Alpiniae Oxyphyllae Fructus

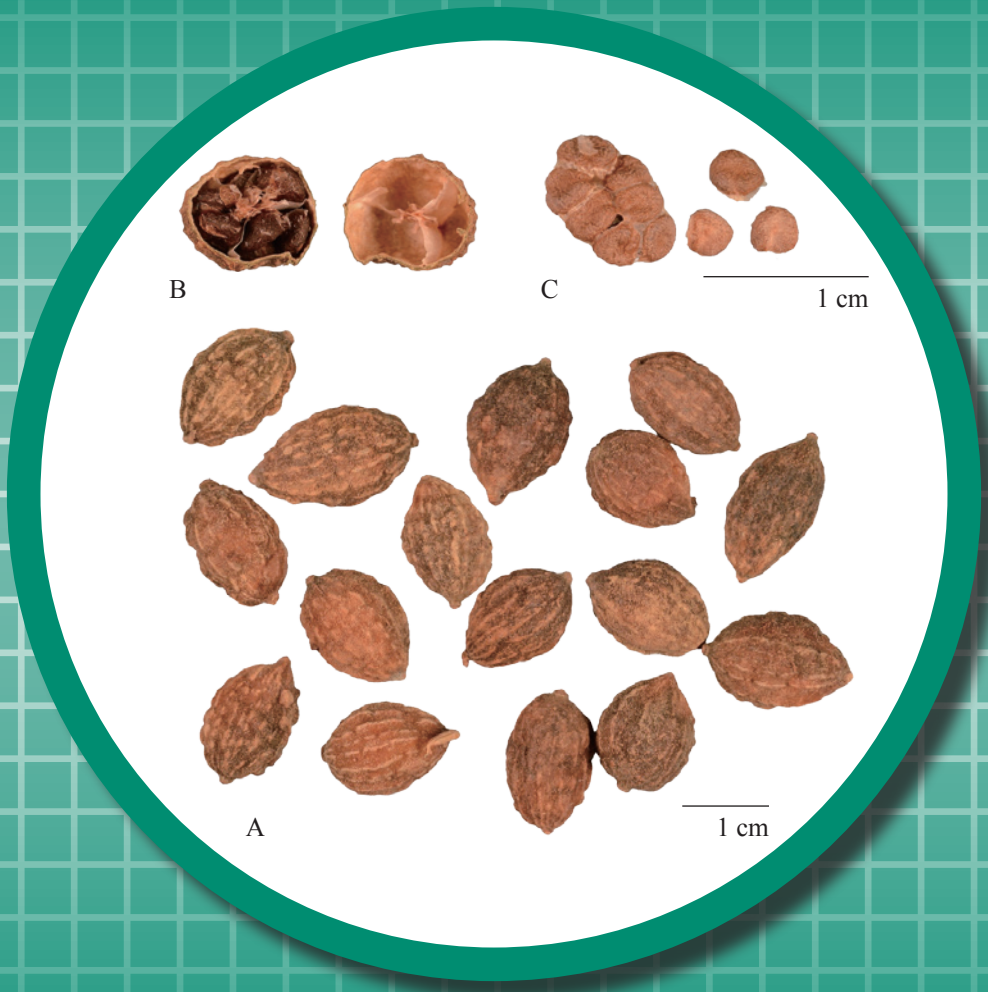

Figure 1 A photograph of Alpiniae Oxyphyllae Fructus

A. Alpiniae Oxyphyllae Fructus
B. Transverse section of fruits showing seeds divided by septa into groups of seeds in 3 valves
C. A group of seeds and separated seeds

1. NAMES

Official Name: Alpiniae Oxyphyllae Fructus

Chinese Name: 益智

Chinese Phonetic Name: Yizhi

2. SOURCE

Alpiniae Oxyphyllae Fructus is the dried ripe fruit of *Alpinia oxyphylla* Miq. (Zingiberaceae). The fruit is collected in summer and autumn when ripe, then dried under the sun to obtain Alpiniae Oxyphyllae Fructus.

3. DESCRIPTION

Ellipsoid, both ends slightly acute, 0.7-2.2 cm long, 4-14 mm in diameter. Externally yellowish-brown to reddish-brown to greyish-brown, with 13-20 uneven and protuberant longitudinal lines on the surface, with remnants of perianth on the apex and fruit stalk at the base. Pericarp thin and slightly tough, adhering closely to the seed. Seeds gathered in groups and divided to 3 valves by septa, 6-11 seeds in each valve. Individual seeds irregularly oblate, with slightly obtuse ribs, about 3 mm in diameter, greyish-brown to greyish-yellow externally, covered with pale brown membranous aril. Texture hard, endosperm white. Odour distinctly aromatic; taste pungent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pericarp: Exocarp consists of 1 layer of subrectangular epidermal cells, covered with cuticle. Mesocarp consists of rectangular or tangentially elongated parenchymatous cells. Parenchymatous cells contain oil cells, vascular bundles scattered, with a fibre bundle outside the phloem. Endocarp consists of 1 layer of tangentially elongated parenchymatous cells. Septum cells relatively thin-walled [Fig. 2 (i)].

Seed: Aril occasionally found, consisting of several layers of parenchymatous cells. Epidermal cells of testa subrounded, subsquare or rectangular, slightly radially elongated, with relatively thick walls. Hypodermis consists of 1 layer of parenchymatous cells with yellowish-brown contents. Oil cell layer consists of 1 layer of oil cells, cells subsquare or rectangular, with yellow oil droplets. Pigment layer consists of several layers of yellowish-brown cells, scattered with 1-3 layers of relatively large subrounded oil cells, with yellow oil droplets. Endotesta consists of 1 layer of palisade sclerenchymatous cells, yellowish-brown or reddish-brown, with heavily thickened inner and lateral walls, lumen small, containing silica bodies. Perisperm cells filled with starch granules and with scattered prisms of calcium oxalate. Endosperm cells contain aleurone grains [Fig. 2 (ii)].

Powder

Colour yellowish-brown. Epidermal cells of testa strip-shaped in surface view, up to 29 μm in diameter, walls slightly thickened, often vertically arranged with hypodermal cells. Hypodermal cells subrectangular or irregular, containing pale yellow or yellowish-brown contents. Oil cells scattered among pigment layer cells, subsquare or rectangular. Cells of pigment layer wrinkled, border indistinct, containing reddish-brown and dark brown contents, most cells broken into irregular fragments of pigment. Sclerenchymatous cells of endotesta yellowish-brown or brown, palisade-like cells appear in 1 layer in lateral view, inner and lateral walls extremely thickened, lumens outward eccentric, containing silica bodies; polygonal in surface view, walls thickened and unlignified, containing silica bodies. Perisperm cells filled with starch masses, aggregated from fine starch granules. Prisms of calcium oxalate 0.6-15 μm in diameter; bright yellowish-white under polarized microscope. Oil cells of pericarp yellowish-brown to reddish-brown, subrounded. Fibres slender, 7-42 μm in diameter, walls thickened, pits distinct. Vessels mainly scalariform, 6-46 μm in diameter (Fig. 3).

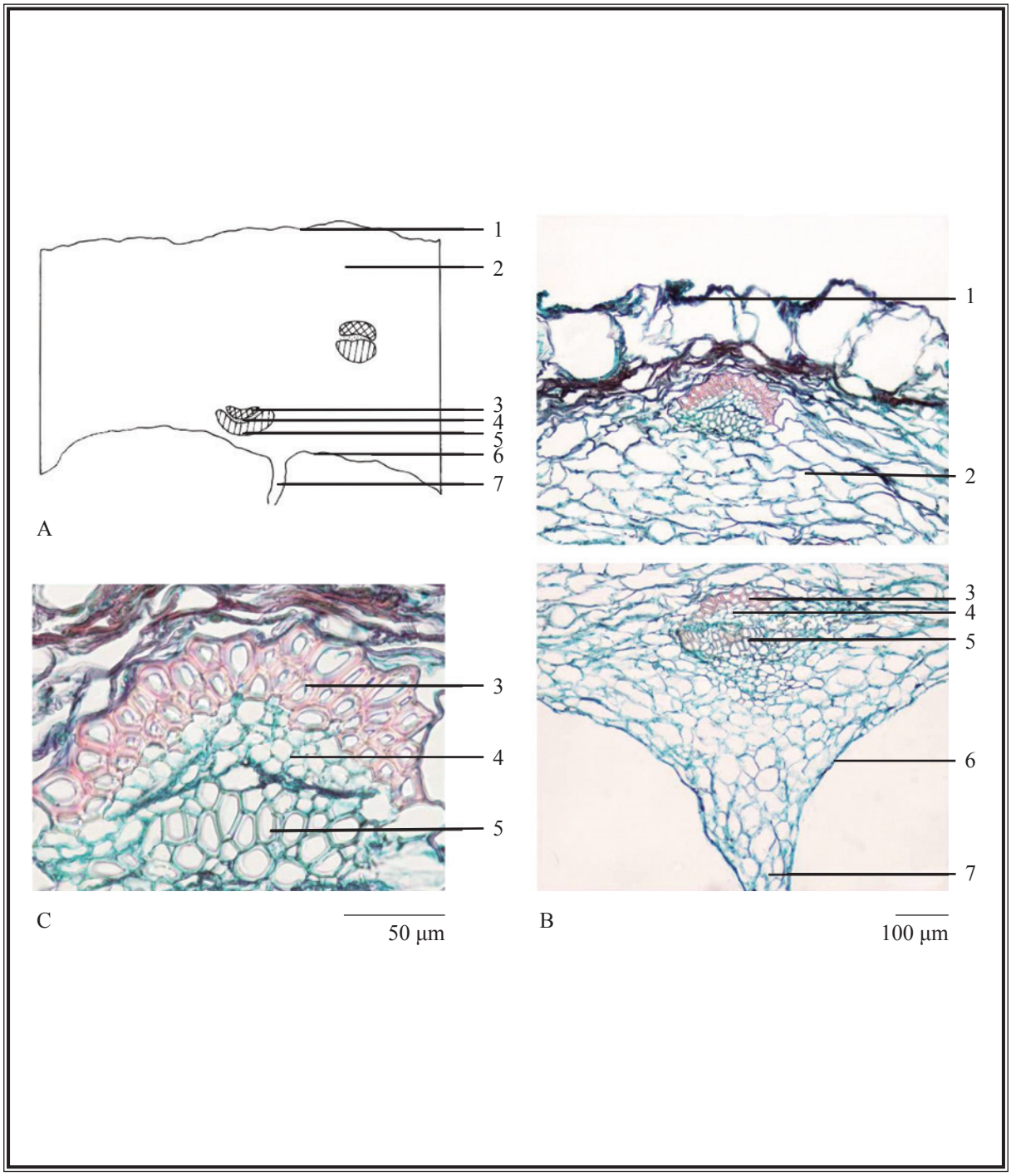

Figure 2 (i) Microscopic features of transverse section of pericarp of Alpiniae Oxyphyllae Fructus

A. Sketch B. Section illustration C. Vascular bundle

1. Exocarp 2. Mesocarp 3. Fibre bundle 4. Phloem 5. Xylem 6. Endocarp 7. Septum

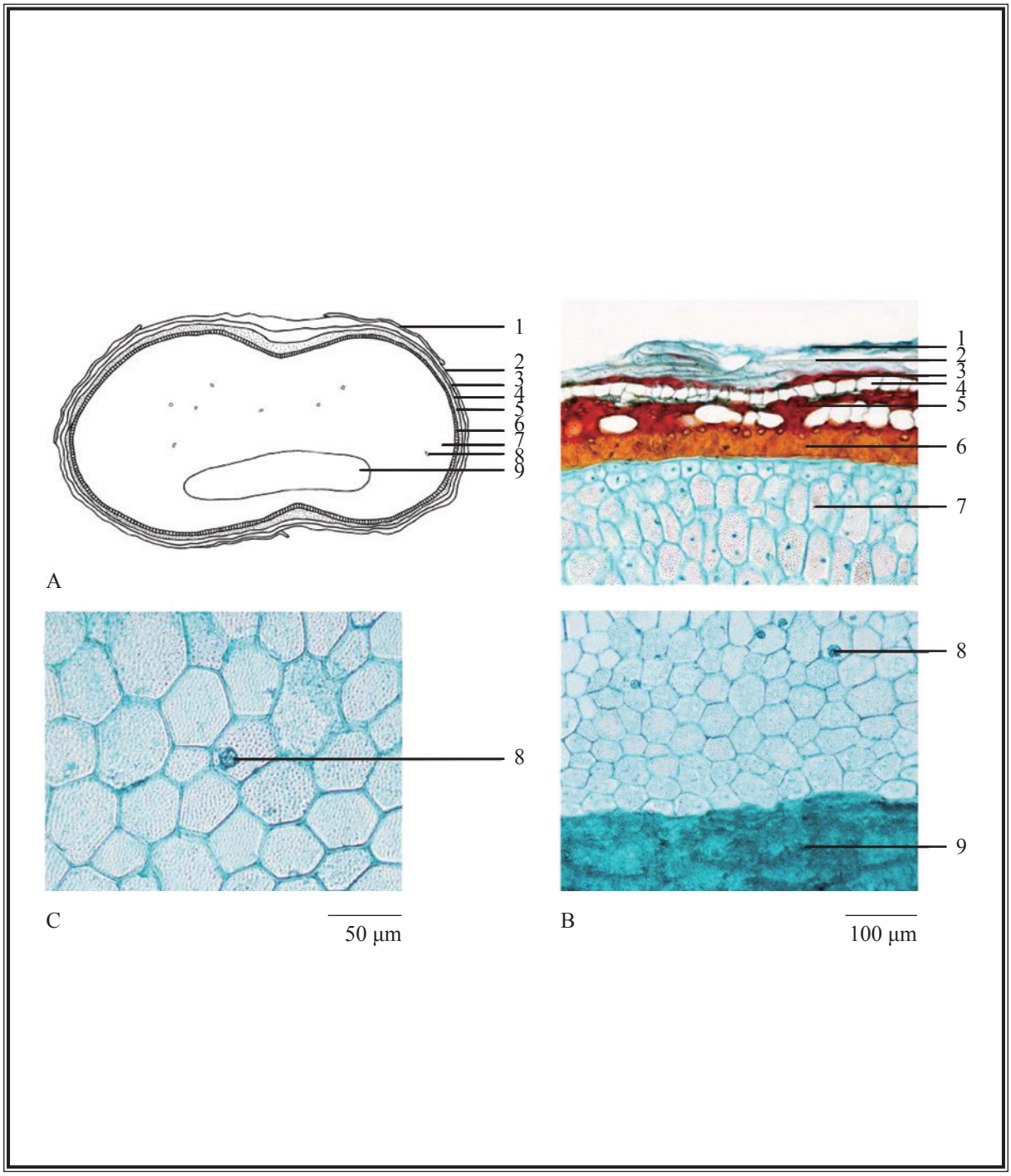

Figure 2 (ii) Microscopic features of transverse section of seed of Alpiniae Oxyphyllae Fructus

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

1. Aril 2. Epidermis of testa 3. Hypodermis 4. Oil cell layer 5. Pigment layer
6. Endotesta 7. Perisperm 8. Prisms of calcium oxalate 9. Endosperm

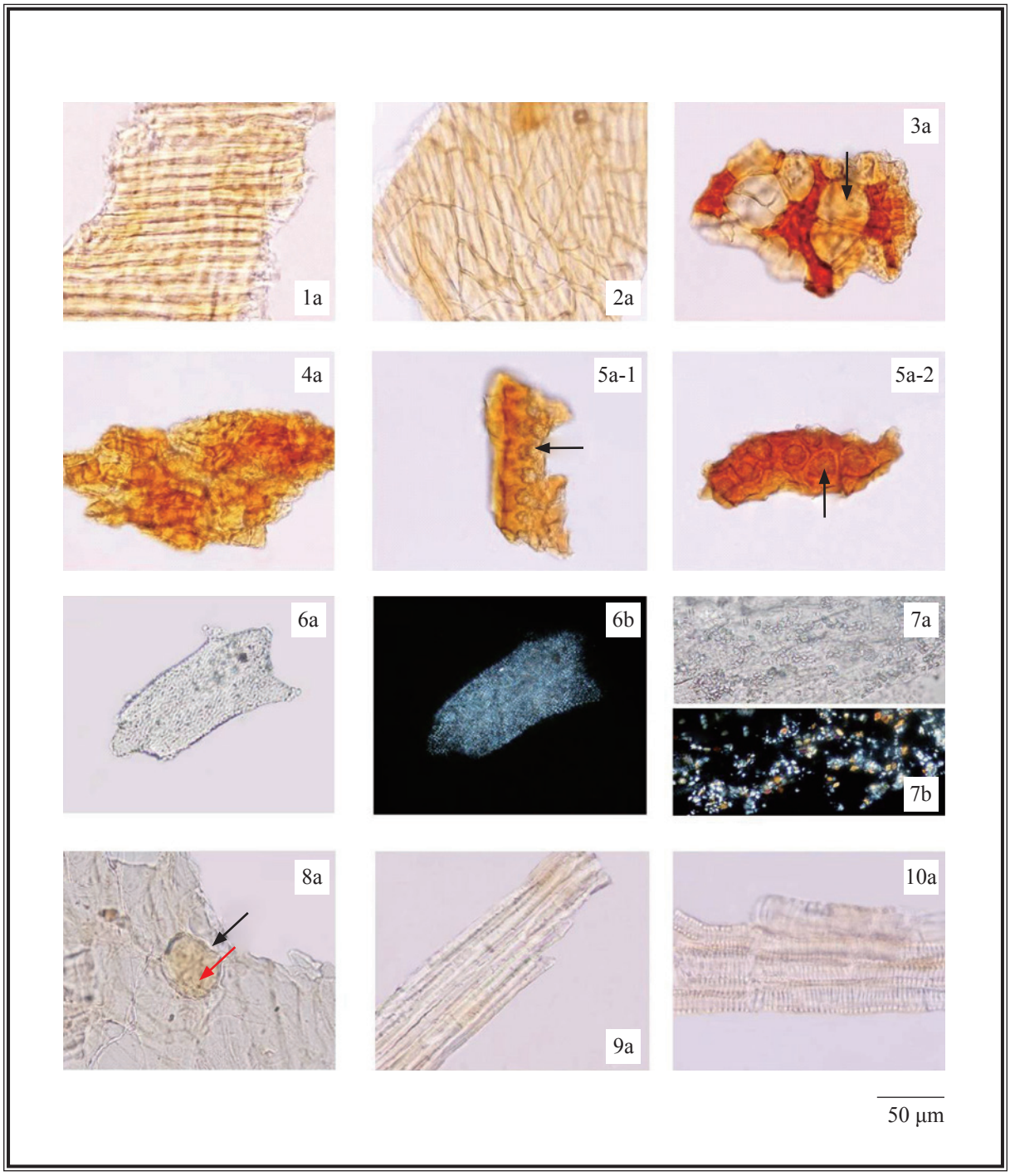

Figure 3 Microscopic features of powder of Alpiniae Oxyphyllae Fructus

1. Epidermal cells of testa 2. Hypodermal cells 3. Oil cells 4. Pigment layer cells
5. Sclerenchymatous cells of endotesta (silica bodies ⟶) (5-1 in lateral view, 5-2 in surface view)
6. Perisperm cells with starch masses 7. Prisms of calcium oxalate 8. Oil cells of pericarp (⟶)
9. Fibres of pericarp 10. Vessels

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

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

Standard solution

Nootkatone standard solution

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

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC) and ethyl acetate (3:2, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 10 mL of methanol. Sonicate (140 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 nootkatone standard solution (5 μL) and the test solution (10 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. Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

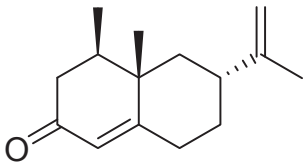

Figure 4 Chemical structure of nootkatone

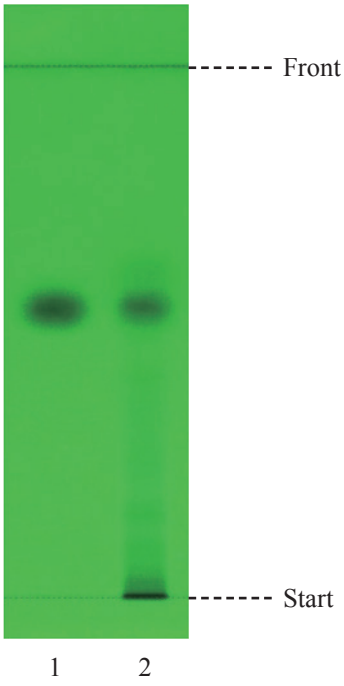

Figure 5 A reference HPTLC chromatogram of Alpiniae Oxyphyllae Fructus extract observed under UV light (254 nm)

1. Nootkatone 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 nootkatone (Fig. 5).

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

Standard solution

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

Weigh 5.0 mg of nootkatone CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 12.5 mL of methanol. Sonicate (400 W) the mixture for 40 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction for two more times. Wash the residue with 5 mL of methanol. Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask 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 (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	36	64	isocratic
10 – 60	36 → 30	64 → 70	linear gradient

System suitability requirements

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

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.92	± 0.03
2 (marker, nootkatone)	1.00	-
3	1.07	± 0.03
4	1.11	± 0.03

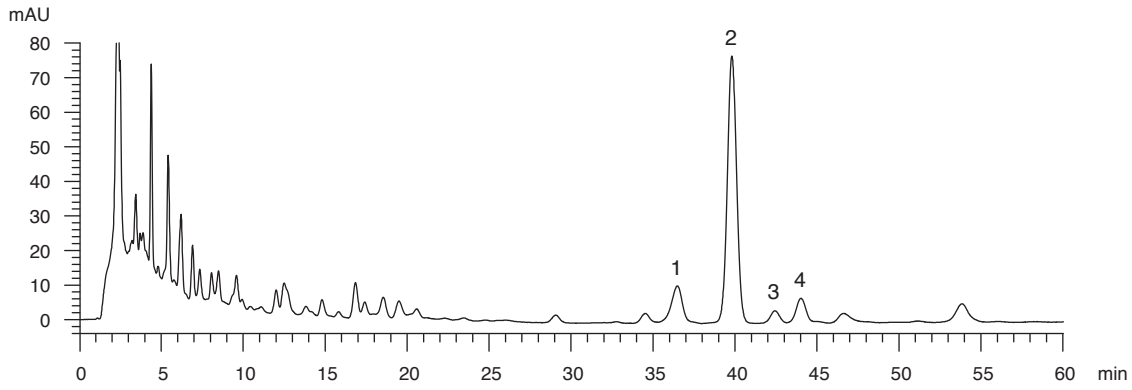

Figure 6 A reference fingerprint chromatogram of Alpiniae Oxyphyllae Fructus extract

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 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 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 7.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

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

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

Weigh accurately 10.0 mg of nootkatone CRS and dissolve in 10 mL of methanol.

Nootkatone standard solution for assay, Std-AS

Measure accurately the volume of the nootkatone Std-Stock, dilute with methanol to produce a series of solutions of 25, 50, 100, 250, 500 mg/L for nootkatone.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 12.5 mL of methanol. Sonicate (400 W) the mixture for 40 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction for two more times. Wash the residue with 5 mL of methanol. Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask 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 (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. The mobile phase is a mixture of 0.1% formic acid and acetonitrile (50:50, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of nootkatone Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of nootkatone should not be more than 5.0%; the RSD of the retention time of nootkatone peak should not be more than 2.0%; the column efficiency determined from nootkatone peak should not be less than 9000 theoretical plates.

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

Limits

The sample contains not less than 0.077% of nootkatone ($C_{15}H_{22}O$), calculated with reference to the dried substance.