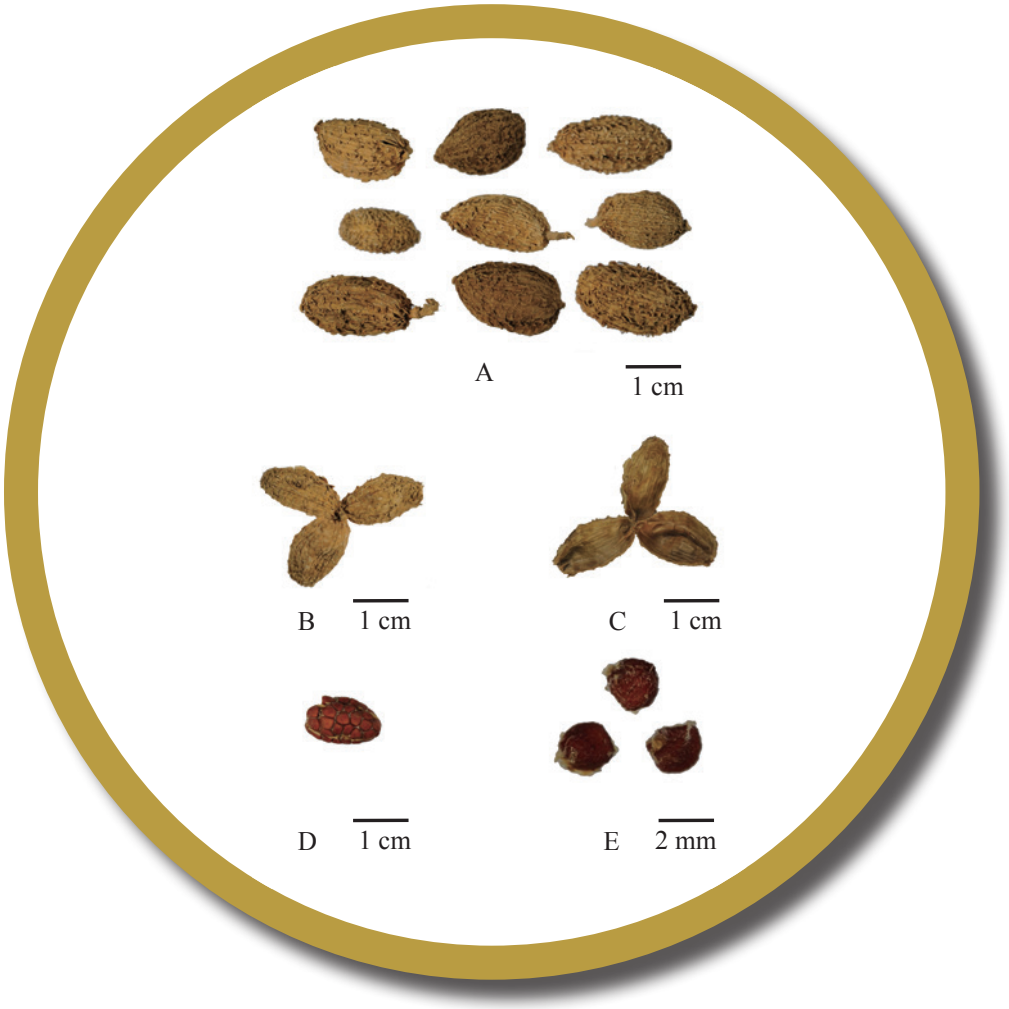


Figure 1 (iii) A photograph of dried ripe fruit of *Amomum longiligulare* T. L. Wu

A. Fruits B. Outer surface of pericarp C. Inner surface of pericarp
D. Seed mass E. Magnified image of seeds

1. NAMES

Official Name: Amomi Fructus

Chinese Name: 砂仁

Chinese Phonetic Name: Sharen

2. SOURCE

Amomi Fructus is the dried ripe fruit of *Amomum villosum* Lour., *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen or *Amomum longiligulare* T. L. Wu (Zingiberaceae). The fruit is collected in summer and autumn when ripe, and then dried under the sun to obtain Amomi Fructus.

Part I Dried ripe fruit of *Amomum villosum* Lour. and *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen

3. DESCRIPTION

***Amomum villosum* Lour.:** Ellipsoid or ovoid, slightly 3-ridged, 0.7-2.3 cm long, 5-16 mm in diameter; externally greyish-brown to dark brown, densely covered with spiny protrudings, with remnants of calyx at apex and a fruit stalk often at base. Pericarp thin and soft. Seeds aggregated into an obtusely 3-ridged mass, divided into 3 segments separated by white septa, each segment consists of 3-26 seeds. Seeds irregularly polyhedral, 2-3 mm in diameter; externally brownish-red to dark brown, finely wrinkled, covered with pale brown membranous aril; texture hard, endosperm greyish-white. Odour strongly aromatic; taste pungent, cool and slightly bitter [Fig. 1 (i)].

***Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen:** Ellipsoid or ovoid, indistinctly 3-ridged, 0.9-2.5 cm long, 6-20 mm in diameter. Seeds agglutinated into an obtusely 3-ridged mass, divided into 3 segments separated by white septa, each segment consists of 4-26 seeds [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

***Amomum villosum* Lour.**

Pericarp: Exocarp consists of 1 layer of flattened rectangular cells. Mesocarp broad; parenchymatous cells relatively large, subrounded to oblong; vascular bundles collateral, arranged in an interrupted ring in the inner side of mesocarp, surrounded by cresent-shaped fibre bundles; sieve tubes and vessels visible; parenchymatous cells scattered with crystals of calcium oxalate. Endocarp consists of 1 layer of rectangular cells, mostly shrunken [Fig. 2 (i)].

Seeds: Aril present sometimes, consisting of several layers of slender cells. Epidermis of testa consists of 1 layer of cells, cells radially elongated, slightly thick-walled. Hypodermis consists of 1 layer of cells, containing brown to reddish-brown contents. Oil cell layer consists of 1 layer of subrectangular cells, with thin walls. Pigment layer consists of several layers of cells, cells polygonal, irregularly arranged, containing dark reddish-brown contents. Endotesta consists of 1 layer of small, palisade-like thick-walled cells, small, containing silica bodies, inner and lateral walls heavily thickened. Perisperm cells filled with small starch granules, some contain crystals of calcium oxalate. Endosperm and embryo cells contain fine aleurone grains. Hilum deeply indented [Fig. 2 (ii)].

***Amomum villosum* Lour. Var. *xanthioides* T. L. Wu et Senjen:** There is no significant difference in features as compared with *Amomum villosum* Lour. [Fig.2 (iii-iv)].

Powder

***Amomum villosum* Lour.:** Colour greyish-brown. Endotesta cells yellowish-brown to reddish-brown, polygonal in surface view, with thickened and non-lignified walls, lumen contains silica bodies. Epidermal cells of testa pale yellow, elongated in surface view. Hypodermal cells rectangular to oblong, containing brown to reddish-brown contents. Oil cells nearly colourless to pale yellow, easily broken, subsquare, 16-96 μm in diameter, sometimes with oil droplets. Clusters and prisms of calcium oxalate scattered in parenchymatous cells, 2-41 μm in diameter; polychromatic under the polarized microscope. Mainly spiral vessels, 8-41μm in diameter. Pigment cells usually shrunken, boundaries unclear, containing reddish-brown to dark brown contents. Perisperm cells subrectangular or irregular in shape, filled with starch masses formed by aggregation of small starch granules [Fig. 3 (i)].

***Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen:** Oil cells 17-76 μm in diameter. Spiral vessels 11-52 μm in diameter. Clusters and prisms of calcium oxalate 2-46 μm in diameter [Fig. 3 (ii)].

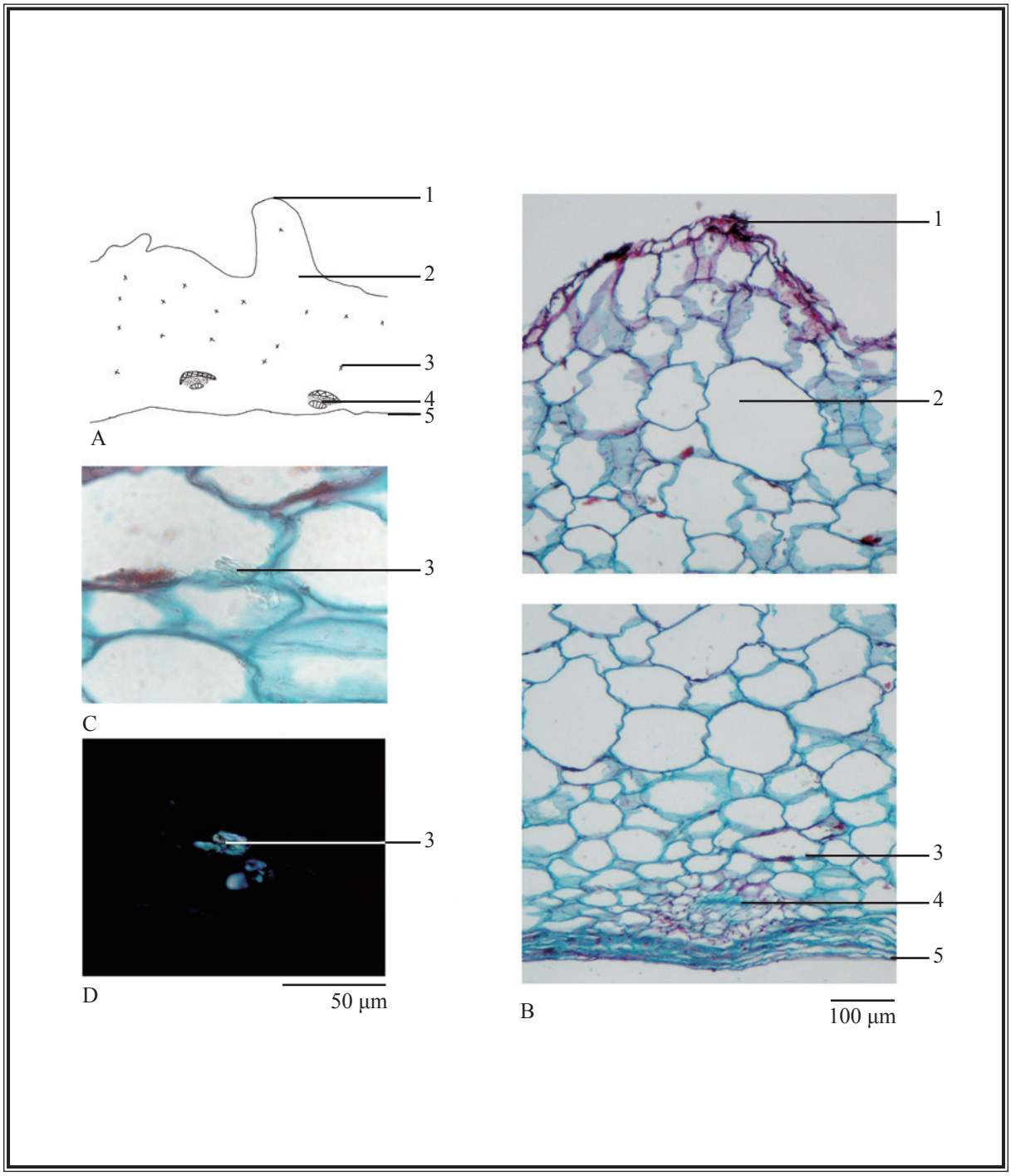


Figure 2 (i) Microscopic features of transverse section of pericarp of *Amomum villosum* Lour.

A. Sketch B. Section illustration C. Crystals of calcium oxalate in mesocarp (under the light microscope)

D. Crystals of calcium oxalate in mesocarp (under the polarized microscope)

1. Exocarp 2. Mesocarp 3. Crystal of calcium oxalate 4. Vascular bundle 5. Endocarp

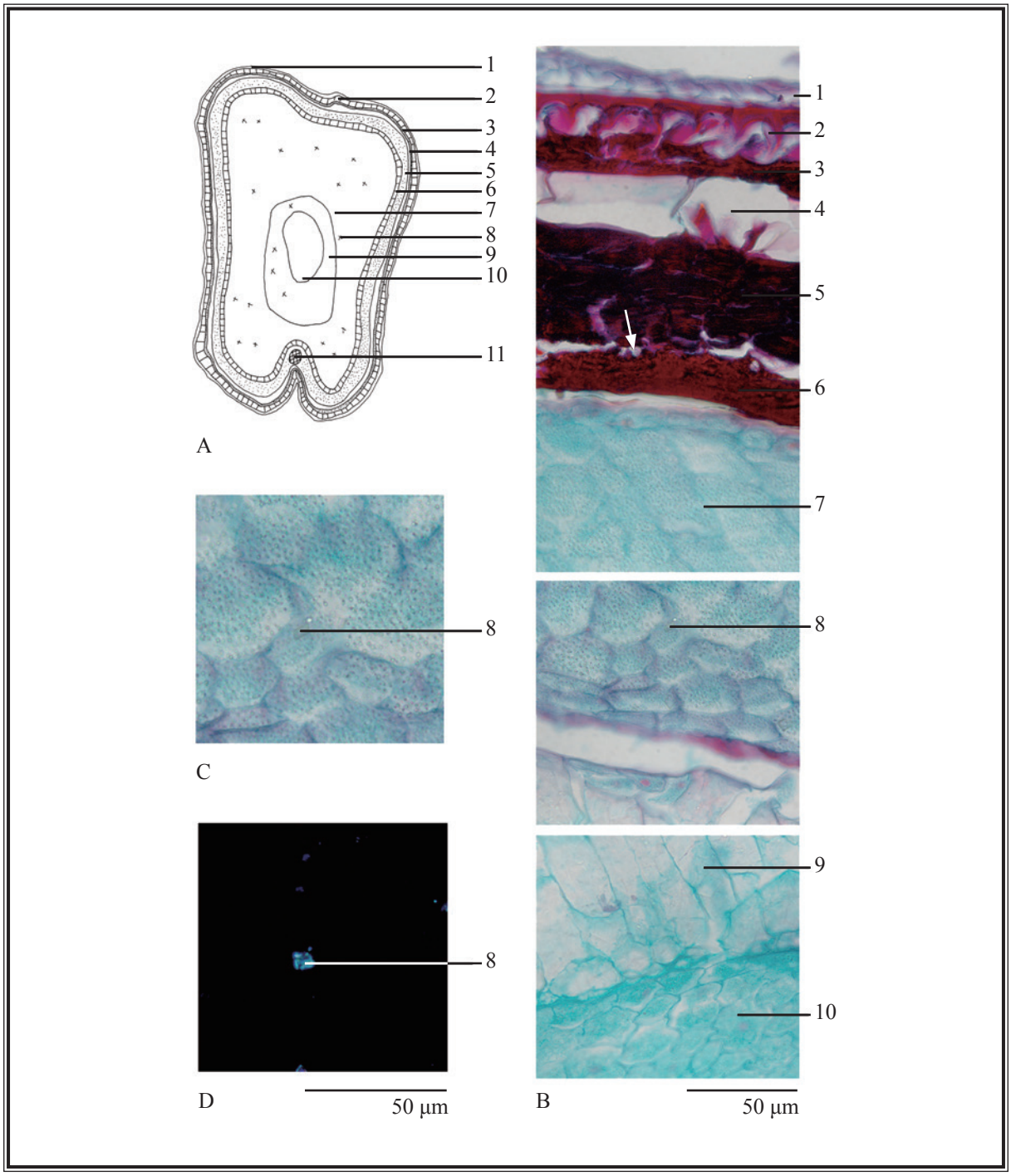


Figure 2 (ii) Microscopic features of transverse section of seed of *Amomum villosum* Lour.

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

1. Aril 2. Epidermis of testa 3. Hypodermis of testa 4. Oil cell 5. Pigment layer
6. Endotesta (silica body→) 7. Perisperm 8. Crystal of calcium oxalate 9. Endosperm
10. Embryo 11. Hilum

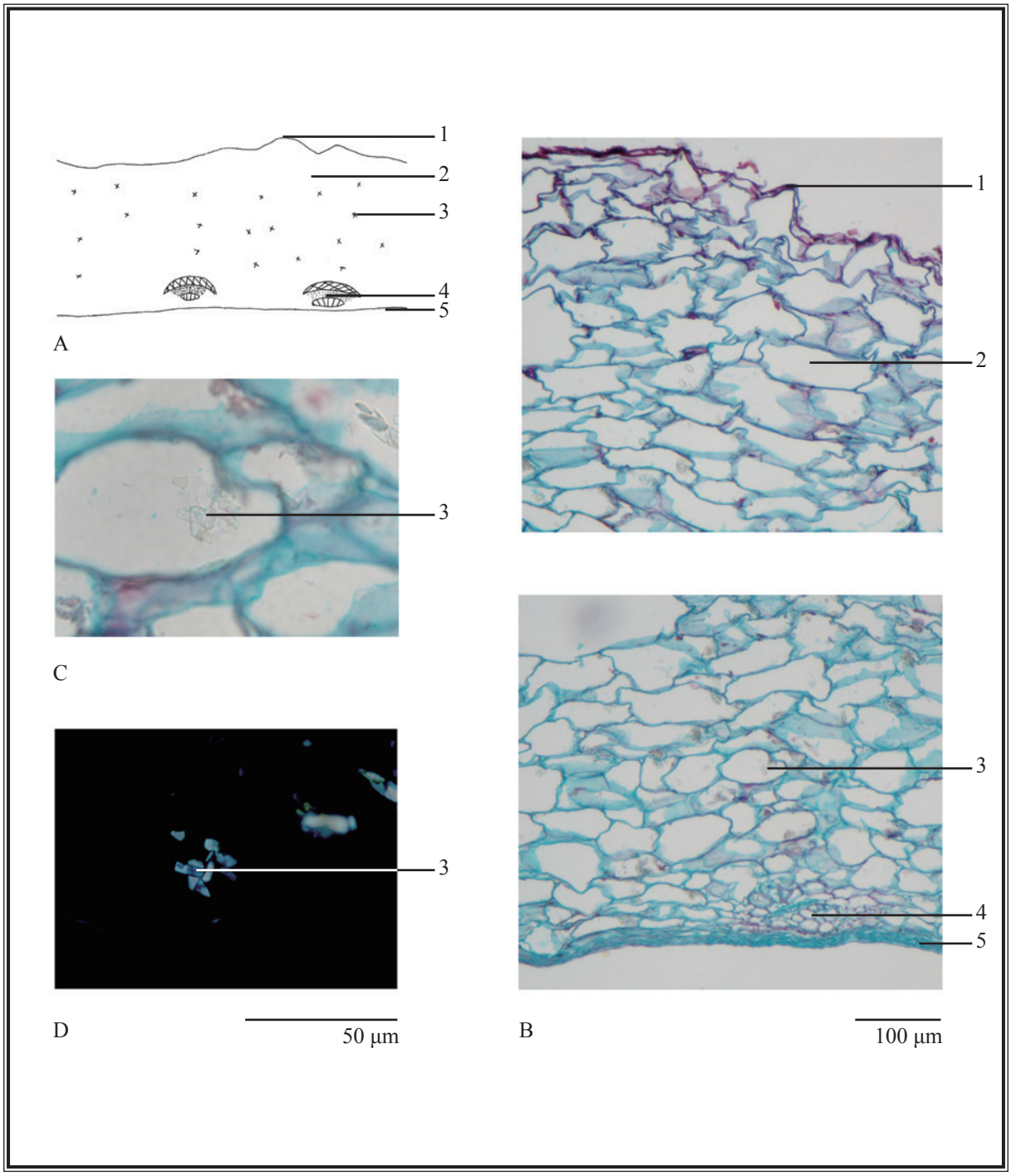


Figure 2 (iii) Microscopic features of transverse section of pericarp of *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen

A. Sketch B. Section illustration C. Crystals of calcium oxalate in mesocarp (under the light microscope)
D. Crystals of calcium oxalate in mesocarp (under the polarized microscope)

1. Exocarp 2. Mesocarp 3. Crystal of calcium oxalate 4. Vascular bundle 5. Endocarp

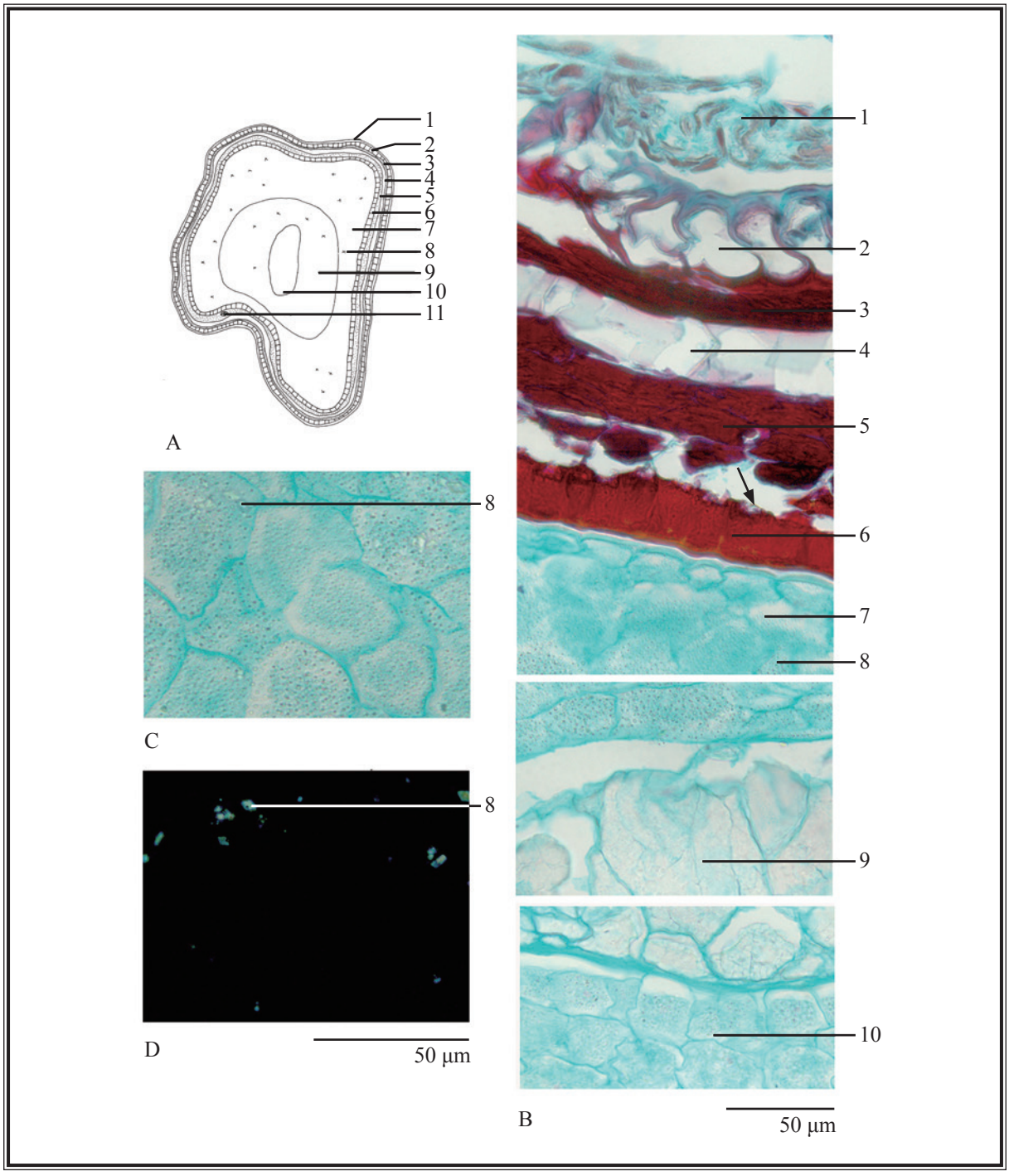


Figure 2 (iv) Microscopic features of transverse section of seed of *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen

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

1. Aril 2. Epidermis of testa 3. Hypodermis of testa 4. Oil cell 5. Pigment layer
6. Endotesta (silica body ⟶) 7. Perisperm 8. Crystal of calcium oxalate 9. Endosperm
10. Embryo 11. Hilum

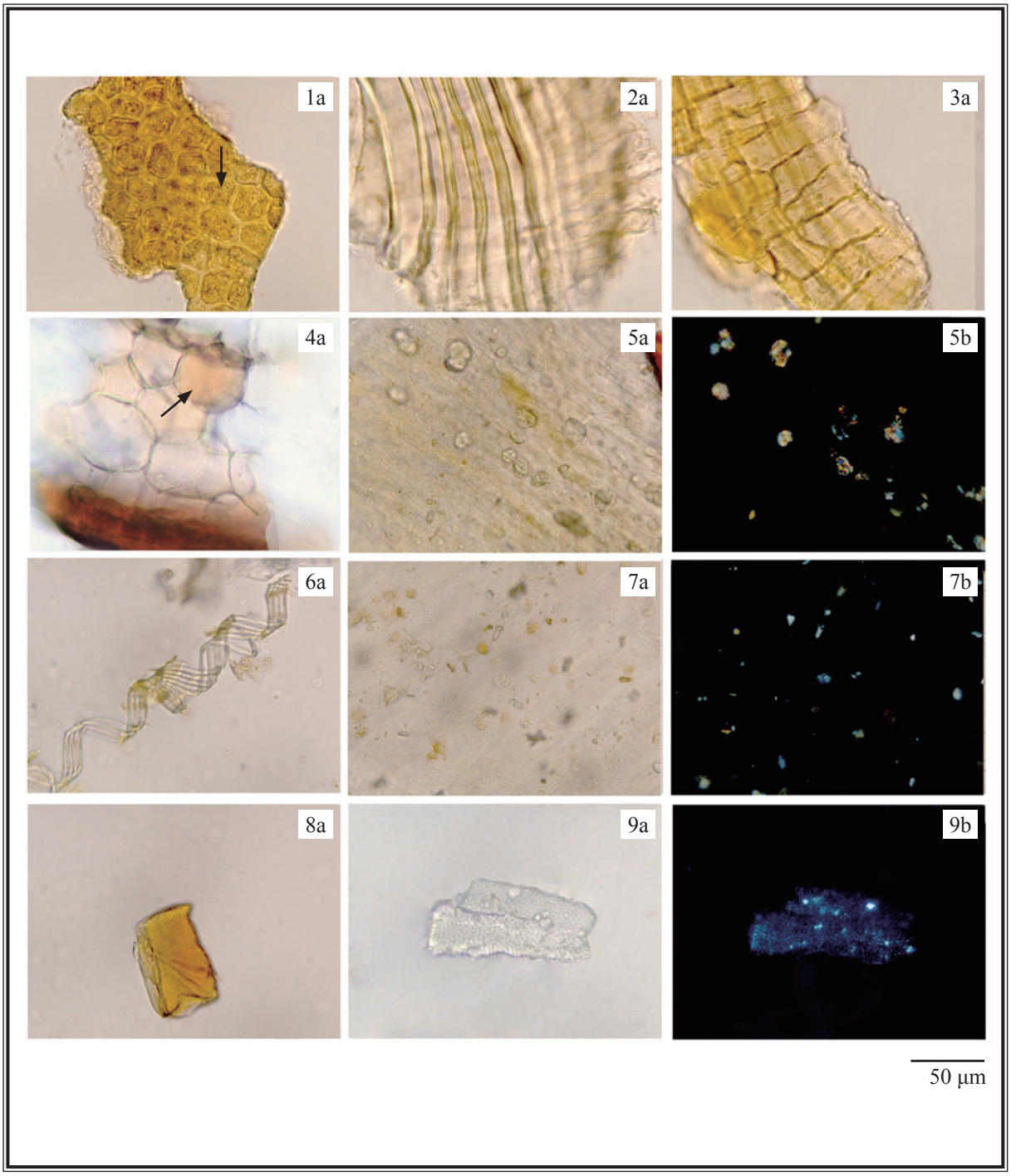


Figure 3 (i) Microscopic features of powder of dried ripe fruit of *Amomum villosum* Lour.

1. Endotesta cells with silica bodies (in surface view, silica bodies→)
2. Epidermal cells of testa 3. Hypodermal cells 4. Oil cells (oil droplets→)
5. Clusters of calcium oxalate 6. Spiral vessels 7. Prisms of calcium oxalate
8. Pigment 9. Perisperm cells

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

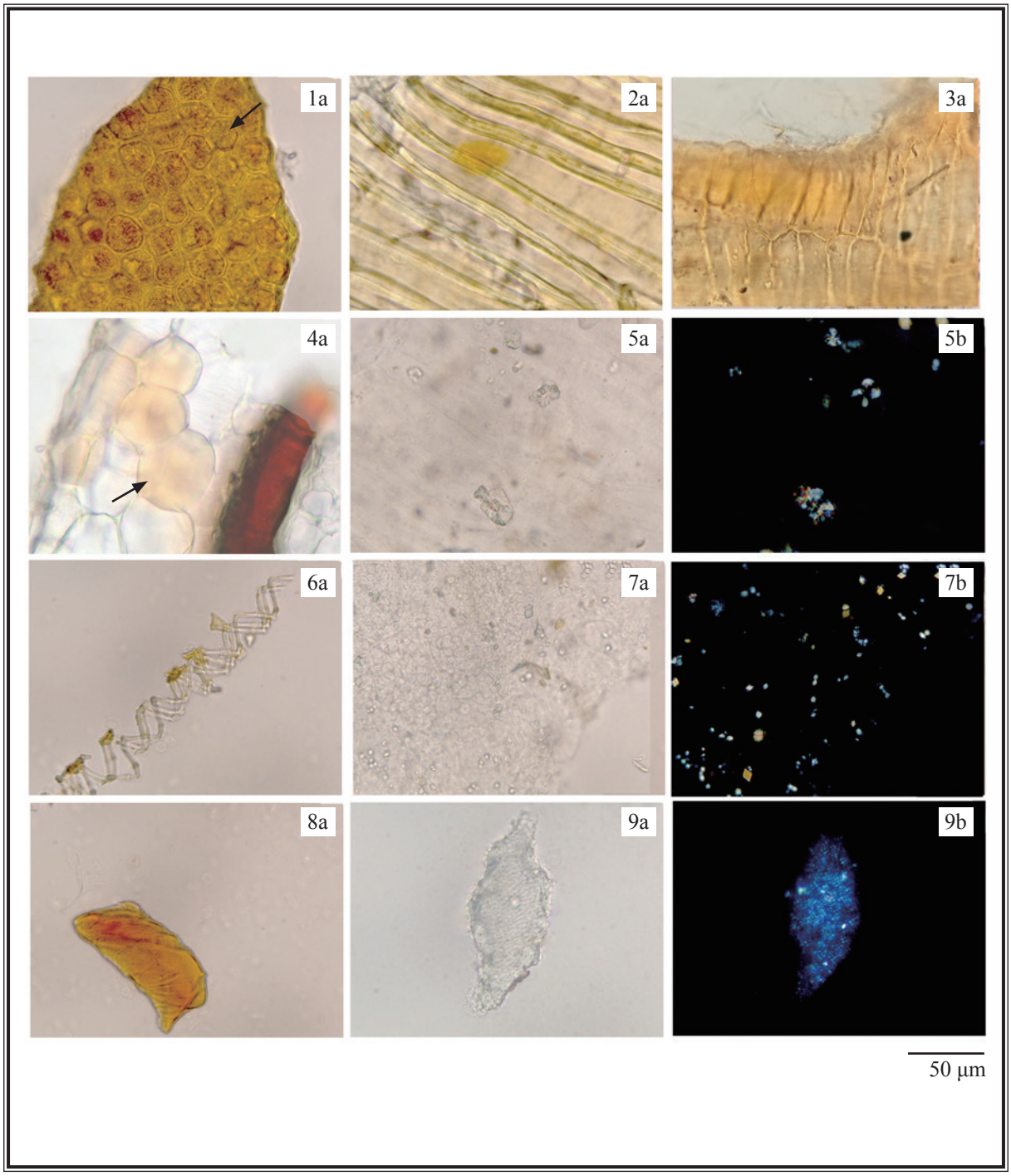


Figure 3 (ii) Microscopic features of powder of dried ripe fruit of *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen

1. Endotesta cells with silica bodies (in surface view, silica bodies ⟶) 2. Epidermal cells of testa
3. Hypodermal cells 4. Oil cells (oil droplets ⟶) 5. Clusters of calcium oxalate 6. Spiral vessels
7. Prisms of calcium oxalate 8. Pigment 9. Perisperm cell

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

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

Standard solution

Bornyl acetate standard solution

Weigh 5.5 mg of bornyl acetate CRS (Fig. 4) and dissolve in 5 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of cyclohexane and ethyl acetate (11:0.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol and dissolve 5 g of vanillin.

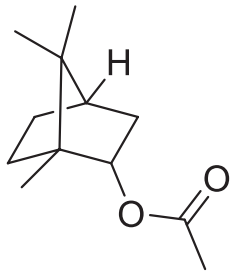
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethyl acetate. Sonicate (150 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 bornyl acetate standard solution and the test solution (10 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 105ºC until the spots or bands become visible (about 3 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

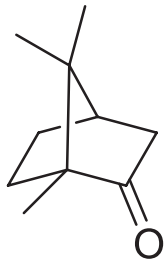


Figure 4 Chemical structures of (i) bornyl acetate and (ii) camphor

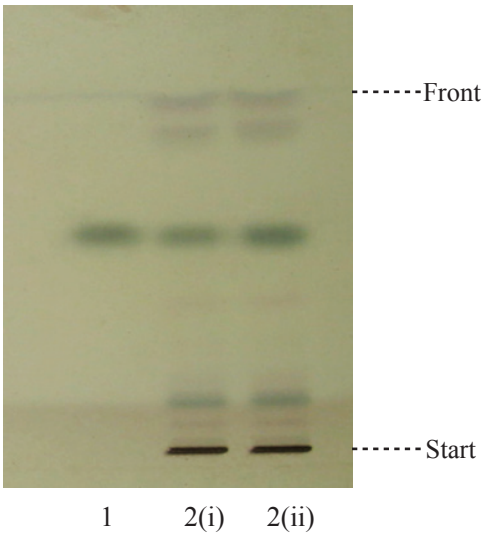


Figure 5 A reference HPTLC chromatogram of Amomi Fructus extract observed under visible light after staining

1. Bornyl acetate standard solution
2. Test solution of
(i) dried ripe fruit of *Amomum villosum* Lour.
(ii) dried ripe fruit of *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen

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 bornyl acetate (Fig. 5).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Bornyl acetate standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 0.6 mg of bornyl acetate CRS and dissolve in 10 mL of ethyl acetate.

Camphor standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of camphor CRS (Fig. 4) and dissolve in 10 mL of ethyl acetate.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethyl acetate. Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-1, 0.25 mm × 30 m) of which the internal wall is covered with dimethylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 230ºC. The detector temperature is at 250ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 10	70	-
10 – 12	70 → 80	5
12 – 17	80	-
17 – 25	80 → 160	10
25 – 30	160	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of bornyl acetate Std-FP and camphor Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bornyl acetate and camphor should not be more than 5.0%; the RSD of the retention times of bornyl acetate and camphor peaks should not be more than 2.0%; the column efficiencies determined from bornyl acetate and camphor peaks should not be less than 2000000 and 300000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and 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 bornyl acetate Std-FP, camphor Std-FP and the test solution (1 µL each) into the GC system and record the chromatograms. Measure the retention times of bornyl acetate and camphor peaks in the chromatograms of bornyl acetate Std-FP, camphor Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify bornyl acetate and camphor peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of bornyl acetate Std-FP and camphor Std-FP. The retention times of bornyl acetate and camphor peaks in the chromatograms of the test solution and the corresponding Std-FP 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 dried ripe fruit of *Amomum villosum* Lour. extract and *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of dried ripe fruit of *Amomum villosum* Lour. extract and *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen extract

Peak No.	RRT	Acceptable Range
1 (camphor)	0.80	± 0.03
2 (borneol)	0.86	± 0.03
3 (marker, bornyl acetate)	1.00	-
4 (caryophyllene)	1.12	± 0.03

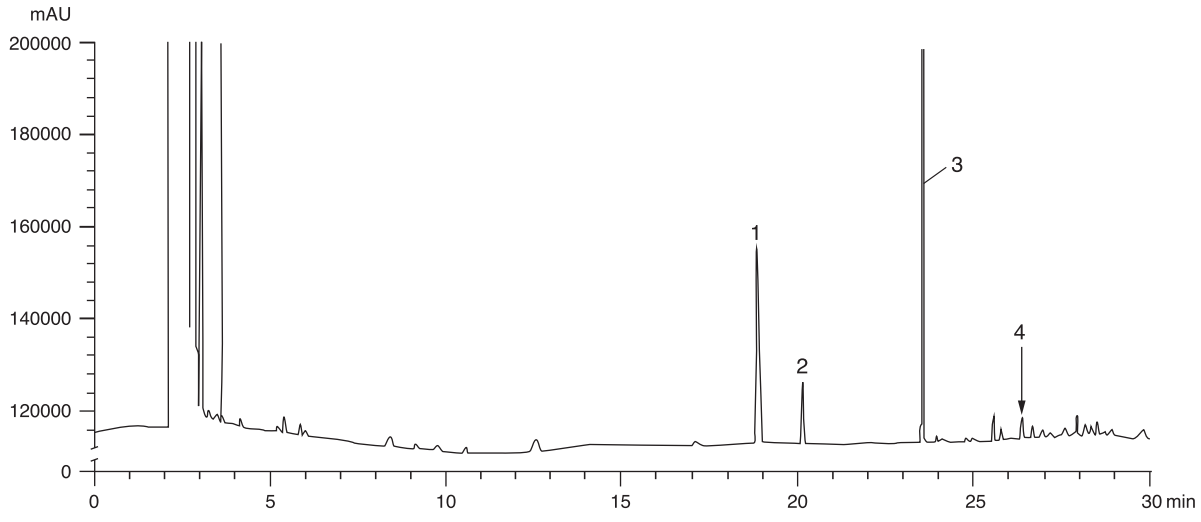


Figure 6 (i) A reference GC fingerprint chromatogram of dried ripe fruit of *Amomum villosum* Lour. extract

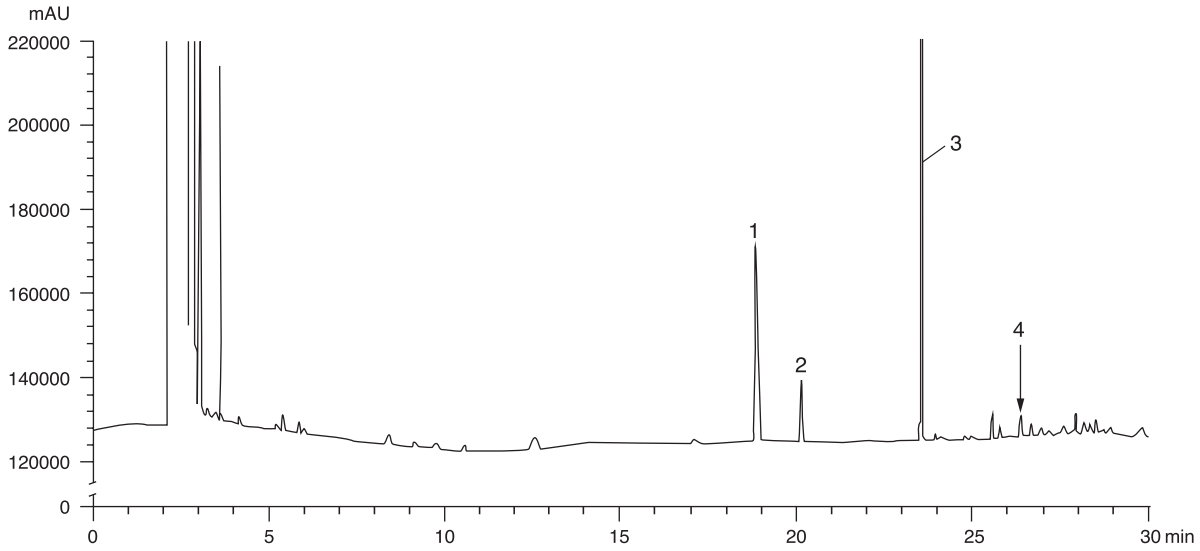


Figure 6 (ii) A reference GC fingerprint chromatogram of dried ripe fruit of *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen extract

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 GC 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)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.5%.
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 (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 6.0%.

7. ASSAY

7.1 Assay of Bornyl acetate and Camphor

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

Standard solution

Mixed bornyl acetate and camphor standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 5.0 mg of bornyl acetate CRS and 5.0 mg of camphor CRS, and dissolve in 10 mL of ethyl acetate.

Mixed bornyl acetate and camphor standard solution for assay, Std-AS

Measure accurately the volume of the mixed bornyl acetate and camphor Std-Stock, dilute with ethyl acetate to produce a series of solutions of 8, 16, 24, 50, 60 mg/L for bornyl acetate and 2, 4, 8, 12, 25 mg/L for camphor.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethyl acetate. Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethyl acetate. Centrifuge at about 4000 × *g* for 5 min. Combine the supernatants and make up to the mark with ethyl acetate. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-1, 0.25 mm × 30 m) of which the internal wall is covered with dimethylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 230ºC. The detector temperature is at 250ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C/min)
0 – 10	70	-
10 – 12	70 → 80	5
12 – 17	80	-
17 – 25	80 → 160	10
25 – 30	160	-

System suitability requirements

Perform at least five replicate injections, each using 1 µL of the mixed bornyl acetate and camphor Std-AS (24 mg/L for bornyl acetate and 8 mg/L for camphor). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bornyl acetate and camphor should not be more than 5.0%; the RSD of the retention times of bornyl acetate and camphor peaks should not be more than 2.0%; the column efficiencies determined from bornyl acetate and camphor peaks should not be less than 2000000 and 300000 theoretical plates respectively.

The *R* value between bornyl acetate peak and the closest peak; and the *R* value between camphor 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 curves

Inject a series of the mixed bornyl acetate and camphor Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of bornyl acetate and camphor against the corresponding concentrations of the mixed bornyl acetate and camphor Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify bornyl acetate and camphor peaks [Fig. 7 (i) or (ii)] in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed bornyl acetate and camphor Std-AS. The retention times of bornyl acetate and camphor peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of bornyl acetate and camphor in the test solution, and calculate the percentage contents of bornyl acetate and camphor in the sample by using the equations as indicated in Appendix IV (B).

Limits

The dried ripe fruit of *Amomum villosum* Lour. and *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen contain not less than 1.4% of the total content of bornyl acetate ($C_{12}H_{20}O_2$) and camphor ($C_{10}H_{16}O$), calculated with reference to the dried substance.

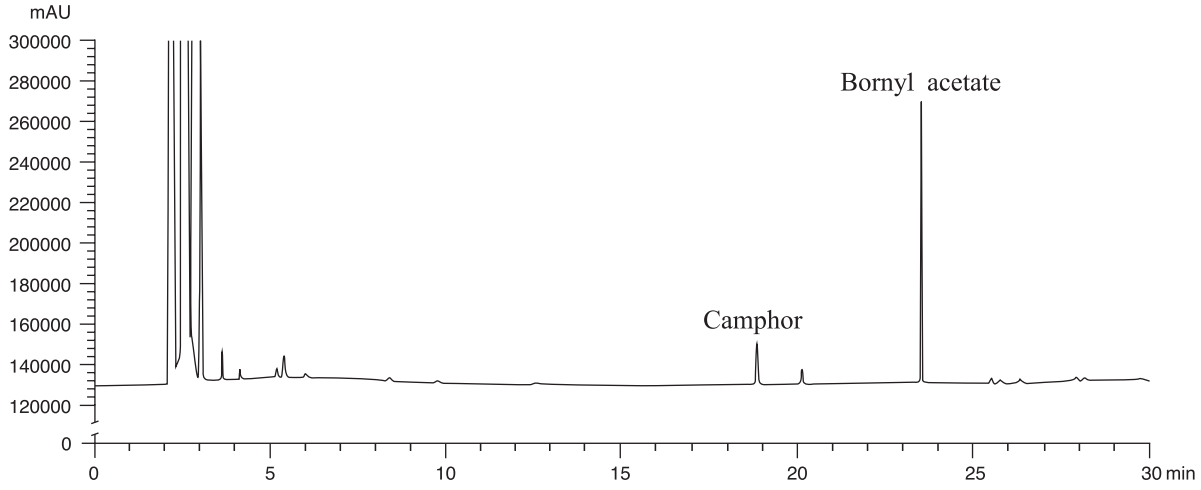


Figure 7 (i) A reference GC assay chromatogram of dried ripe fruit of *Amomum villosum* Lour. extract

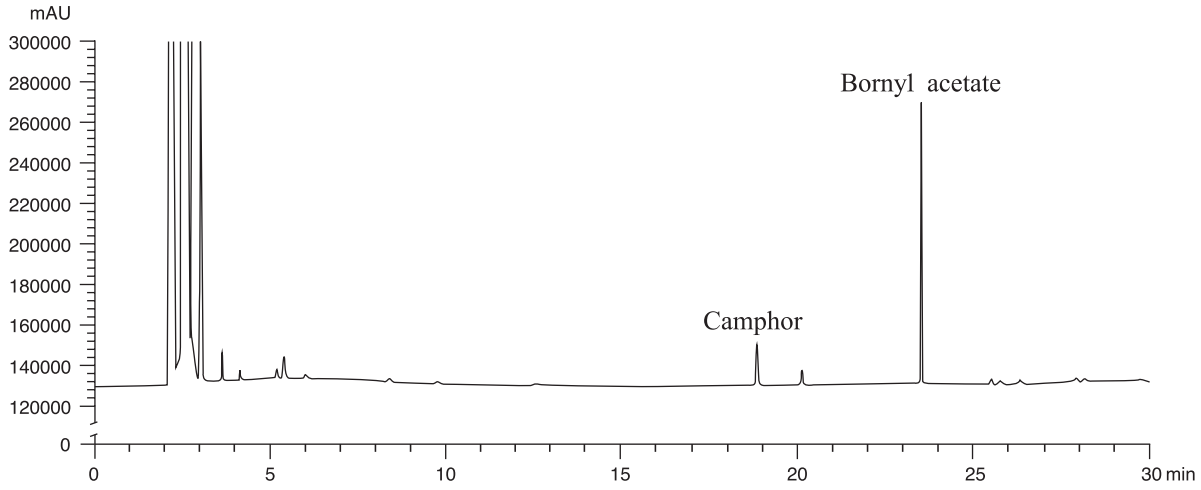


Figure 7 (ii) A reference GC assay chromatogram of dried ripe fruit of *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen extract

7.2 Assay of Volatile Oil

Weigh accurately 50 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 dried ripe fruit of *Amomum villosum* Lour. and *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen contain not less than 1.9% (v/w) of volatile oil.

Part II Dried ripe fruit of *Amomum longiligulare* T. L. Wu

3. DESCRIPTION

Long ellipsoid or ovoid, distinctly 3-ridged, 0.8-2.8 cm long, 6-23 mm in diameter. Externally with laminar and branched soft spiny protrudings, base bearing fruit stalk or showing a scar of fruit stalk. Pericarp thickened and hard. Seeds aggregated into an obtusely 3-ridged mass, divided into 3 segments separated by white septa, masses of seeds relatively small, in 3 segments, each segment consists of 2-28 seeds. Seeds irregularly polyhedral, 1-4 mm in diameter; externally brownish-red to dark brown, finely wrinkled, covered with pale brown membranous aril; texture hard, endosperm greyish-white. Odour aromatic; taste pungent, cool and slightly bitter [Fig. 1 (iii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pericarp: Exocarp consists of 1 layer of flattened rectangular cells. Mesocarp broad; parenchymatous cells relatively large, subrounded to oblong; vascular bundles collateral, arranged in an interrupted ring in the inner side of mesocarp, surrounded by cresent-shaped fibre bundles; sieve tubes and vessels visible; parenchymatous cells scattered with crystals of calcium oxalate. Endocarp consists of 1 layer of rectangular cells, mostly shrunken [Fig. 8 (i)].

Seeds: Aril present sometimes, consisting of several layers of slender cells. Epidermis of testa consists of 1 layer of cells, cells radially elongated, slightly thick-walled. Hypodermis consists of 1 layer of cells, containing brown to reddish-brown contents. Oil cell layer consists of 1 layer of subrectangular cells, with thin walls. Pigment layer consists of several layers of

cells, cells polygonal, irregularly arranged, containing dark reddish-brown contents. Endotesta consists of 1 layer of small, palisade-like thick-walled cells, small, containing silica bodies, inner and lateral walls heavily thickened. Perisperm cells filled with small starch granules, some contain crystals of calcium oxalate. Endosperm and embryo cells contain fine aleurone grains. Hilum deeply indented [Fig. 8 (ii)].

Powder

Colour greyish-brown. Endotesta cells yellowish-brown to reddish-brown, polygonal in surface view, with thickened and non-lignified walls, lumen contains silica bodies. Epidermal cells of testa pale yellow, elongated in surface view. Hypodermal cells rectangular to oblong, containing brown to reddish-brown contents. Oil cells nearly colourless to pale yellow, easily broken, subsquare, 4-76 μm in diameter, sometimes with oil droplets. Clusters and prisms of calcium oxalate scattered in parenchymatous cells, 2-39 μm in diameter; polychromatic under the polarized microscope. Mainly spiral vessels, 5-68 μm in diameter. Pigment cells usually shrunken, boundaries unclear, containing reddish-brown to dark brown contents. Perisperm cells subrectangular or irregular in shape, filled with starch masses formed by aggregation of small starch granules (Fig. 9).

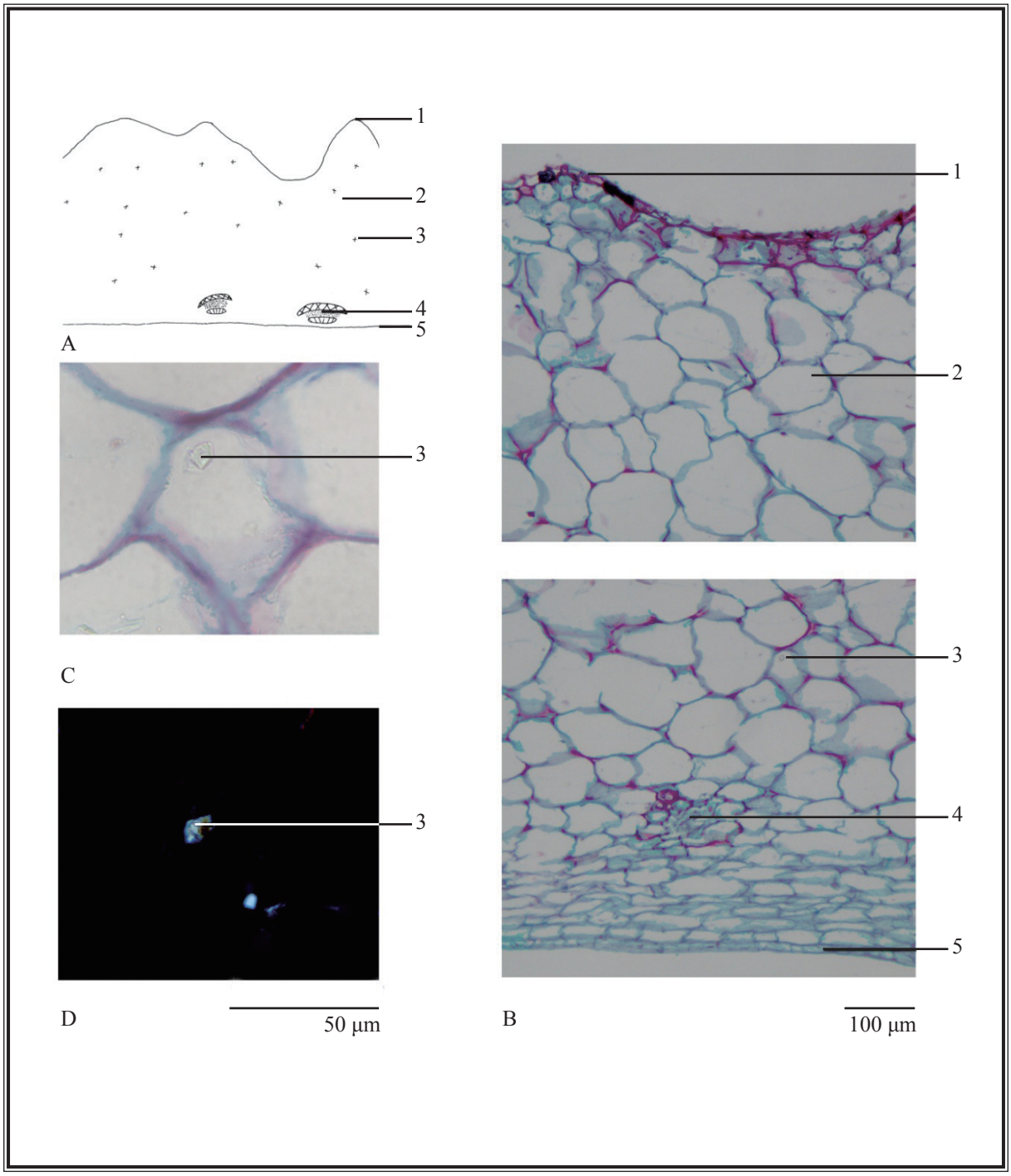


Figure 8 (i) Microscopic features of transverse section of pericarp of *Amomum longiligulare* T. L. Wu

A. Sketch B. Section illustration

C. Crystals of calcium oxalate in mesocarp (under the light microscope)

D. Crystals of calcium oxalate in mesocarp (under the polarized microscope)

1. Exocarp 2. Mesocarp 3. Crystal of calcium oxalate 4.Vascular bundle 5.Endocarp

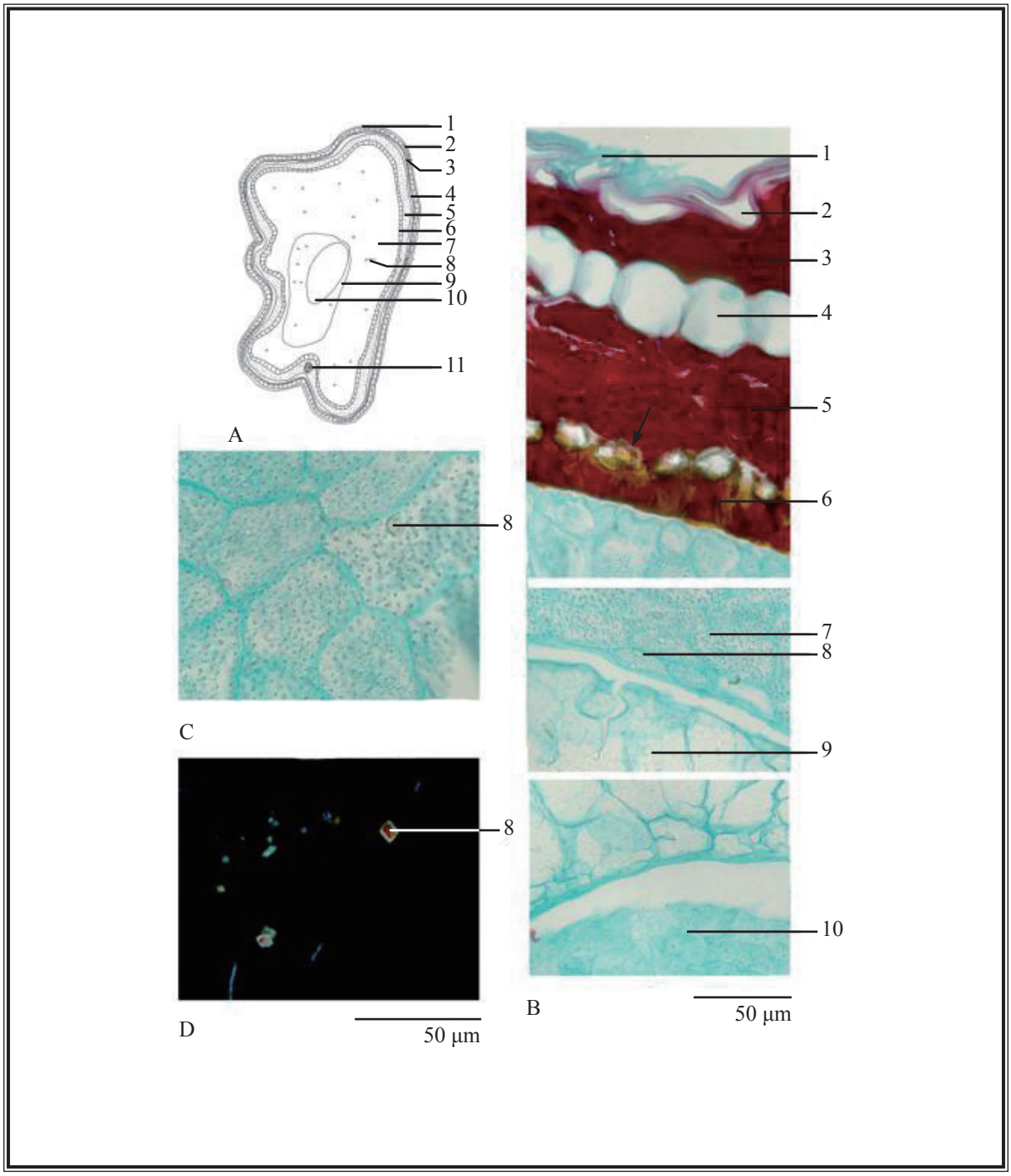


Figure 8 (ii) Microscopic features of transverse section of seed of *Amomum longiligulare* T. L. Wu

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

1. Aril 2. Epidermis of testa 3. Hypodermis of testa 4. Oil cell 5. Pigment layer
6. Endotesta (silica body ⟶) 7. Perisperm 8. Crystal of calcium oxalate 9. Endosperm
10. Embryo 11. Hilum

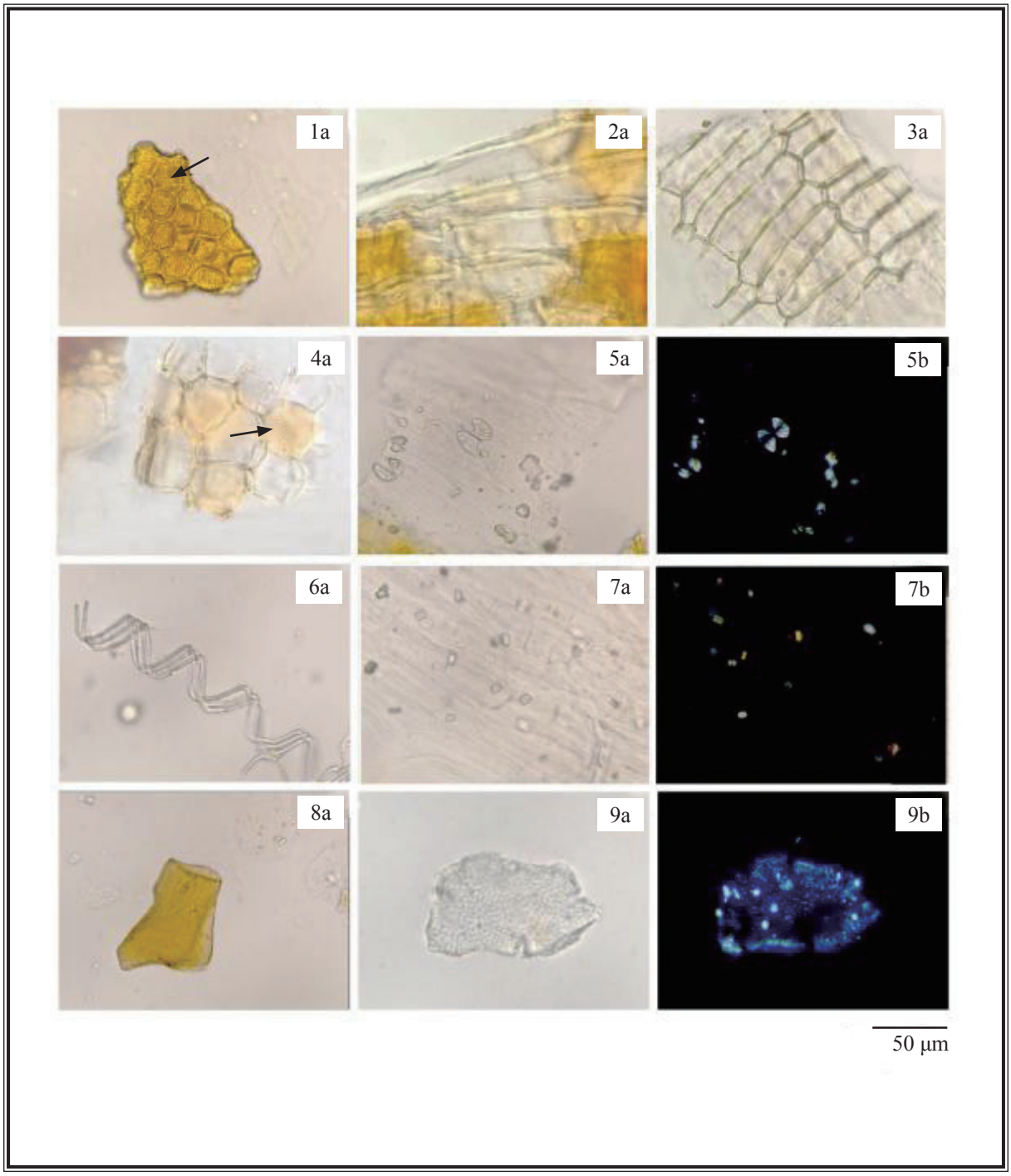


Figure 9 Microscopic features of powder of dried riped fruit of *Amomum longiligulare* T. L. Wu

1. Endotesta cells with silica bodies (in surface view, silica bodies ⟶)
2. Epidermal cells of testa 3. Hypodermal cells 4. Oil cells (oil droplets ⟶)
5. Clusters of calcium oxalate 6. Spiral vessels 7. Prisms of calcium oxalate
8. Pigment 9. Perisperm cell

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

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

Standard solution

Bornyl acetate standard solution

Weigh 5.5 mg of bornyl acetate CRS (Fig. 10) and dissolve in 5 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of cyclohexane and ethyl acetate (11:0.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol and dissolve 5 g of vanillin.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethyl acetate. Sonicate (150 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 bornyl acetate standard solution and the test solution (10 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 105ºC until the spots or bands become visible (about 3 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

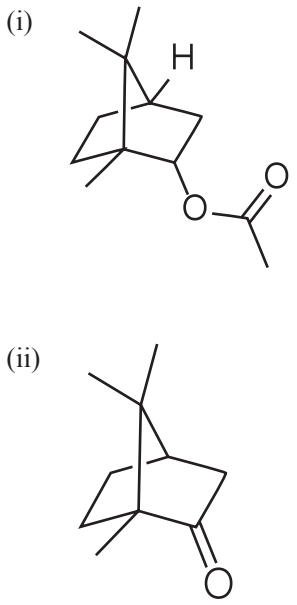


Figure 10 Chemical structures of (i) bornyl acetate and (ii) camphor

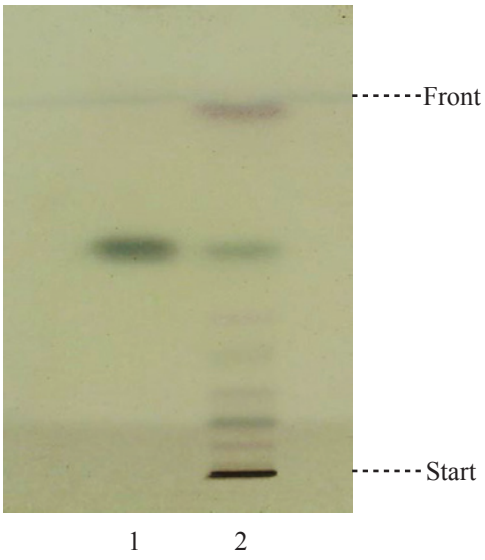


Figure 11 A reference HPTLC chromatogram of dried ripe fruit of *Amomum longiligulare* T. L. Wu extract observed under visible light after staining

1. Bornyl acetate 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 bornyl acetate (Fig. 11).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Bornyl acetate standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 0.6 mg of bornyl acetate CRS and dissolve in 10 mL of ethyl acetate.

Camphor standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of camphor CRS (Fig. 10) and dissolve in 10 mL of ethyl acetate.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethyl acetate. Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-1, 0.25 mm × 30 m) of which the internal wall is covered with dimethylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 230ºC. The detector temperature is at 250ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 4) –

Table 4 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 10	70	-
10 – 12	70 → 80	5
12 – 17	80	-
17 – 25	80 → 160	10
25 – 30	160	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of bornyl acetate Std-FP and camphor Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bornyl acetate and camphor should not be more than 5.0%; the RSD of the retention times of bornyl acetate and camphor peaks should not be more than 2.0%; the column efficiencies determined from bornyl acetate and camphor peaks should not be less than 2000000 and 300000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and 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. 12).

Procedure

Separately inject bornyl acetate Std-FP, camphor Std-FP and the test solution (1 μL each) into the GC system and record the chromatograms. Measure the retention times of bornyl acetate and camphor peaks in the chromatograms of bornyl acetate Std-FP, camphor Std-FP and the retention times of the four characteristic peaks (Fig. 12) in the chromatogram of the test solution. Identify bornyl acetate and camphor peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of bornyl acetate Std-FP and camphor Std-FP. The retention times of bornyl acetate and camphor peaks in the chromatograms of the test solution and the corresponding Std-FP 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 dried ripe fruit of *Amomum longiligulare* T. L. Wu extract are listed in Table 5.

Table 5 The RRTs and acceptable ranges of the four characteristic peaks of dried ripe fruit of *Amomum longiligulare* T. L. Wu extract

Peak No.	RRT	Acceptable Range
1 (camphor)	0.80	± 0.03
2 (borneol)	0.85	± 0.03
3 (marker, bornyl acetate)	1.00	-
4 (caryophyllene)	1.12	± 0.03

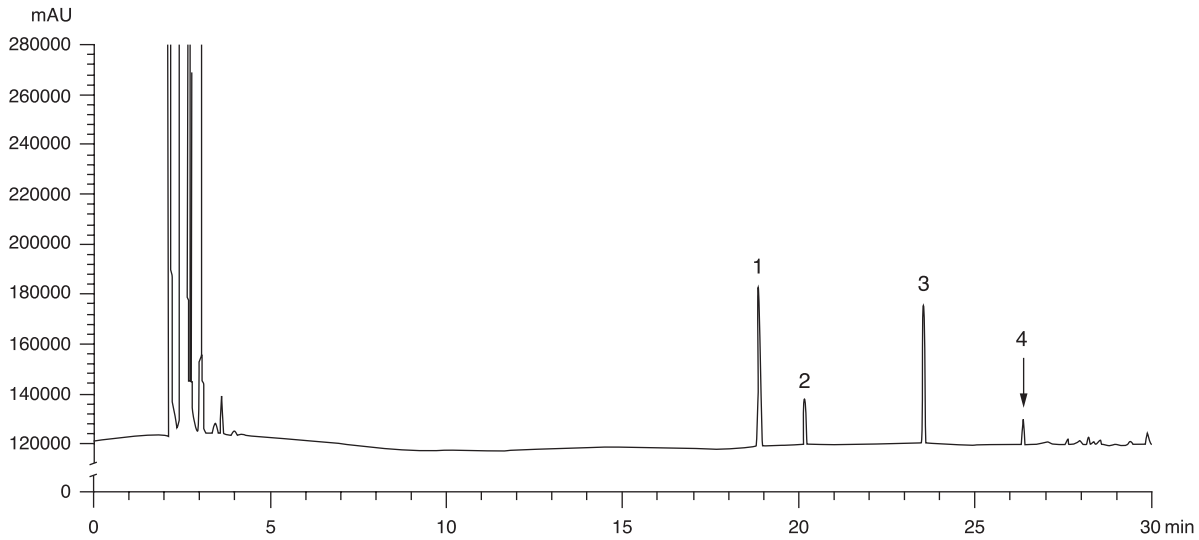


Figure 12 A reference GC fingerprint chromatogram of dried ripe fruit of *Amomum longiligulare* T. L. Wu extract

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 reference GC fingerprint chromatograms (Fig. 12).

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 10.0%.
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 (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

7.1 Assay of Bornyl acetate and Camphor

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

Standard solution

Mixed bornyl acetate and camphor standard stock solution, Std-Stock (500 mg/L each)
Weigh accurately 5.0 mg of bornyl acetate CRS and 5.0 mg of camphor CRS, and dissolve in 10 mL of ethyl acetate.
Mixed bornyl acetate and camphor standard solution for assay, Std-AS
Measure accurately the volume of the mixed bornyl acetate and camphor Std-Stock, dilute with ethyl acetate to produce a series of solutions of 1, 2, 4, 8, 16 mg/L for bornyl acetate and 2, 4, 8, 12, 25 mg/L for camphor.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethyl acetate. Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethyl acetate. Centrifuge at about 4000 × *g* for 5 min. Combine the supernatants and make up to the mark with ethyl acetate. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-1, 0.25 mm × 30 m) of which the internal wall is covered with dimethylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 230ºC. The detector temperature is at 250ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 6) –

Table 6 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 10	70	-
10 – 12	70 → 80	5
12 – 17	80	-
17 – 25	80 → 160	10
25 – 30	160	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of the mixed bornyl acetate and camphor Std-AS (4 mg/L for bornyl acetate and 8 mg/L for camphor). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bornyl acetate and camphor should not be more than 5.0%; the RSD of the retention times of bornyl acetate and camphor peaks should not be more than 2.0%; the column efficiencies determined from bornyl acetate and camphor peaks should not be less than 2000000 and 300000 theoretical plates respectively.

The *R* value between bornyl acetate peak and the closest peak; and the *R* value between camphor peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 13).

Calibration curves

Inject a series of the mixed bornyl acetate and camphor Std-AS (1 μL each) into the GC system and record the chromatograms. Plot the peak areas of bornyl acetate and camphor against the corresponding concentrations of the mixed bornyl acetate and camphor Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify bornyl acetate and camphor peaks (Fig. 13) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed bornyl acetate and camphor Std-AS. The retention times of bornyl acetate and camphor peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of bornyl acetate and camphor in the test solution, and calculate the percentage contents of bornyl acetate and camphor in the sample by using the equations as indicated in Appendix IV (B).

Limits

The dried ripe fruit of *Amomum longiligulare* T. L. Wu contains not less than 0.58% of the total content of bornyl acetate ($C_{12}H_{20}O_2$) and camphor ($C_{10}H_{16}O$), calculated with reference to the dried substance.

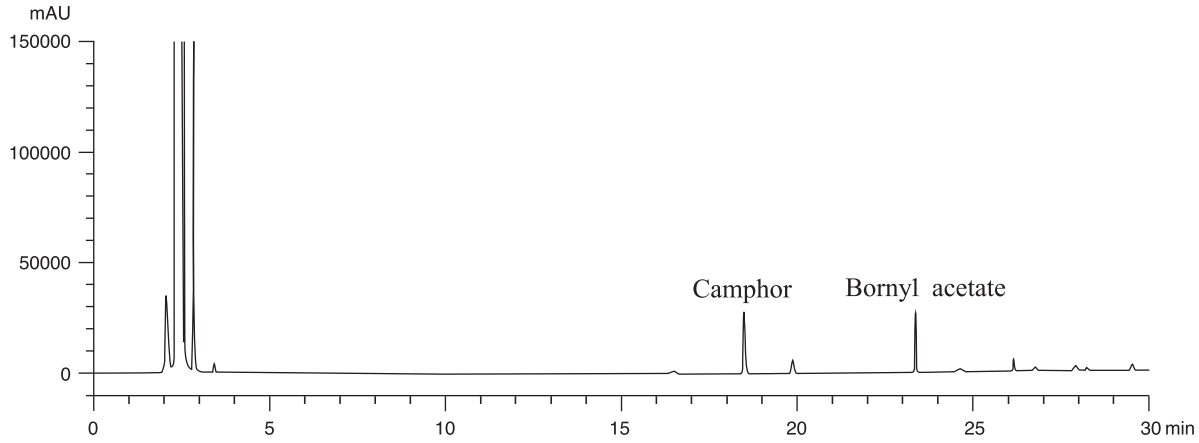


Figure 13 A reference GC assay chromatogram of dried ripe fruit of *Amomum longiligulare* T. L. Wu extract

7.2 Assay of Volatile Oil

Weigh accurately 50 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 dried ripe fruit of *Amomum longiligulare* T. L. Wu contains not less than 1.0% (v/w) of volatile oil.