Piperis Fructus

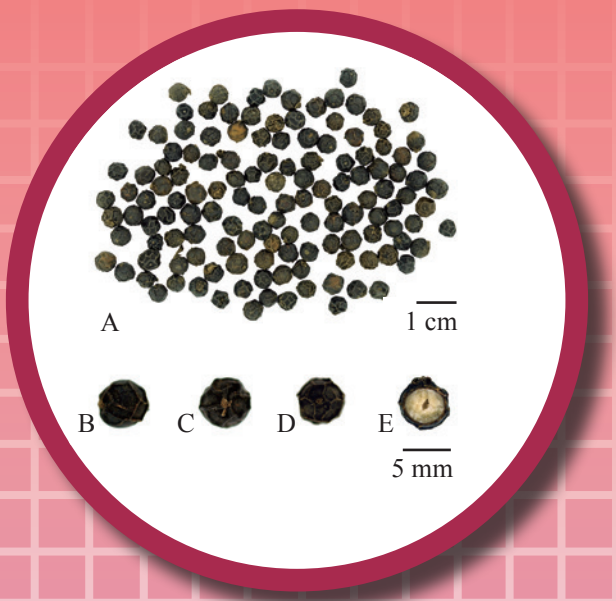

Figure 1 (i) A photograph of Piperis Fructus – Black Pepper

A. Black Pepper B. Magnified image of fruit C. Apex D. Base
E. Magnified image of transverse section of fruit

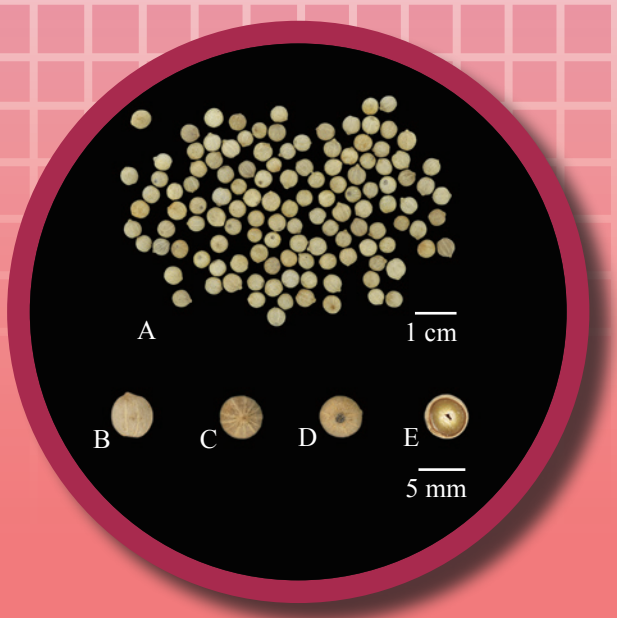

Figure 1 (ii) A photograph of Piperis Fructus – White Pepper

A. White Pepper B. Magnified image of fruit C. Apex D. Base
E. Magnified image of transverse section of fruit

1. NAMES

Official Name: Piperis Fructus

Chinese Name: 胡椒

Chinese Phonetic Name: Hujiao

2. SOURCE

Piperis Fructus is the dried and almost ripe or ripe fruit of *Piper nigrum* L. (Piperaceae). The fruit is collected when it is dark green (almost ripe), then dried under the sun to obtain "Black Pepper". The fruit is also collected when it turns red (ripe), macerate in water for several days, followed by removal of sarcocarp, then dried under the sun to obtain "White Pepper".

3. DESCRIPTION

Black Pepper: Spheroidal, 3.5-5.5 mm in diameter. Externally blackish-brown, with raised reticulated wrinkles, a small remnant of style at the apex and a scar of fruit stalk at the base. Texture hard, exocarp may be stripped, endocarp greyish-white to pale yellow. Transverse section yellowish-white, starchy, with a small hollowed space at the centre. Odour aromatic; taste pungent [Fig.1(i)].

White Pepper: 3-4.5 mm in diameter, externally greyish-white to pale yellowish-white, smooth, with numerous linear striations of light colour between apex and base [Fig.1(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Black Pepper: Exocarp consists of 1 layer of epidermal cells and 2-3 layers of stone cells. Mesocarp consists of parenchymatous cells, scattered with oil cells and vascular bundles. Endocarp consists of 1 layer of stone cells (beaker cells), inner tangential wall strongly thickened, sometimes containing crystals of calcium oxalate. Testa consists of 2-3 layers of brown to dark brown compressed cells on the outer side, hyaline layer consists of 1 layer of transparent cells on the inner side. Perisperm consists of thin-walled polygonal cells, filled with small starch granules [Fig. 2(i)].

White Pepper: Exocarp absent. Mesocarp incomplete, consisting of parenchymatous cells, scattered with oil cells [Fig. 2(ii)].

Powder

Black Pepper: Colour dark grey. Stone cells of exocarp subsquare, rectangular or irregular in shape, 8-78 μm in diameter, walls relatively thickened. Stone cells of endocarp (beaker cells) subpolygonal in surface view, 10-37 μm in diameter, square in lateral view, one side of cell wall thin, strongly thickened on the other side of the cell, sometimes embedded with crystals of calcium oxalate. Cells of testa brown to reddish-brown, polygonal. Cells of hyaline layer transparent, rectangular or subpolygonal. Perisperm cells polygonal, filled with small starch granules. Starch granules small, scattered singly or aggregated in mass, subrounded; black and cruciate-shaped under the polarized microscope [Fig. 3(i)].

White Pepper: Colour yellowish-white. Cells of endocarp, testa, hyaline layer and perisperm can be observed in white pepper and showing the same features as described in black pepper [Fig. 3(ii)].

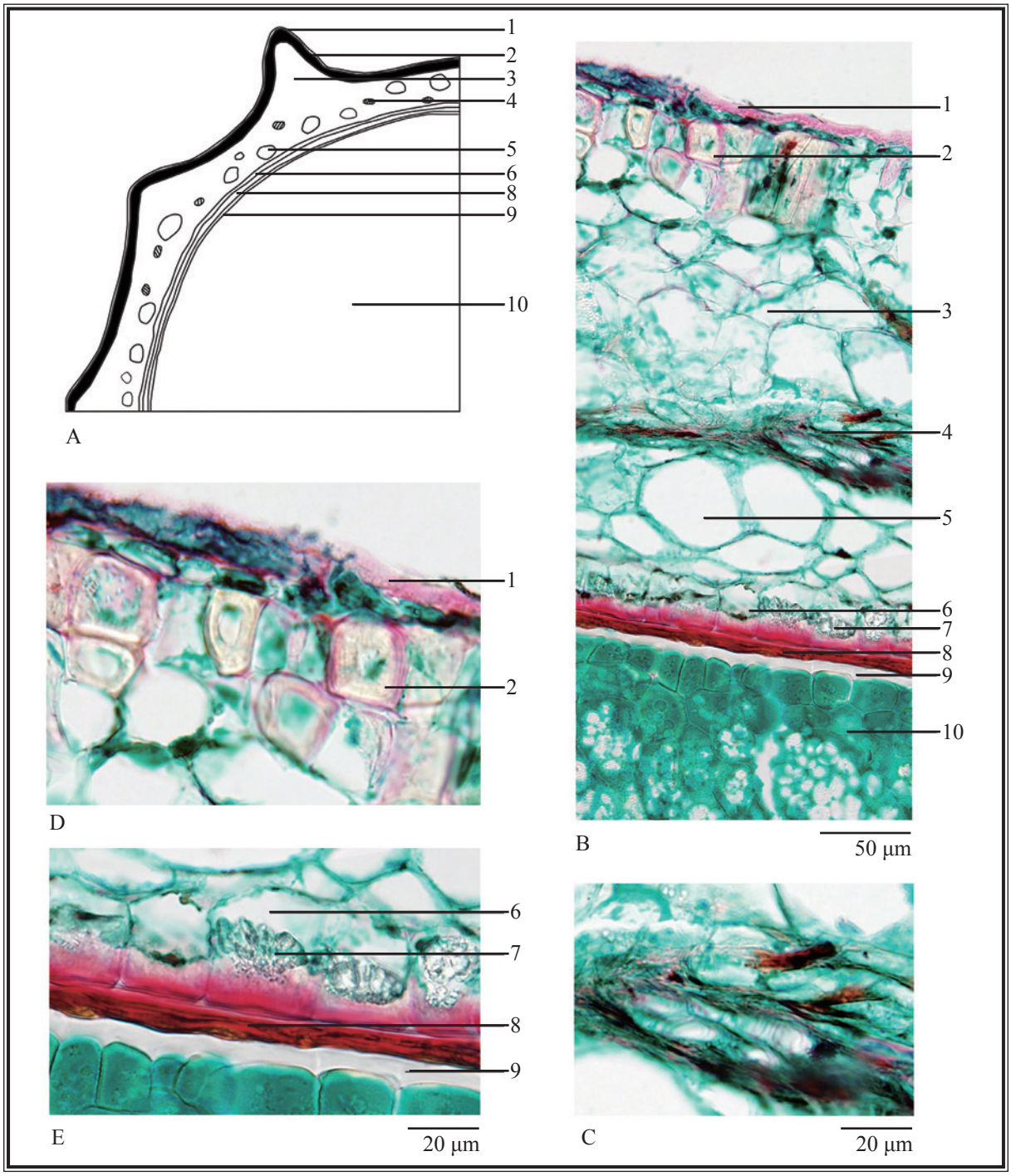

Figure 2 (i) Microscopic features of transverse section of Piperis Fructus – Black Pepper

A. Sketch B. Section illustration C. Vascular bundles D-E. Section magnified

1. Epidermis of exocarp 2. Stone cells 3. Mesocarp 4. Vascular bundles
5. Oil cell 6. Endocarp stone cells (beaker cells) 7. Crystals of calcium oxalate
8. Testa 9. Hyaline layer 10. Perisperm

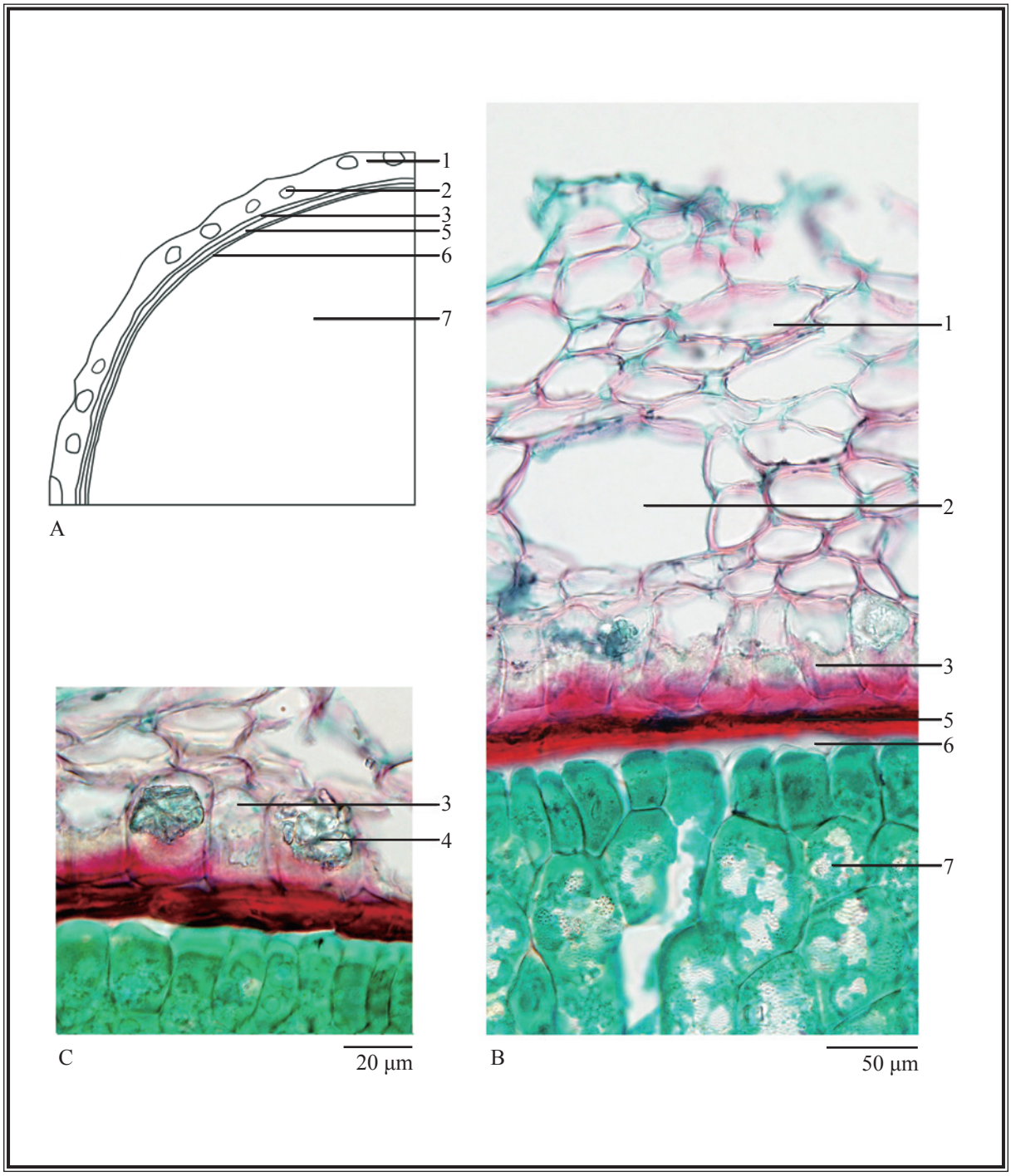

Figure 2 (ii) Microscopic features of transverse section of Piperis Fructus – White Pepper

A. Sketch B. Section illustration C. Section magnified

1. Mesocarp 2. Oil cell 3. Endocarp stone cells (beaker cells)
4. Crystals of calcium oxalate 5. Testa 6. Hyaline layer 7. Perisperm

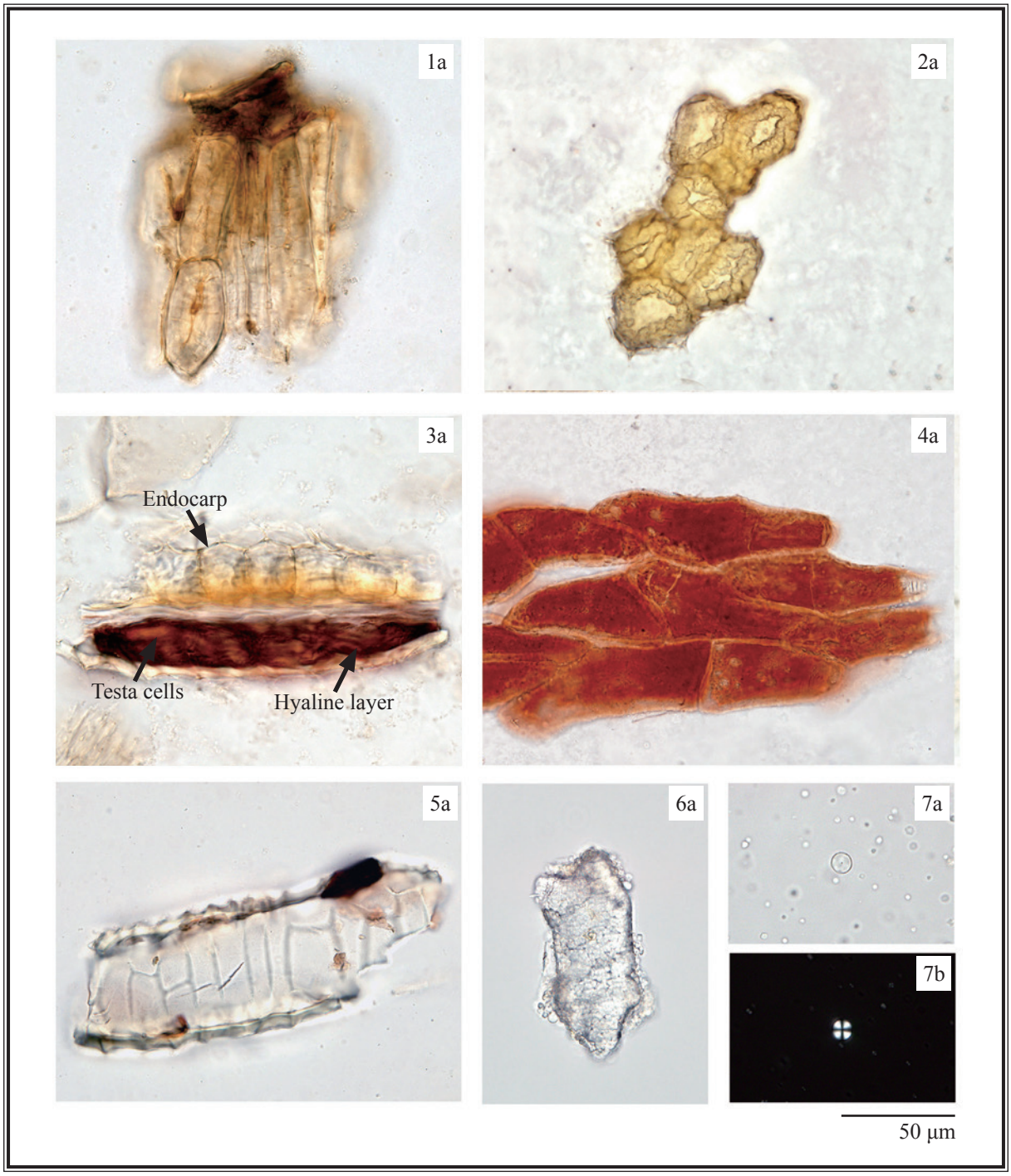

Figure 3 (i) Microscopic features of powder of Piperis Fructus – Black Pepper

1. Stone cells of exocarp 2. Stone cells of endocarp (beaker cells)
3. Lateral view of endocarp, testa cells and hyaline layer 4. Cells of testa
5. Cells of hyaline layer 6. Perisperm cell 7. Starch granule

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

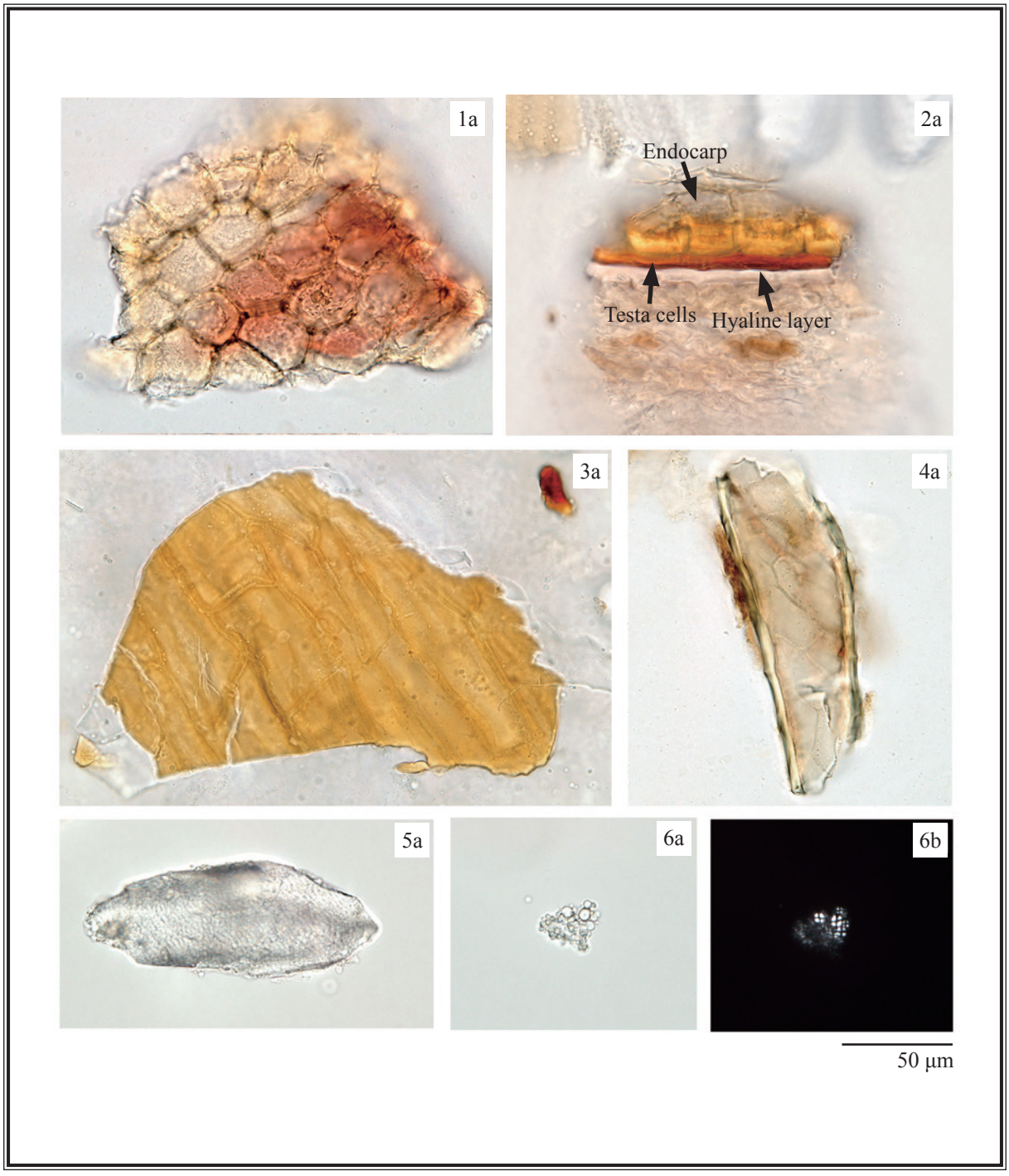

Figure 3 (ii) Microscopic features of powder of Piperis Fructus – White Pepper

1. Stone cells of endocarp (beaker cells)
2. Lateral view of endocarp, testa cells and hyaline layer
3. Cells of testa 4. Cells of hyaline layer 5. Perisperm cell 6. Starch granules

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

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

Standard solution

Piperine standard solution

Weigh 5.0 mg of piperine CRS (Fig. 4) and place it in a 5-mL amber glass volumetric flask. Make up to the mark with ethanol. Freshly prepare the standard solution.

Developing solvent system

Prepare a mixture of *n*-hexane, ethyl acetate and acetone (8:4:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask wrapped in aluminium foil, then add 10 mL of ethanol. Sonicate (220 W) the mixture for 30 min. Filter the mixture. Freshly prepare the test solution.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately piperine standard solution and the test solution (1 μL each) to the plate. 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).

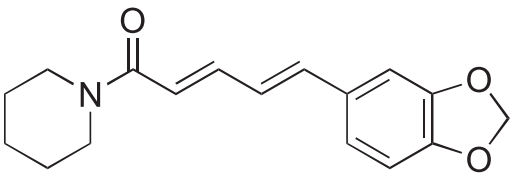

Figure 4 Chemical structure of piperine

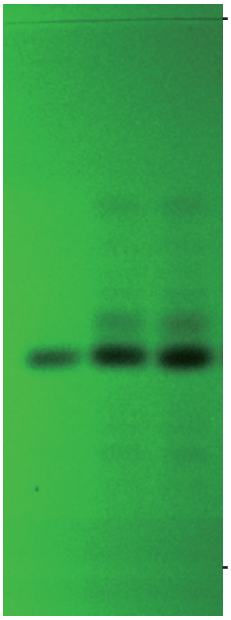

Figure 5 A reference HPTLC chromatogram of Piperis Fructus extract observed under UV light (254 nm)

1. Piperine standard solution
2. Test solution of
(i) White Pepper
(ii) Black Pepper

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 piperine (Fig. 5).

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

Standard solution

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

Weigh 1.0 mg of piperine CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol. Freshly prepare the standard solution.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask wrapped in aluminium foil, then add 10 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL amber glass volumetric flask. Make up to the mark with methanol. Filter through a 0.45-µm PTFE filter. Freshly prepare the test solution.

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)	Acetonitrile (%, v/v)	Water (%, v/v)	Elution
0 – 15	50	50	isocratic
15 – 30	50 → 55	50 → 45	linear gradient
30 – 60	55 → 80	45 → 20	linear gradient

System suitability requirements

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

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 6 (i) or (ii)].

Procedure

Separately inject piperine Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of piperine peak in the chromatogram of piperine Std-FP and the retention times of the six characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify piperine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of piperine Std-FP. The retention times of piperine 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 six characteristic peaks of Piperis Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Piperis Fructus extract

Peak No.	RRT	Acceptable Range
1	0.71	± 0.03
2 (marker, piperine)	1.00	-
3	1.53	± 0.03
4	1.80	± 0.04
5	2.57	± 0.04
6	3.45	± 0.06

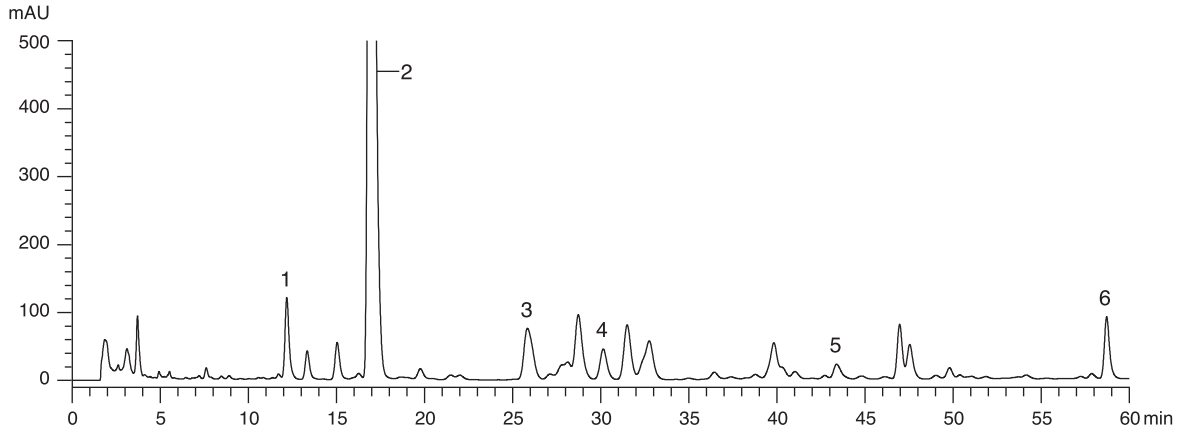

Figure 6 (i) A reference fingerprint chromatogram of Piperis Fructus – Black Pepper extract

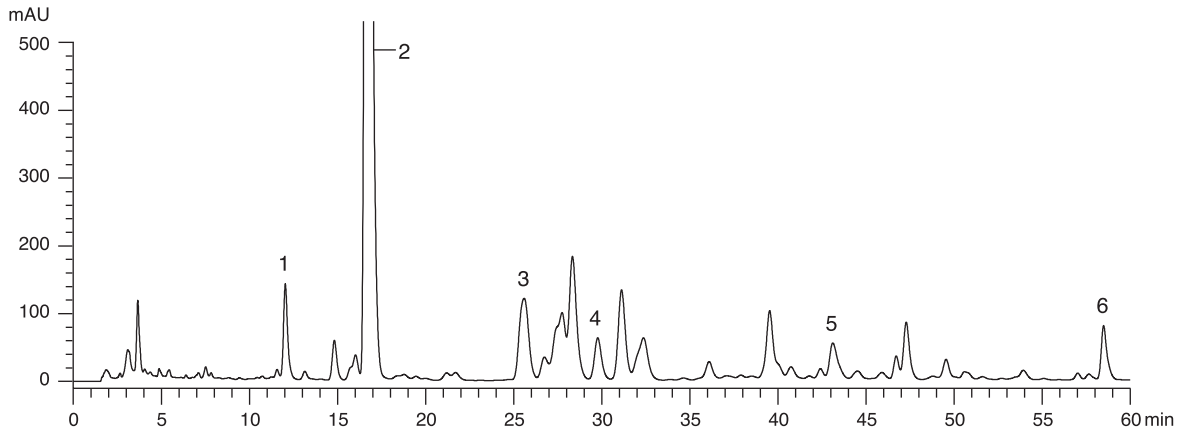

Figure 6 (ii) A reference fingerprint chromatogram of Piperis Fructus – White Pepper extract

For positive identification, the sample must give the above six characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference 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 – Aflatoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 1.0%.

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

7. ASSAY

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

Standard solution

Piperine standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 mg of piperine CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol. Freshly prepare the standard solution.

Piperine standard solution for assay, Std-AS

Measure accurately the volume of the piperine Std-Stock, dilute with methanol to produce a series of solutions of 10, 20, 40, 50, 80 mg/L for piperine. Store in amber glass volumetric flasks.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL conical flask wrapped in aluminium foil, then add 40 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL amber glass volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter. Freshly prepare the test solution.

Chromatographic system

The liquid chromatograph is equipped with a DAD (343 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. The mobile phase is a mixture of water and acetonitrile (52: 48, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of piperine Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of piperine should not be more than 5.0%; the RSD of the retention time of piperine peak should not be more than 2.0%; the column efficiency determined from piperine peak should not be less than 10000 theoretical plates.

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

Limits

The sample contains not less than 3.3% of piperine ($C_{17}H_{19}NO_3$), calculated with reference to the dried substance.

Piperis Fructus（胡椒）

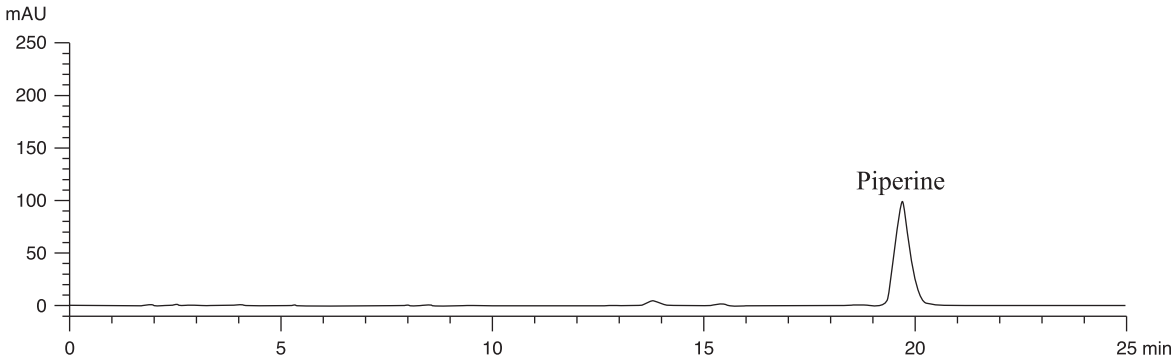

Figure 1 (i) A reference assay chromatogram of Piperis Fructus - Black Pepper extract

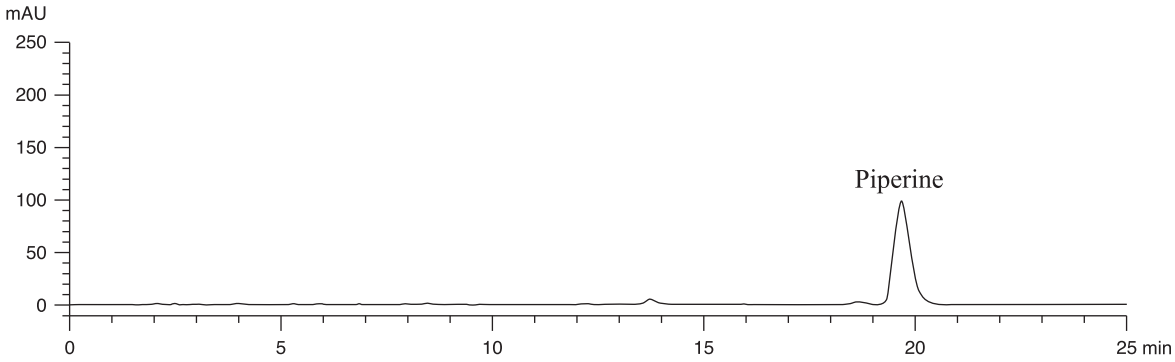

Figure 1 (ii) A reference assay chromatogram of Piperis Fructus - White Pepper extract