Arecae Pericarpium

Figure 1 A photograph of Arecae Pericarpium

1. NAMES

Official Name: Arecae Pericarpium

Chinese Name: 大腹皮

Chinese Phonetic Name: Dafupi

2. SOURCE

Arecae Pericarpium is the dried pericarp of *Areca catechu* L. (Palmae). The unripe fruit is collected in winter to next spring, dried under the sun after boiled, cut longitudinally into two valves; the pericarp is peeled off to obtain Arecae Pericarpium.

3. DESCRIPTION

Slightly elliptical or elongated-ovate, gourd-shaped, 3.2-9.1 cm long, 1.6-6.6 cm wide, 2-5 mm thick. Exocarp dark brown to black, with irregular longitudinal wrinkles and prominent transverse striations on the surface; style scars at apex, a fruit stalk and remnants of calyx at the base. Mesocarp fibres present when pericarp stripped off longitudinally. Endocarp dented, brown or dark brown, smooth and hard shell-shaped. Texture hard and light in weight, easily stripped off longitudinally. Odour slight; taste slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp cells arranged relatively densely, covered with cuticle. Mesocarp relatively thick, parenchymatous cells contain raphides of calcium oxalate; stone cells scattered in mescocarp, subrounded, subrectangular or oblong. Fibre bundles scattered, lignified, scattered with vascular bundles. Endocarp cells irregularly polygonal, with thick wall, lignified (Fig. 2).

Powder

Colour yellowish-white or yellowish-brown. Stone cells subrounded, subrectangular or oblong, 12-76 µm in diameter, 22-241 µm long, sometimes clearly laminated. Exocarp cells polygonal or long polygonal. Endocarp cells irregularly polygonal, subrounded or elliptical, wall thick and lignified, pits distinct. Mesocarp fibres in bundles, fine and long, slightly lignified, pits distinct, cells surrounding the fibres containing round-clustered silica bodies. Vessels mainly spiral or scalariform, 4-30 µm in diameter. Raphides of calcium oxalate scattered singly or in bundles, bright orange, 22-109 µm long; polychromatic under the polarized microscope (Fig. 3).

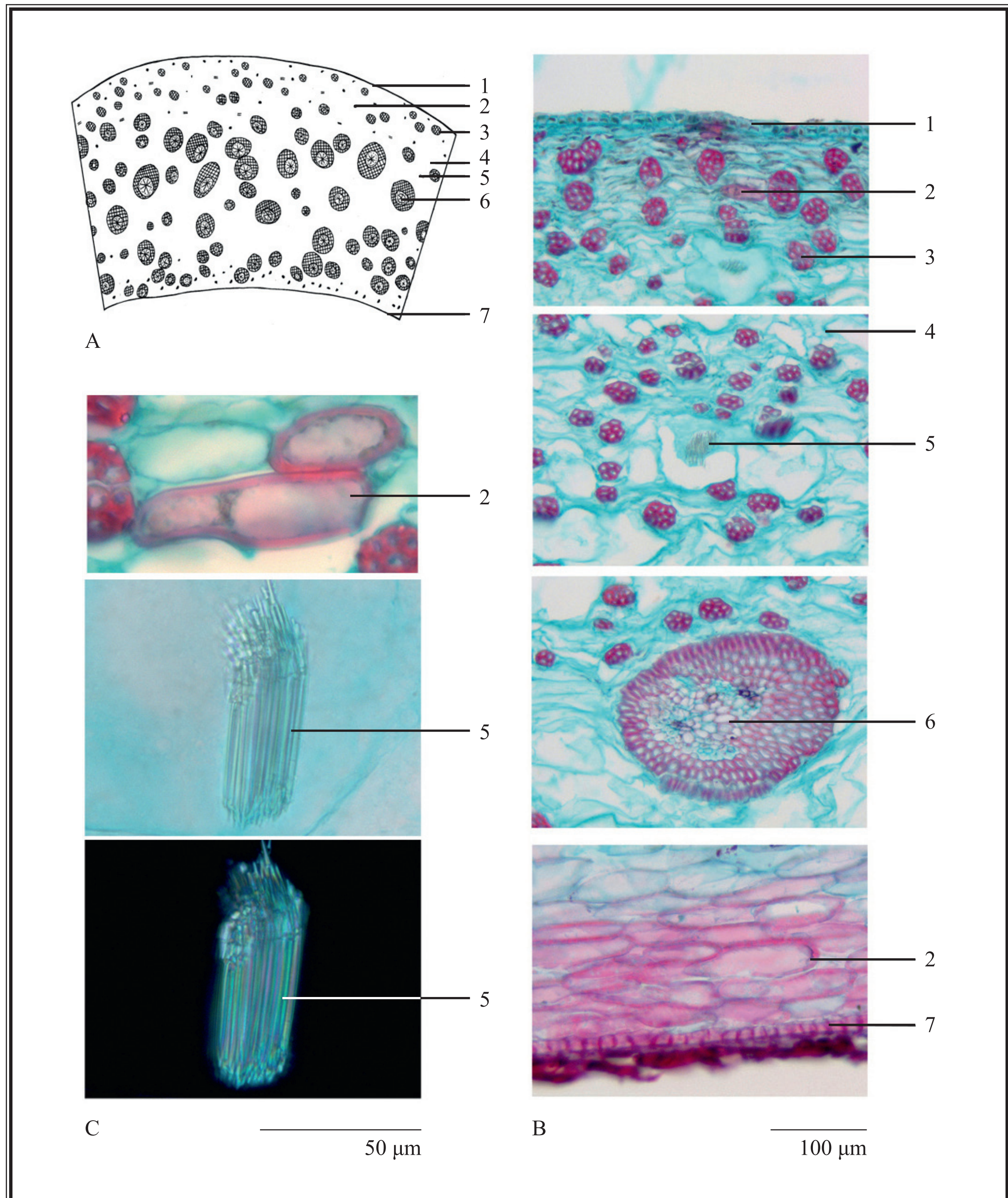

Figure 2 Microscopic features of transverse section of Arecae Pericarpium

A. Sketch B. Section illustration
C. Stone cells and raphides of calcium oxalate

1. Exocarp 2. Stone cells 3. Fibre bundles 4. Mesocarp
5. Raphides of calcium oxalate 6. Vascular bundles 7. Endocarp

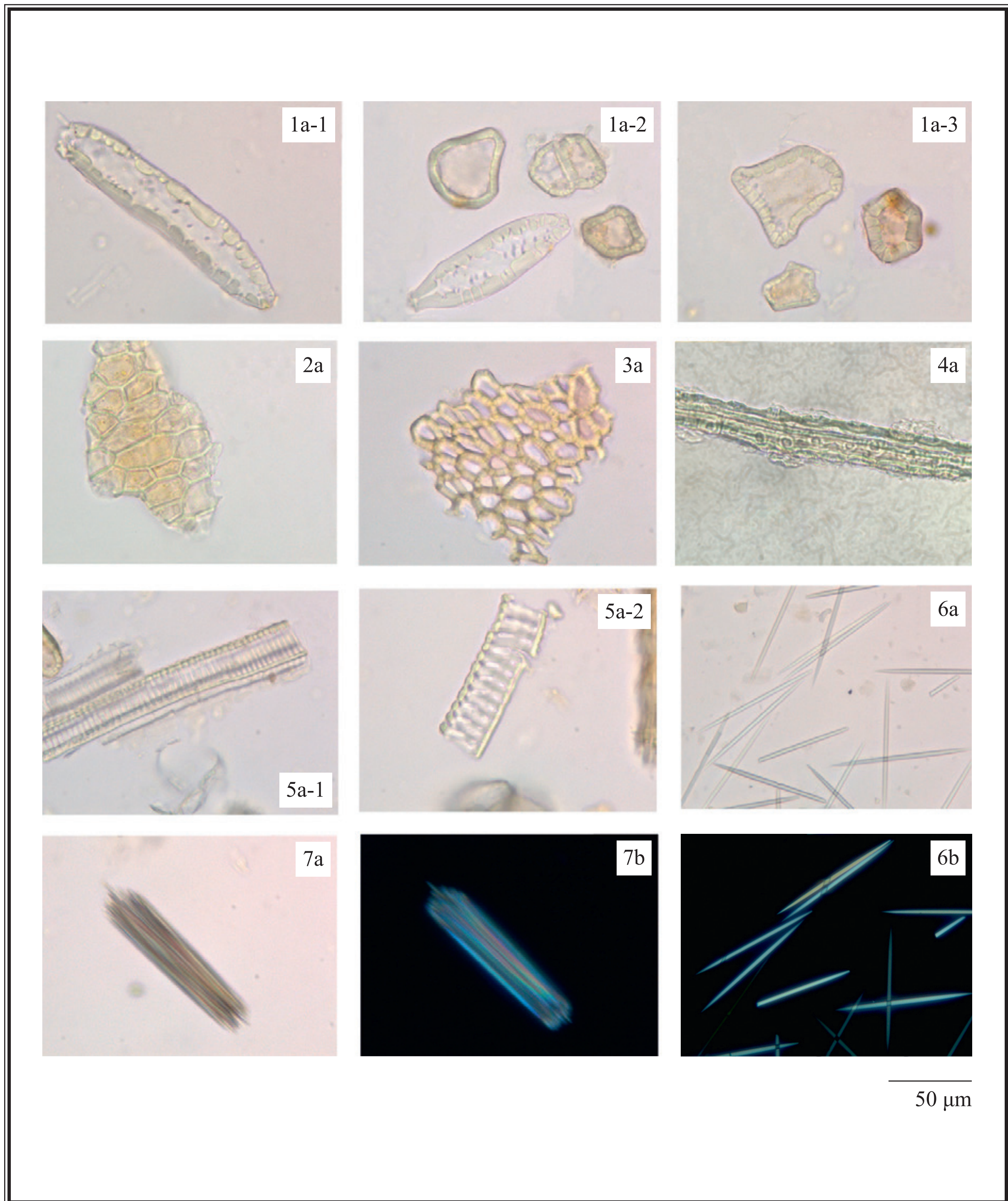

Figure 3 Microscopic features of powder of Arecae Pericarpium

1. Stone cells 2. Exocarp cells 3. Endocarp cells 4. Mesocarp fibres
5a-1. Spiral vessels 5a-2. Scalariform vessel
6. Scattered raphides of calcium oxalate 7. Raphides of calcium oxalate in bundle

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

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

Standard solution

Arecoline hydrobromide standard solution

Weigh 2.0 mg of arecoline hydrobromide CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), methanol and ethyl acetate (0.2:0.4:8.5, v/v).

Staining reagent

Iodine.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of ethanol (95%). Sonicate (150 W) the mixture for 45 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. 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 arecoline hydrobromide standard solution and the test solution (5 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. Fumigate the plate with iodine vapor chamber for about 1 h until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

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 arecoline hydrobromide.

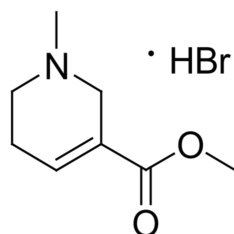

Figure 4 Chemical structure of arecoline hydrobromide

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

Standard solution

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

Weigh 1.0 mg of arecoline hydrobromide CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (200 W) the mixture for 1 h. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (215 nm) and a column (4.6 × 250 mm) packed with strong cation exchanger bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.2% phosphoric acid* and acetonitrile (35:65, v/v). The elution time is about 30 min.

*Adjust the pH to 3.8 with ammonium hydroxide solution (25%, v/v)

System suitability requirements

Perform at least five replicate injections, each using 10 µL of arecoline hydrobromide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of arecoline hydrobromide should not be more than 5.0%; the RSD of the retention time of arecoline hydrobromide peak should not be more than 2.0%; the column efficiency determined from arecoline hydrobromide peak should not be less than 8500 theoretical plates.

The *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject arecoline hydrobromide Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of arecoline hydrobromide peak in the chromatogram of arecoline hydrobromide Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify arecoline hydrobromide peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of arecoline hydrobromide Std-FP. The retention times of arecoline hydrobromide 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 Arecae Pericarpium extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the five characteristic peaks of Arecae Pericarpium extract

Peak No.	RRT	Acceptable Range
1	0.43	± 0.03
2	0.49	± 0.03
3	0.56	± 0.03
4	0.80	± 0.03
5 (marker, arecoline hydrobromide)	1.00	-

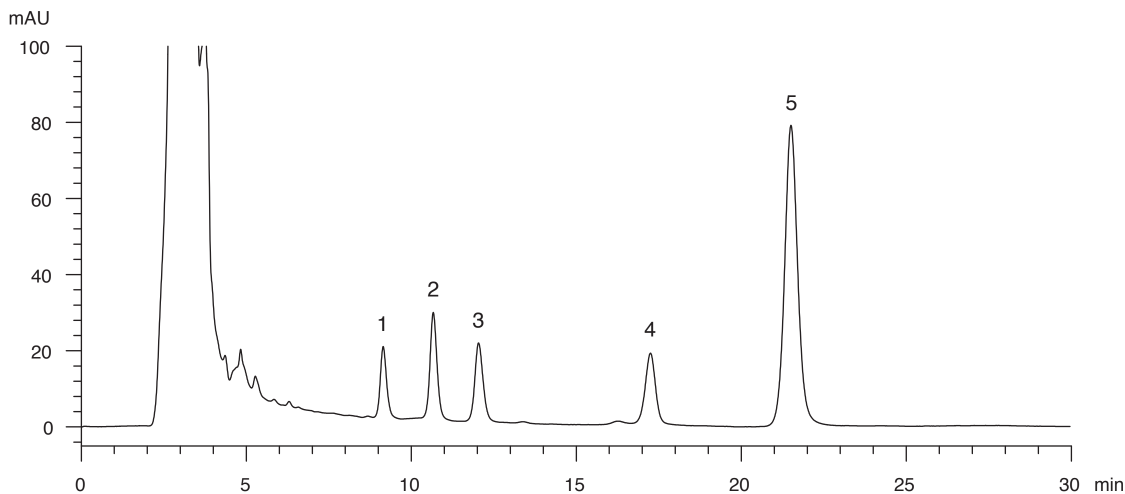

Figure 5 A reference fingerprint chromatogram of Arecae Pericarpium extract

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 fingerprint chromatogram (Fig. 5).

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 4.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 2.0 mg of arecoline hydrobromide CRS and dissolve in 2 mL of methanol.

Arecoline hydrobromide standard solution for assay, Std-AS

Measure accurately the volume of the arecoline hydrobromide Std-Stock, dilute with methanol to produce a series of solutions of 10, 20, 30, 40, 50 mg/L for arecoline hydrobromide.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (200 W) the mixture for 1 h. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol. Combine the solutions and make up to the mark with methanol. Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with strong cation exchanger bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.2% phosphoric acid* and acetonitrile (35:65, v/v). The elution time is about 30 min.

*Adjust the pH to 3.8 with ammonium hydroxide solution (25%, v/v)

System suitability requirements

Perform at least five replicate injections, each using 10 μL of arecoline hydrobromide Std-AS (30 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of arecoline hydrobromide should not be more than 5.0%; the RSD of the retention time of arecoline hydrobromide peak should not be more than 2.0%; the column efficiency determined from arecoline hydrobromide peak should not be less than 9000 theoretical plates.

The *R* value between arecoline hydrobromide 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 arecoline hydrobromide Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of arecoline hydrobromide against the corresponding concentrations of arecoline hydrobromide 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 arecoline hydrobromide peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of arecoline hydrobromide Std-AS. The retention times of arecoline hydrobromide peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of arecoline hydrobromide in the test solution, and calculate the percentage content of arecoline (the percentage content of arecoline hydrobromide × 0.657) in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.078% of arecoline ($C_8H_{13}NO_2$), calculated with reference to the dried substance.