Fraxini Cortex

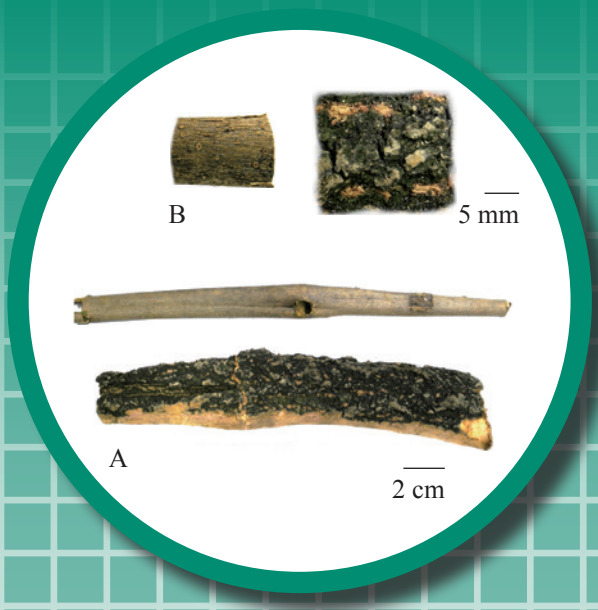


Figure 1 (i) A photograph of dried branch bark and stem bark of *Fraxinus chinensis* Roxb.

A. Branch bark (upper) and stem bark (lower)
B. Magnified branch bark (left) and stem bark (right)

Figure 1 (ii) A photograph of dried branch bark and stem bark of *Fraxinus szaboana* Lingelsh.

A. Branch bark (upper) and stem bark (lower)
B. Magnified branch bark (left) and stem bark (right)

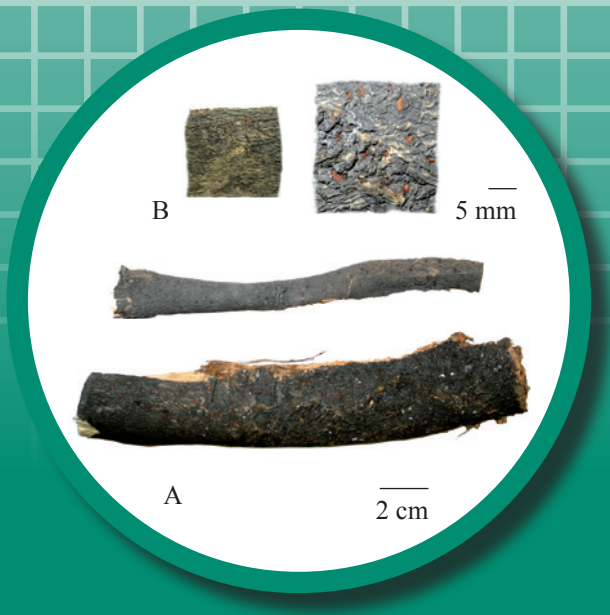


Figure 1 (iii) A photograph of dried branch bark and stem bark of *Fraxinus stylosa* Lingelsh.

A. Branch bark (upper) and stem bark (lower)
B. Magnified branch bark (left) and stem bark (right)

1. NAMES

Official Name: Fraxini Cortex

Chinese Name: 秦皮

Chinese Phonetic Name: Qinpi

2. SOURCE

Fraxini Cortex is the dried branch bark or stem bark of *Fraxinus chinensis* Roxb., *Fraxinus szaboana* Lingelsh. or *Fraxinus stylosa* Lingelsh. (Oleanaceae). The branch bark or stem bark is collected in spring and autumn, then dried under the sun to obtain Fraxini Cortex.

3. DESCRIPTION

Branch bark:

***Fraxinus chinensis* Roxb.:** Quilled or channeled, 10-60 cm long, 1-3 mm thick. Externally greenish-brown to blackish-brown, not showing greyish-white patches of lichens. Purplish-red in the centre of the lenticels. Crescent leaf-scars visible. The inner surface yellowish-white to brown, smooth. Texture hard and fragile; fracture fibrous, yellowish-white. Odour slight; taste bitter [Fig. 1 (i)].

***Fraxinus szaboana* Lingelsh.:** Quilled or channeled, 17-49 cm long, 1-3 mm thick. Externally greyish-white to greyish-brown, with distinct fine longitudinal wrinkles, showing greyish-white patches of lichens. Branch-scars opposite. Crescent leaf-scars visible. Odour slight; taste bitter [Fig. 1 (ii)].

***Fraxinus stylosa* Lingelsh.:** Quilled or channeled, 15-40 cm long, 1-2.9 mm thick. Externally greenish-brown to blackish-brown, occasionally showing greyish-white patches of lichens. Odour slight; taste relatively bitter [Fig. 1 (iii)].

Stem bark

***Fraxinus chinensis* Roxb.:** Slat-shaped pieces or channeled, 2-6 mm thick. Externally shrunken obviously, the thick stem bark with numerous ramous cracks. Lenticels dotted or ovate, reddish-brown. Texture hard; fracture relatively fibrous [Fig. 1 (i)].

***Fraxinus szaboana* Lingelsh.:** Slat-shaped pieces, 2-6 mm thick. Externally greyish-brown, with rimose furrows. Lenticels dotted or ovate, greyish-white in surrounding and reddish-brown in centre [Fig. 1 (ii)].

***Fraxinus stylosa* Lingelsh.:** Slat-shaped pieces, 2-6 mm thick. Externally greyish-brown, with rimose furrows and reddish-brown, rounded or transversely slender lenticels [Fig. 1 (iii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Branch bark or stem bark:

***Fraxinus chinensis* Roxb.:** Cork consists of 5-10 layers of cells, cells rectangular or squared. Phelloderm consists of several layers of polygonal collenchymatous cells. Cortex relatively broad, fibres and stone cells scattered singly or in groups. Phloem relatively broad, ring of sclerenchymatous cells composed of stone cells and fibre bundles located on the outside, occasionally interrupted; stone cells in the band relatively large, with distinct pit canals. Some bands composed of fibres and stone cells, located in parenchymatous cells of phloem and sieve tubes; two bands arranged alternately, interrupted by phloem rays. Phloem rays 1-4 rows of cells wide. Parenchymatous cells contain crystals of calcium oxalate [Fig. 2 (i)].

***Fraxinus szaboana* Lingelsh.:** Cortex relatively narrow. Groups of stone cells in the ring of sclerenchymatous cells located in the outside of phloem relatively well developed. Parenchymatous cells of phloem and sieve tubes relatively broad [Fig. 2 (ii)].

***Fraxinus stylosa* Lingelsh.:** Groups of stone cells in the ring of sclerenchymatous cells located in the outside of phloem relatively well developed [Fig. 2 (iii)].

Powder

Colour greenish-brown or yellowish-white. Stone cells relatively abundant, scattered singly or in groups, subrounded, subsquare, subrectangular, elliptical, subfusiform or shortly and irregularly branched, 22-79 μm in diameter, 125-190 μm long, walls extremely thickened, pit canals distinct. Cork cells polygonal, walls slightly thickened and lignified, pits relatively sparse. Fibres scattered singly or in bundles, extremely long, mostly broken, straight or slightly curved, 10-37 μm in diameter, margin undulate or lumpy, walls extremely thickened and lignified, pits indistinct, lumens linear; bright brownish-white or polychromatic under the polarized microscope. Phloem ray 1-4 cells wide, lumens filled with microcrystals of calcium

oxalate; bright white or yellowish-white under the polarized microscope. Crystals of calcium oxalate occur in parenchymatous cells of cork, phloem and ray cells, slightly fine fusiform or granular, up to 3 μm in diameter; bright white under the polarized microscope [Fig. 3 (i), (ii) and (iii)].

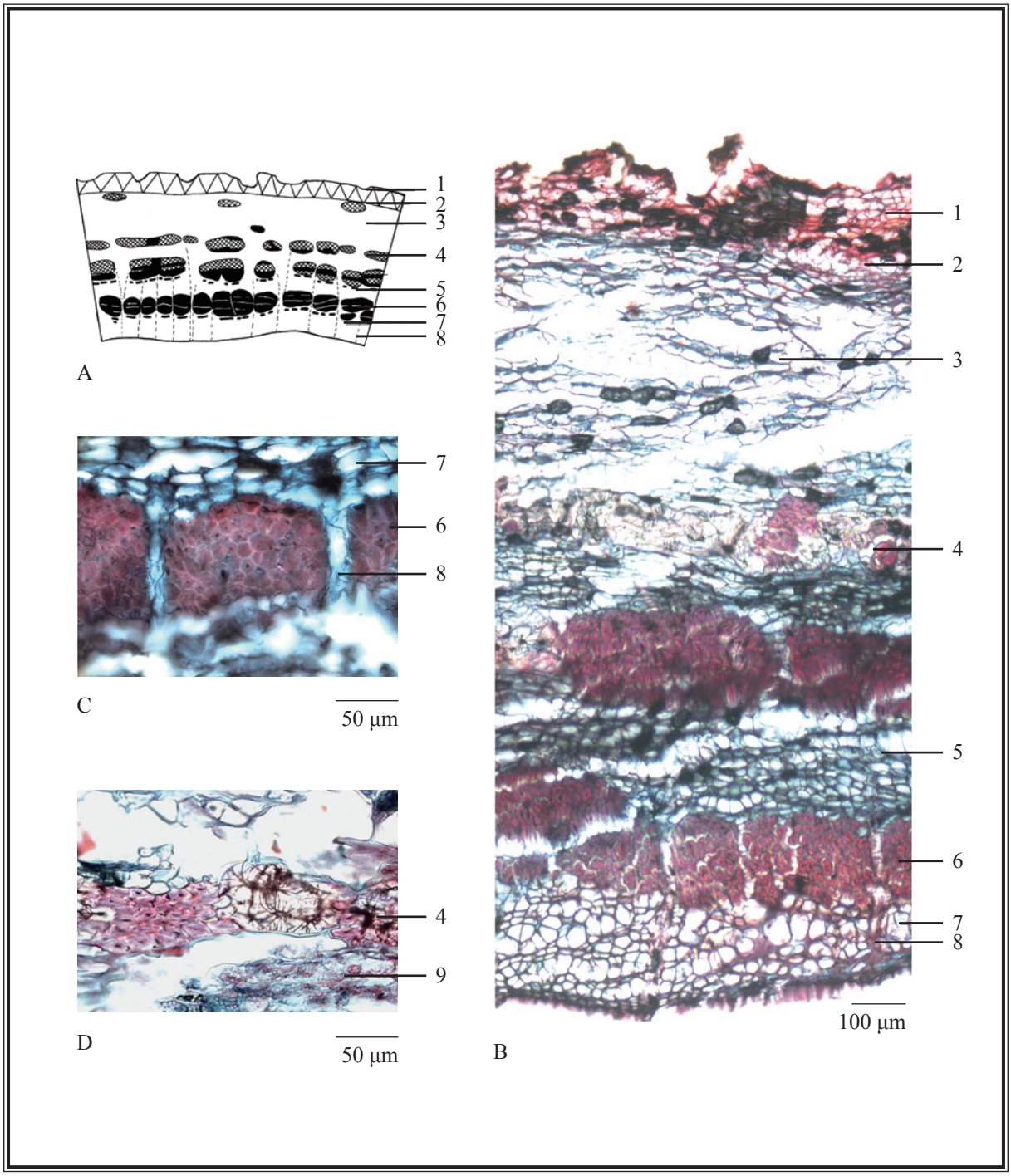


Figure 2 (i) Microscopic features of transverse section of dried branch bark or stem bark of *Fraxinus chinensis* Roxb.

A. Sketch B. Section illustration C. Phloem D. Ring of sclerenchymatous cells

1. Cork 2. Phelloderm 3. Cortex 4. Ring of sclerenchymatous cells
5. Phloem 6. Fibres 7. Sieve tubes and parenchymatous cells 8. Phloem rays
9. Crystals of calcium oxalate

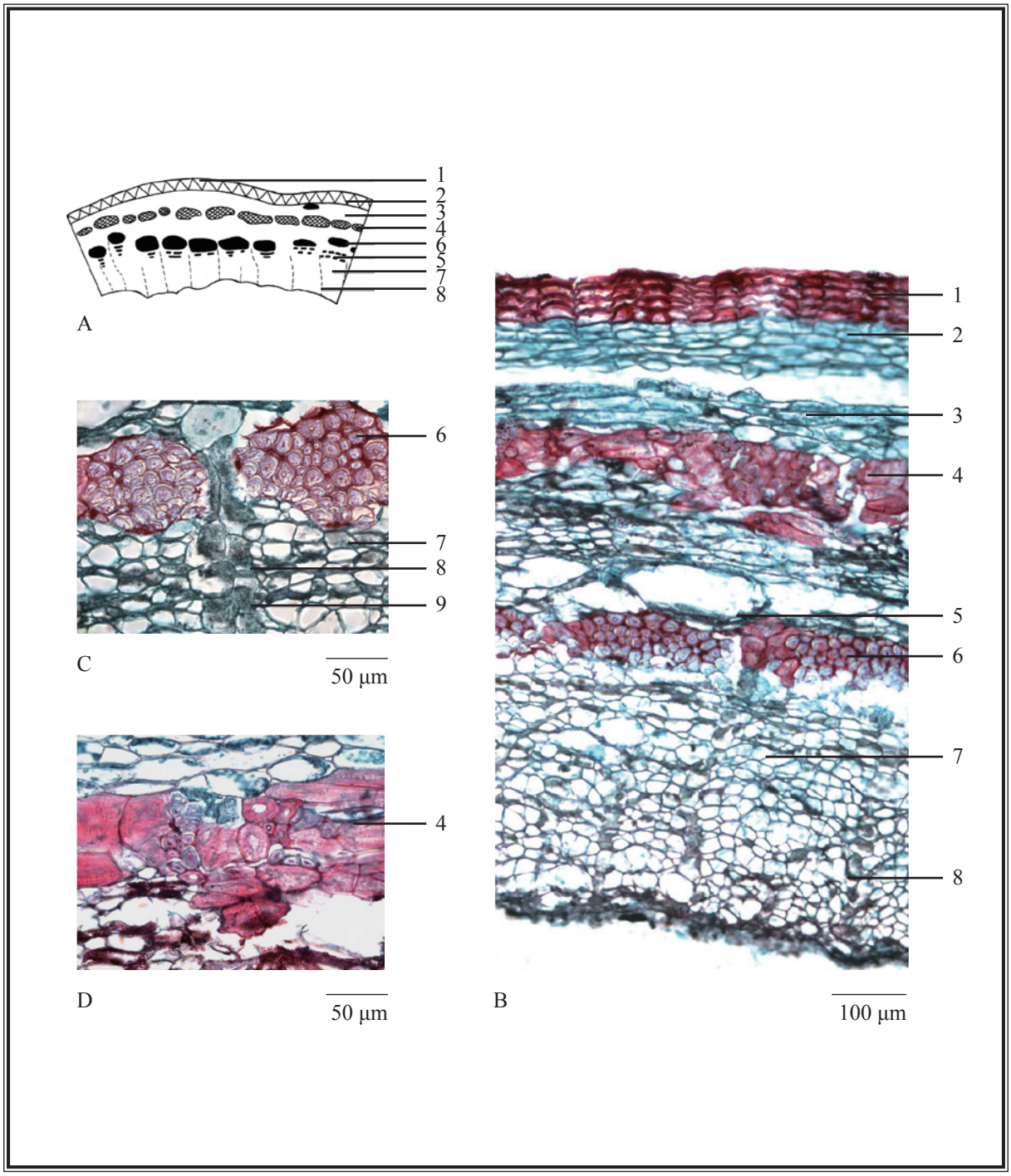


Figure 2 (ii) Microscopic features of transverse section of dried branch bark or stem bark of *Fraxinus szaboana* Lingelsh.

A. Sketch B. Section illustration C. Phloem D. Ring of sclerenchymatous cells

1. Cork 2. Phelloderm 3. Cortex 4. Ring of sclerenchymatous cells
5. Phloem 6. Fibres 7. Sieve tubes and parenchymatous cells 8. Phloem rays
9. Crystals of calcium oxalate

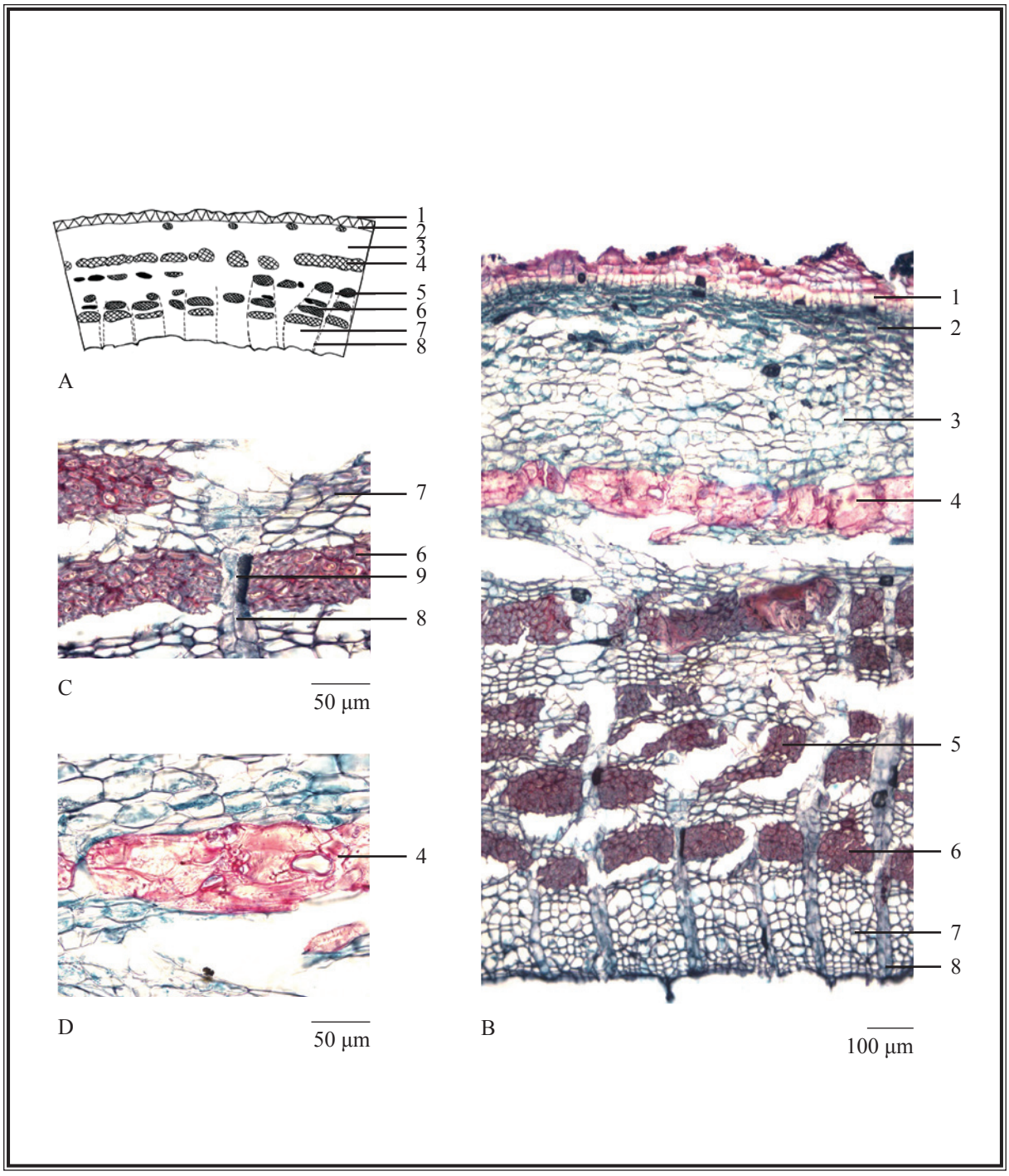


Figure 2 (iii) Microscopic features of transverse section of dried branch bark or stem bark of *Fraxinus stylosa* Lingelsh.

A. Sketch B. Section illustration C. Phloem D. Ring of sclerenchymatous cells

1. Cork 2. Phelloderm 3. Cortex 4. Ring of sclerenchymatous cells
5. Phloem 6. Fibres 7. Sieve tubes and parenchymatous cells 8. Phloem rays
9. Crystals of calcium oxalate

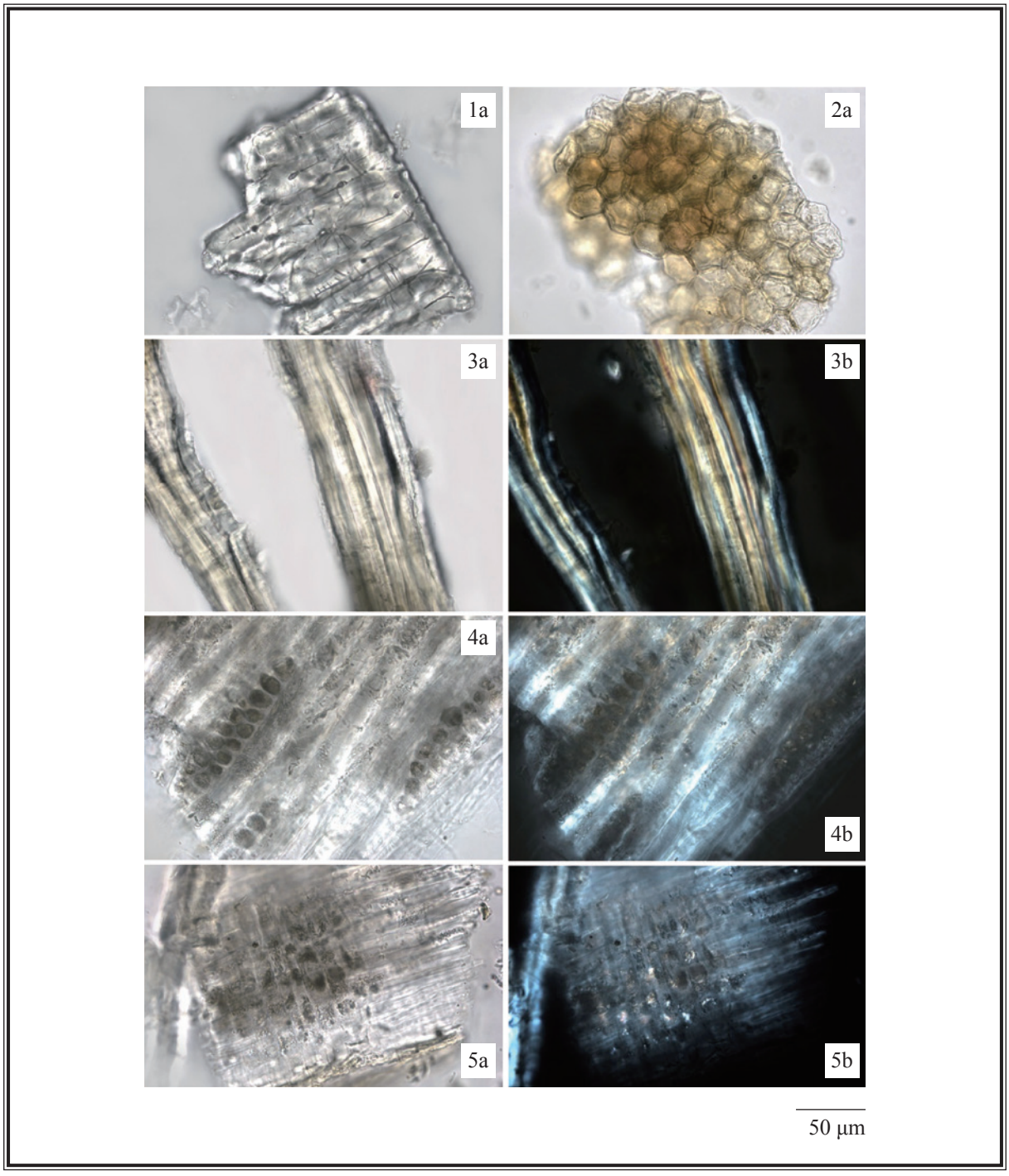


Figure 3 (i) Microscopic features of powder of dried branch bark or stem bark of *Fraxinus chinensis* Roxb.

1. Stone cells 2. Cork cells 3. Fibres 4. Phloem ray cells
5. Crystals of calcium oxalate

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

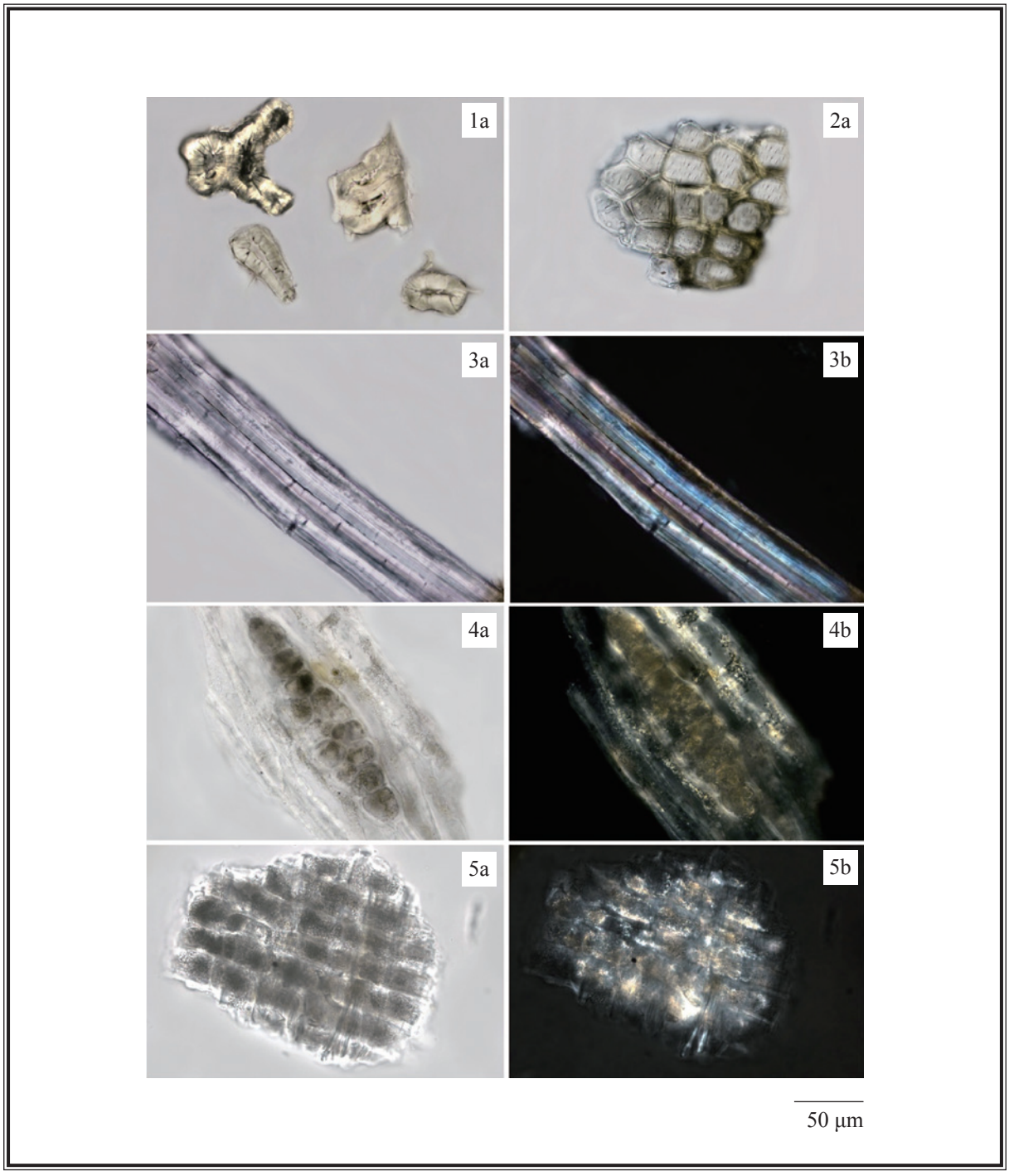


Figure 3 (ii) Microscopic features of powder of dried branch bark or stem bark of *Fraxinus szaboana* Lingelsh.

1. Stone cells 2. Cork cells 3. Fibres 4. Phloem ray cells 5. Crystals of calcium oxalate

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

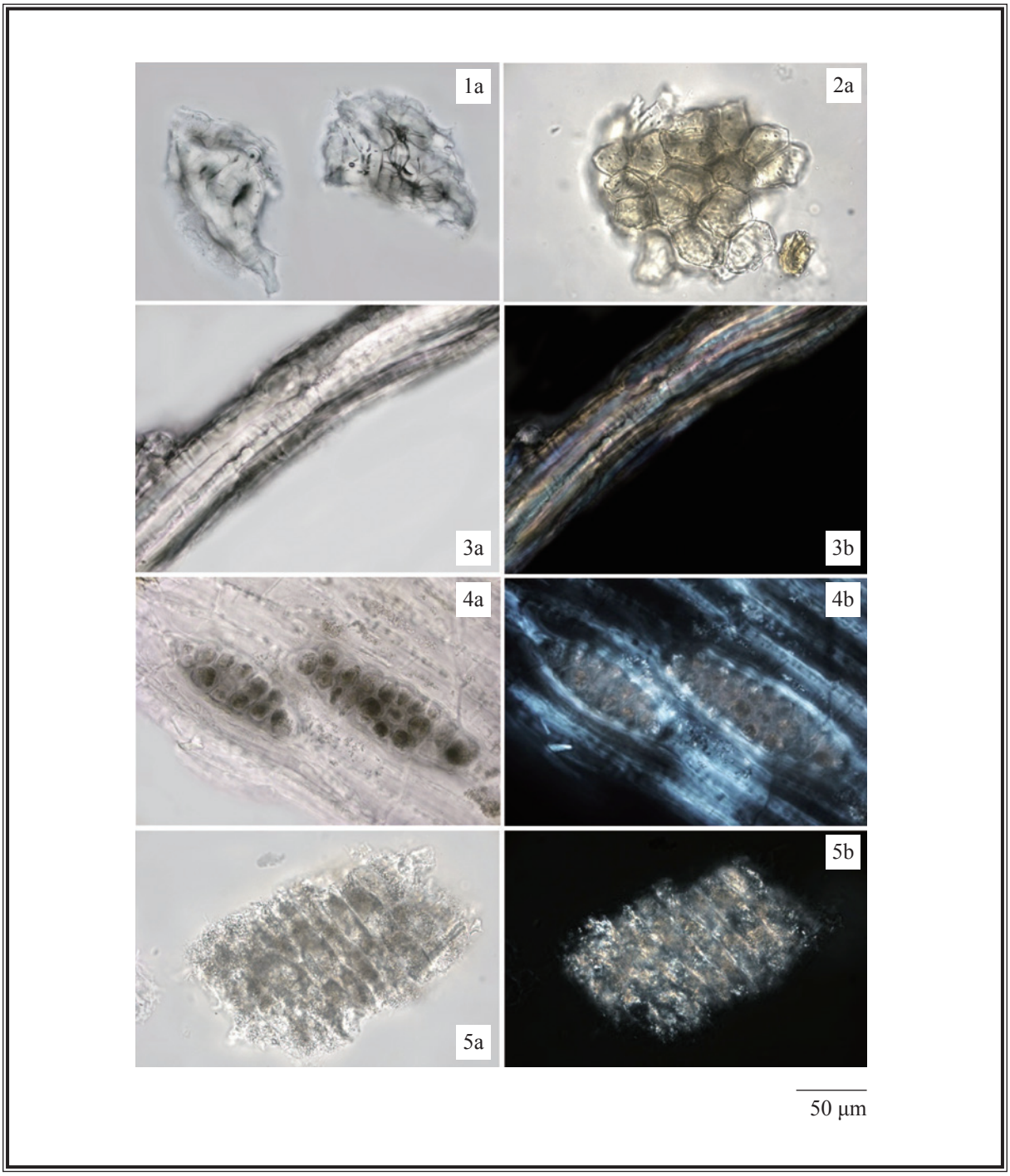


Figure 3 (iii) Microscopic features of powder of dried branch bark or stem bark of *Fraxinus stylosa* Lingelsh.

1. Stone cells 2. Cork cells 3. Fibres 4. Phloem ray cells 5. Crystals of calcium oxalate

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

Footnote: Microscopic features of powder have no significant differences between the dried branch bark or stem bark of *Fraxinus chinensis* Roxb., *Fraxinus szaboana* Lingelsh. and *Fraxinus stylosa* Lingelsh.

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

Standard solutions

Esculetin standard solution

Weigh 0.2 mg of esculetin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Esculin standard solution

Weigh 0.2 mg of esculin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and formic acid (8:1:0.5, v/v).

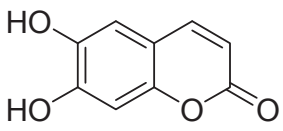
Test solution

Weigh 0.1 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of ethanol. Sonicate (100 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 esculetin standard solution (1 µL), esculin standard solution (1 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. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

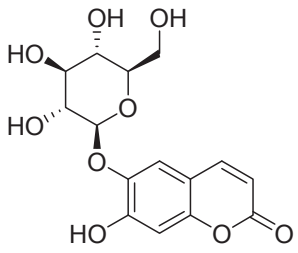


Figure 4 Chemical structures of (i) esculetin and (ii) esculin

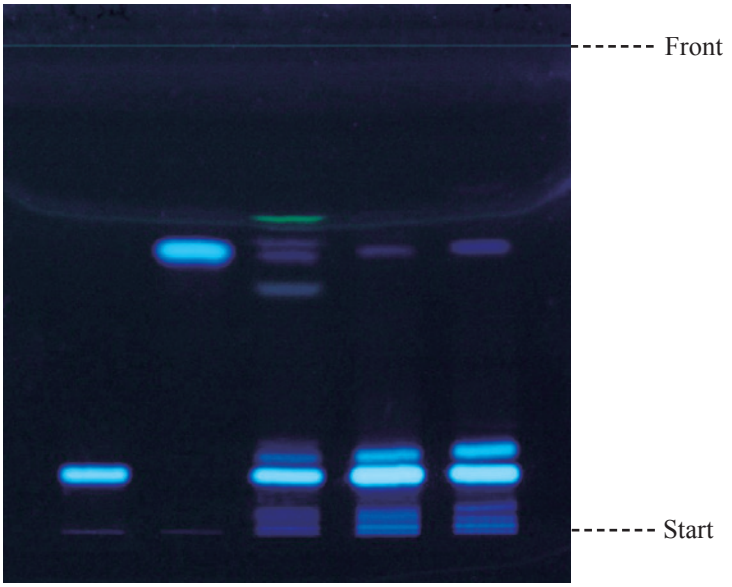


Figure 5 A reference HPTLC chromatogram of Fraxini Cortex extract observed under UV light (366 nm)

1. Esculin standard solution
2. Esculetin standard solution
3. Test solution of

(i) dried branch bark or stem bark of *Fraxinus chinensis* Roxb.
(ii) dried branch bark or stem bark of *Fraxinus szaboana* Lingelsh.
(iii) dried branch bark or stem bark of *Fraxinus stylosa* Lingelsh.

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of esculetin and esculin (Fig. 5).

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

Standard solutions

Esculetin standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.6 mg of esculetin CRS and dissolve in 20 mL of methanol (50%).

Esculin standard solution for fingerprinting, Std-FP (105 mg/L)

Weigh 2.1 mg of esculin CRS and dissolve in 20 mL of methanol (50%).

Test solution

Weigh 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (334 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 28°C during the separation. 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)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 55	5 → 25	95 → 75	linear gradient
55 – 60	25 → 55	75 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of esculetin Std-FP and esculin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of esculetin and esculin should not be more than 5.0%; the RSD of the retention times of esculetin and esculin peaks should not be more than 2.0%; the column efficiencies determined from esculetin and esculin peaks should not be less than 40000 and 30000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and 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), (ii) or (iii)].

Procedure

Separately inject esculetin Std-FP, esculin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of esculetin and esculin peaks in the chromatograms of esculetin Std-FP, esculin Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i), (ii) or (iii)] in the chromatogram of the test solution. Identify esculetin and esculin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of esculetin Std-FP and esculin Std-FP. The retention times

of esculetin and esculin 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 Fraxini Cortex extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Fraxini Cortex extract

Peak No.	RRT	Acceptable Range
1 (esculin)	0.73	± 0.03
2 (marker, esculetin)	1.00	-
3	1.03	± 0.03
4	1.75	± 0.03

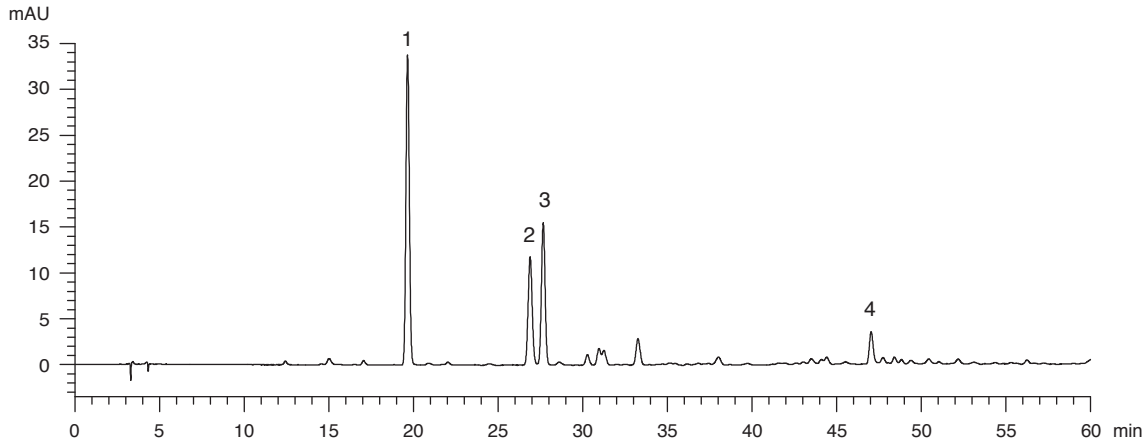


Figure 6 (i) A reference fingerprint chromatogram of dried branch bark or stem bark of *Fraxinus chinensis* Roxb. extract

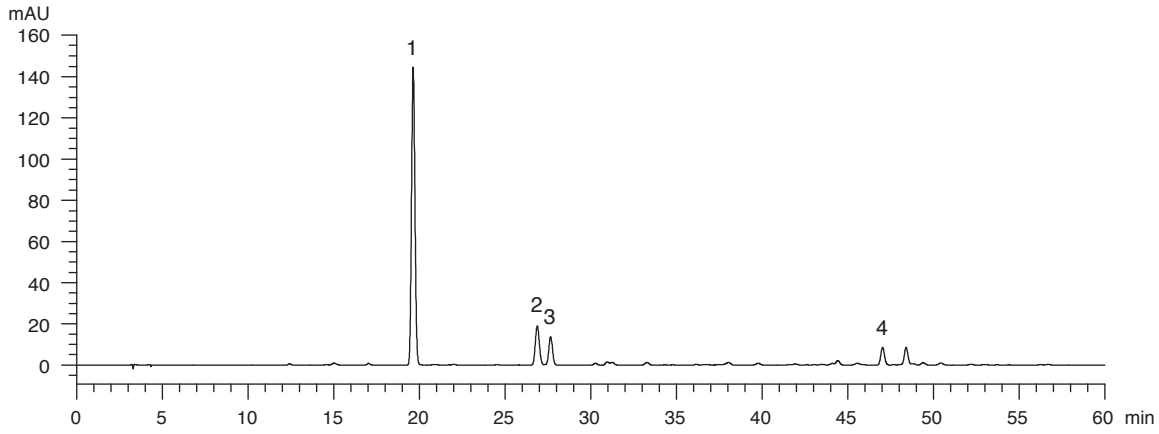


Figure 6 (ii) A reference fingerprint chromatogram of dried branch bark or stem bark of *Fraxinus szaboana* Lingelsh. extract

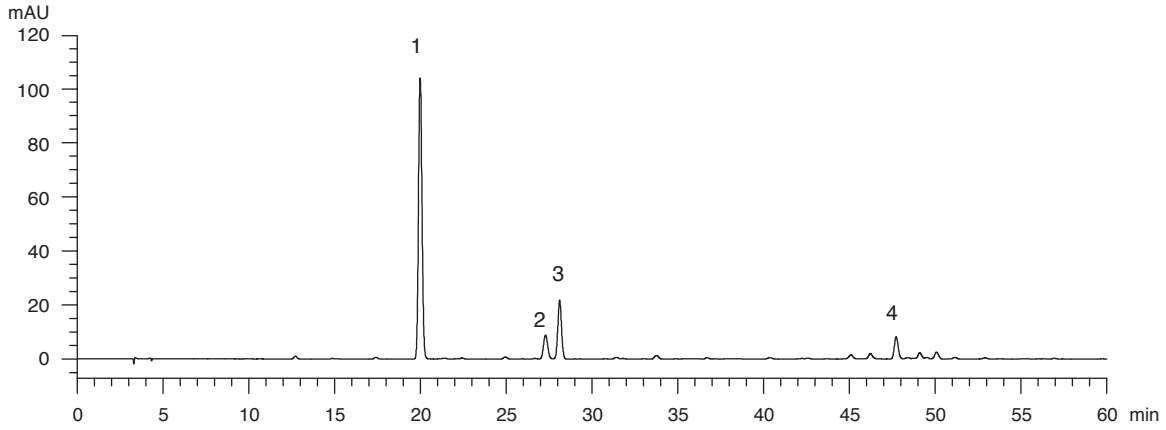


Figure 6 (iii) A reference fingerprint chromatogram of dried branch bark or stem bark of *Fraxinus stylosa* Lingelsh. extract

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 fingerprint chromatograms [Fig. 6 (i), (ii) or (iii)].

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 7.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 9.0%
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

Mixed esculetin and esculin standard stock solution, Std-Stock (600 mg/L for esculetin and 2000 mg/L for esculin)

Weigh accurately 1.2 mg of esculetin CRS and 4.0 mg of esculin CRS, and dissolve in 2 mL of methanol (50%).

Mixed esculetin and esculin standard solution for assay, Std-AS

Measure accurately the volume of the mixed esculetin and esculin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 1.5, 15, 30, 60, 150 mg/L for esculetin and 5, 50, 100, 200, 500 mg/L for esculin.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (334 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 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 30	8 → 13	92 → 87	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of the mixed esculetin and esculin Std-AS (30 mg/L for esculetin and 100 mg/L for esculin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of esculetin and esculin should not be more than 5.0%; the RSD of the retention times of esculetin and esculin peaks should not be more than 2.0%; the column efficiencies determined from esculetin and esculin peaks should not be less than 20000 and 8000 theoretical plates respectively.

The *R* value between esculetin peak and the closest peak; and the *R* value between esculin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

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

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify esculetin and esculin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed esculetin and esculin Std-AS. The retention times of esculetin and esculin 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 esculetin and esculin in the test solution, and calculate the percentage contents of esculetin and esculin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.0% of the total content of esculetin ($C_9H_6O_4$) and esculin ($C_{15}H_{16}O_9$), calculated with reference to the dried substance.