Pyrrosiae Folium

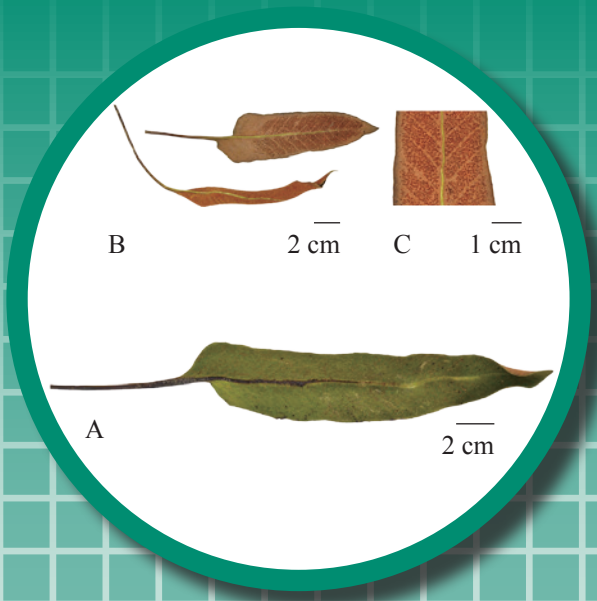

Figure 1 (i) A photograph of dried leaf of *Pyrrosia sheareri* (Bak.) Ching

A. Upper surface of leaf B. Lower surface of leaf C. Magnified lower surface of leaf

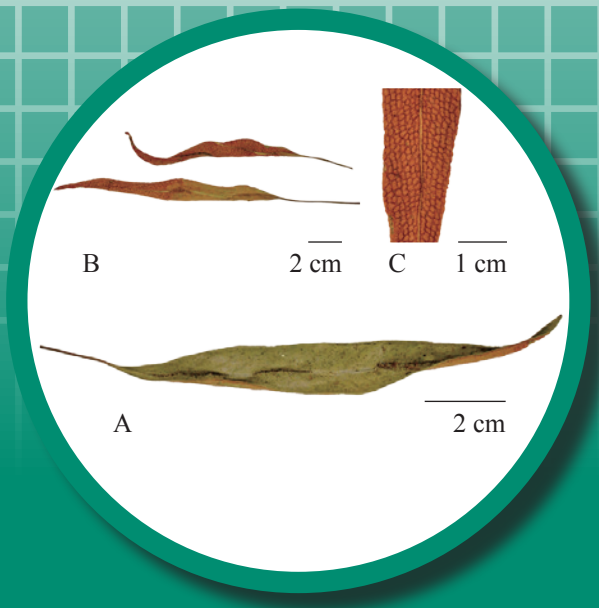

Figure 1 (ii) A photograph of dried leaf of *Pyrrosia lingua* (Thunb.) Farwell

A. Upper surface of leaf B. Lower surface of leaf
C. Magnified lower surface of leaf

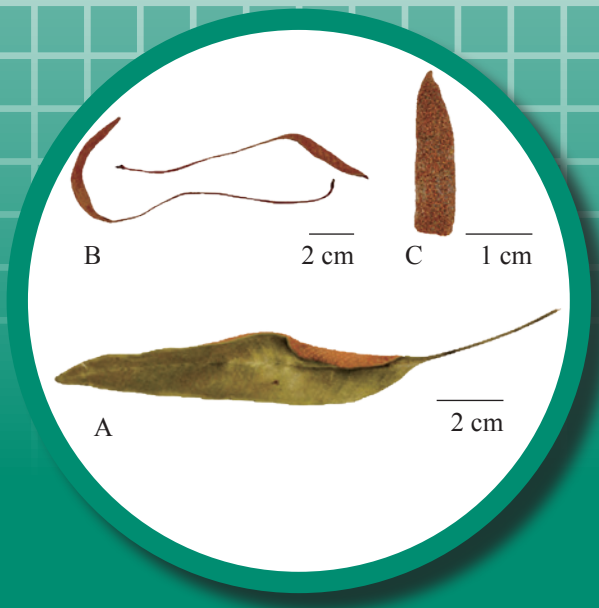

Figure 1 (iii) A photograph of dried leaf of *Pyrrosia petiolosa* (Christ) Ching

A. Upper surface of leaf B. Lower surface of leaf
C. Magnified lower surface of leaf

1. NAMES

Official Name: Pyrrosiae Folium

Chinese Name: 石韋

Chinese Phonetic Name: Shiwei

2. SOURCE

Pyrrosiae Folium is the dried leaf of *Pyrrosia sheareri* (Bak.) Ching, *Pyrrosia lingua* (Thunb.) Farwell or *Pyrrosia petiolosa* (Christ) Ching (Polypodiaceae). The leaf is collected all year round, rhizome and root removed, then dried under the sun or in a shaded area to obtain Pyrrosiae Folium.

3. DESCRIPTION

***Pyrrosia sheareri* (Bak.) Ching:** Leathery and slightly crumpled, when intact flattened out, frond lanceolate, 8-31 cm long, 3-9.5 cm wide, apex acuminate, base auriculate oblique, margins entire, edges usually rolled inwards. The upper surface yellowish-green to greyish-green, sparsely covered with black and rounded pits; the lower surface densely covered with reddish-brown stellate hairs, sometimes the area between the lateral veins densely covered with brown rounded and spotted sori. Stipes with 4 ribs, 8-25 cm long, 1.5-3.5 mm in diameter, slightly twisted, with longitudinal furrows. Odour slight; taste slightly astringent and bitter [Fig. 1 (i)].

***Pyrrosia lingua* (Thunb.) Farwell:** Lanceolate to oblong-lanceolate, 7-18 cm long, 1.5-3.5 cm wide, the base cuneate and symmetrical. Sori densely and regularly arranged between the lateral veins. Stipes 5.5-16 cm long, 0.5-1.5 mm in diameter [Fig. 1 (ii)].

***Pyrrosia petiolosa* (Christ) Ching:** Mostly rolled into a tubular shape, when intact flattened out, frond oblong to ovate-oblong, 4-9 cm long, 0.6-2.2 cm wide, the base cuneate and symmetrical, the lower surface with indistinct lateral veins and densely covered with sori. Stipes 3.7-13 cm long, 0.5-1.2 mm in diameter [Fig. 1 (iii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Frond:

***Pyrrosia sheareri* (Bak.) Ching:** Upper epidermis consists of 1 layer of cells, cells subsquare to subrounded. Hypodermis underneath upper epidermis, consisting of 1-2 layers of rectangular cells, cell wall slightly thickened. Palisade tissue consists of 4-5 layers of rectangular palisade cells. Sponge tissue cells relatively small, arranged loosely, occupied about 1/3 of the frond. Sclerenchyma found on the inner side of the epidermis, above and below the midrib. Endodermis consists of inner and lateral wall thickened cells, subsquare to subrectangle, blackish-brown or colourless. Vascular bundles 6-12, amphicribral, surrounded by endodermis. Vascular bundles locate on the upper central part, relatively large, with a Y-shaped xylem. Lower epidermis consists of 1 layer of subrounded cells, with stellate hairs, sporangia and spores [Fig. 2 (i)].

***Pyrrosia lingua* (Thunb.) Farwell:** Palisade tissue consists of 3-4 layers of rectangular palisade cells. Sponge tissue cells relatively small, arranged loosely, occupied about half of the frond. Sclerenchyma found on the inner side of the epidermis above and below the midrib, relatively less sclerenchyma among the inner side of the upper epidermis. Vascular bundles 2-5, amphicribral, surrounded by inner and lateral wall thickened endodermal cells [Fig. 2 (ii)].

***Pyrrosia petiolosa* (Christ) Ching:** Palisade tissue consists of 3-4 layers of rectangular palisade cells. Sponge tissue cells relatively small, arranged packed, occupied about 1/3 of the frond. Sclerenchyma found on the inner side of the epidermis above and below the midrib, relatively less sclerenchyma among the inner side of the upper epidermis. Vascular bundles 1-2, amphicribral, surrounded by inner and lateral wall thickened endodermal cells [Fig. 2 (iii)].

Stipe:

***Pyrrosia sheareri* (Bak.) Ching:** Epidermis consists of 1 layer of cells, cells subsquare to subrounded. Sclerenchyma underneath epidermis, consisting of about 10 layers of cells, arranged in a ring. Cortex consists of parenchymatous cells. Vascular bundles scattered among cortex, amphicribral, surrounded by endodermis; 11-15 vascular bundles arranged in a U shape. Endodermal cells, inner and lateral wall thickened, subsquare to subrectangular, colourless or blackish-brown [Fig. 3 (i)].

***Pyrrosia lingua* (Thunb.) Farwell:** Sclerenchyma consists of 4-6 layers of cells. Vascular bundles 4-8 [Fig. 3 (ii)].

***Pyrrosia petiolosa* (Christ) Ching:** Sclerenchyma consists of 4-6 layers of cells. Vascular bundles 6-9 [Fig. 3 (iii)].

Powder

***Pyrrosia sheareri* (Bak.) Ching:** Colour yellowish-green, yellowish-brown or reddish-brown. Stellate hairs numerous, consisting of 7- to 12-celled body, radially arranged in upper and lower layers, cells lanceolate, apex acute, occasionally with longitudinal or irregular reticulate striations on the surface; stalk of stellate hair 3- to 11-celled. Sporangia yellowish-brown, rectangular in surface view, subsquare in lateral view, inner and lateral walls thickened. Spores numerous, ellipsoidal in polar view and reniform in equatorial view, 46-99 μm long, 29-72 μm in diameter, walls with warty protuberance. Upper epidermal cells polygonal, walls slightly thickened, anticlinal walls sinuous. Lower epidermal cells polygonal, stomata actinocytic. Fibres mostly in bundles, golden-yellow to yellowish-brown, long fusiform, filling with reddish-brown to brown masses. Endodermal cells rectangular to long fusiform, inner and lateral walls thickened, outer walls relatively thin, colourless or reddish-brown, with dense pits [Fig. 4 (i)].

***Pyrrosia lingua* (Thunb.) Farwell:** Upper epidermal cells subrectangular [Fig. 4 (ii)].

***Pyrrosia petiolosa* (Christ) Ching:** Colour yellowish-brown to reddish-brown. Upper epidermal cells long subpolygonal. Anticlinal walls of lower epidermal cells of leaf relatively straight [Fig. 4 (iii)].

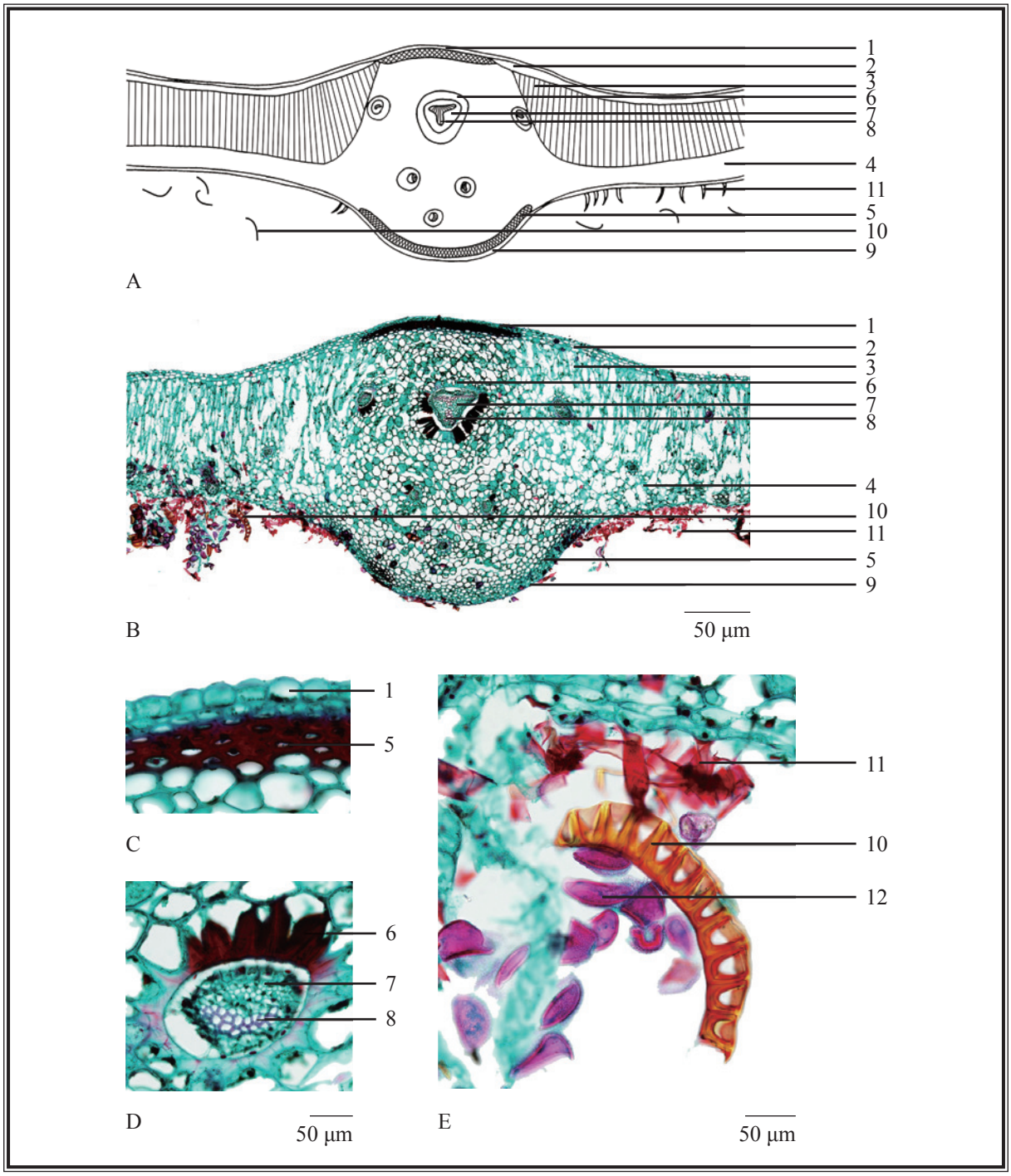

Figure 2 (i) Microscopic features of transverse section of frond of *Pyrrosia sheareri* (Bak.) Ching

A. Sketch B. Section illustration C. Sclerenchyma D. Vascular bundle E. Sporangium

1. Upper epidermis 2. Hypodermis 3. Palisade tissue 4. Sponge tissue
5. Sclerenchyma 6. Endodermis 7. Phloem 8. Xylem 9. Lower epidermis
10. Sporangium 11. Stellate hair 12. Spore

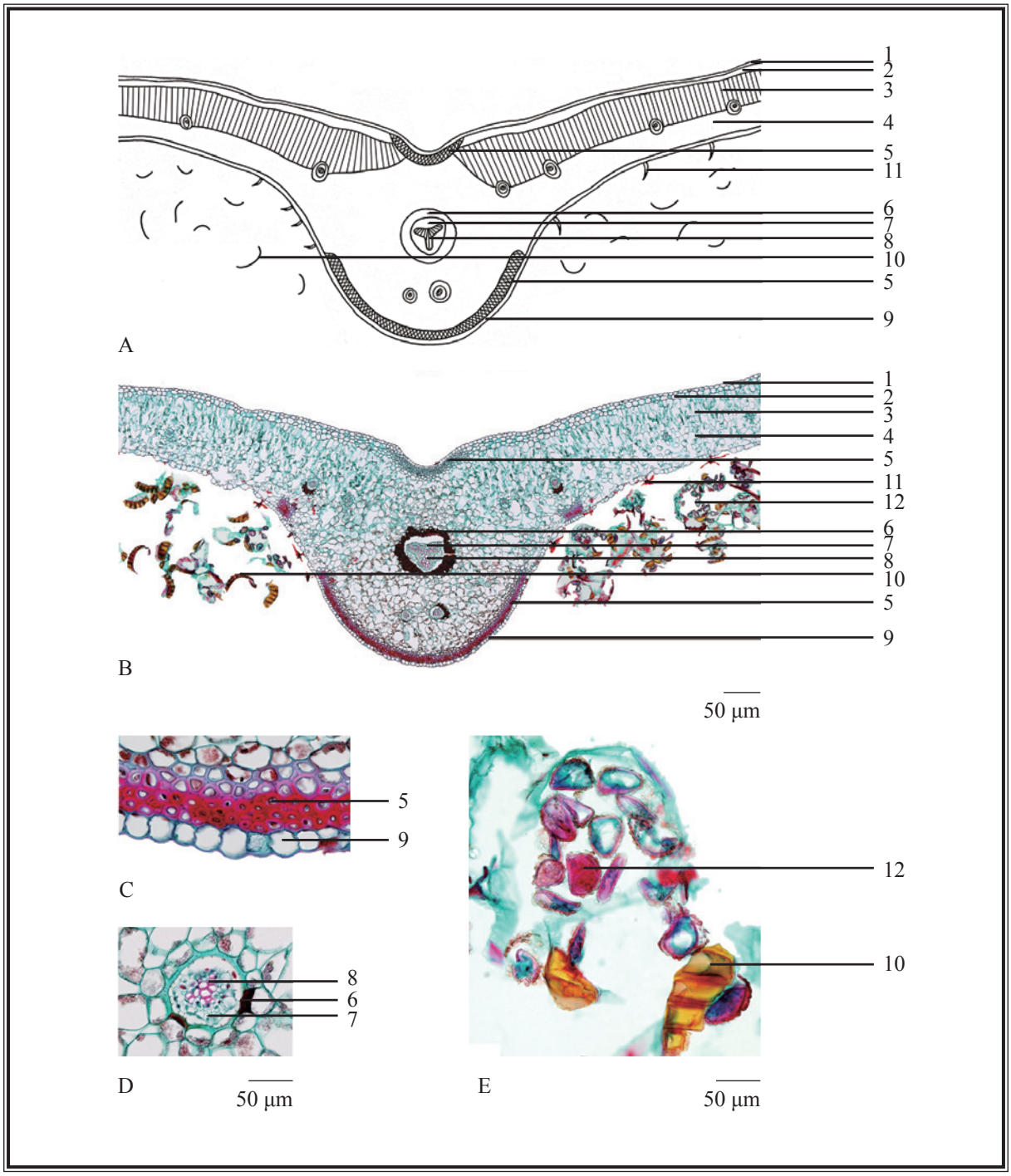

Figure 2 (ii) Microscopic features of transverse section of frond of *Pyrrosia lingua* (Thunb.) Farwell

A. Sketch B. Section illustration C. Sclerenchyma D. Vascular bundle E. Sporangium

1. Upper epidermis 2. Hypodermis 3. Palisade tissue 4. Sponge tissue
5. Sclerenchyma 6. Endodermis 7. Phloem 8. Xylem 9. Lower epidermis
10. Sporangium 11. Stellate hair 12. Spore

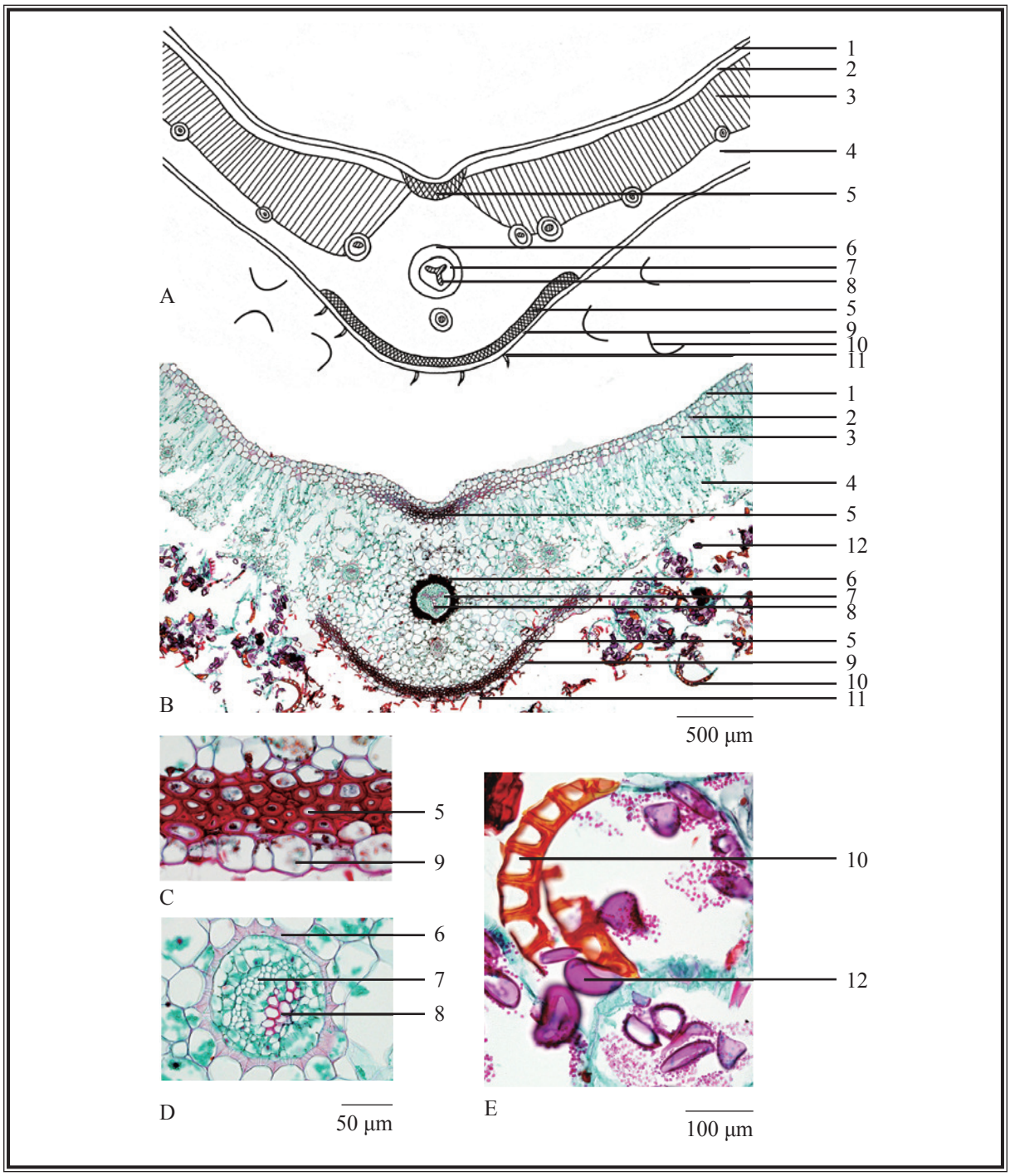

Figure 2 (iii) Microscopic features of transverse section of frond of *Pyrrosia petiolosa* (Christ) Ching

A. Sketch B. Section illustration C. Sclerenchyma D. Vascular bundle E. Sporangium

1. Upper epidermis 2. Hypodermis 3. Palisade tissue 4. Sponge tissue
5. Sclerenchyma 6. Endodermis 7. Phloem 8. Xylem 9. Lower epidermis
10. Sporangium 11. Stellate hair 12. Spore

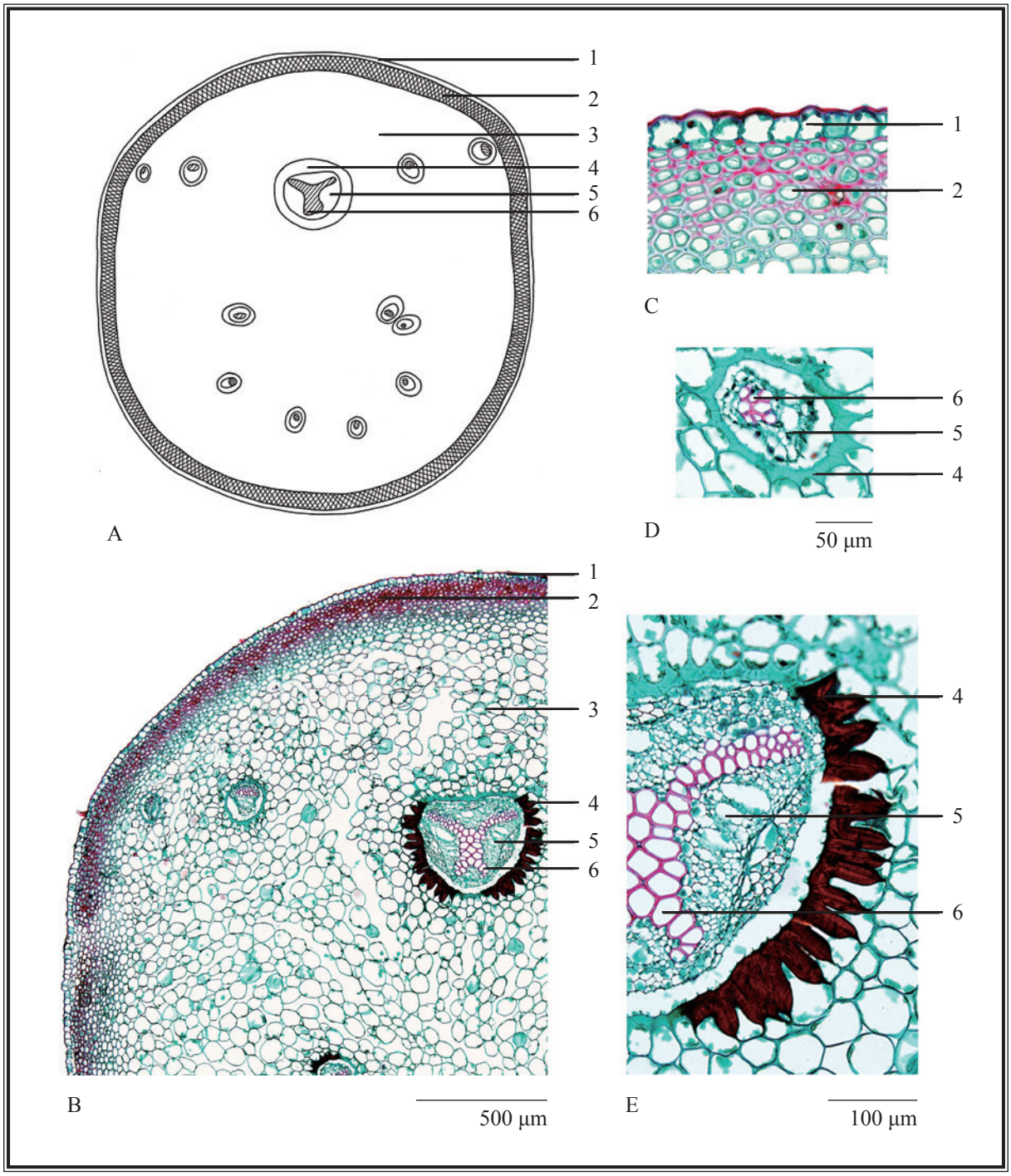

Figure 3 (i) Microscopic features of transverse section of stipe of *Pyrrosia sheareri* (Bak.) Ching

A. Sketch B. Section illustration C. Epidermis D, E. Vascular bundle

1. Epidermis 2. Sclerenchyma 3. Cortex 4. Endodermis 5. Phloem 6. Xylem

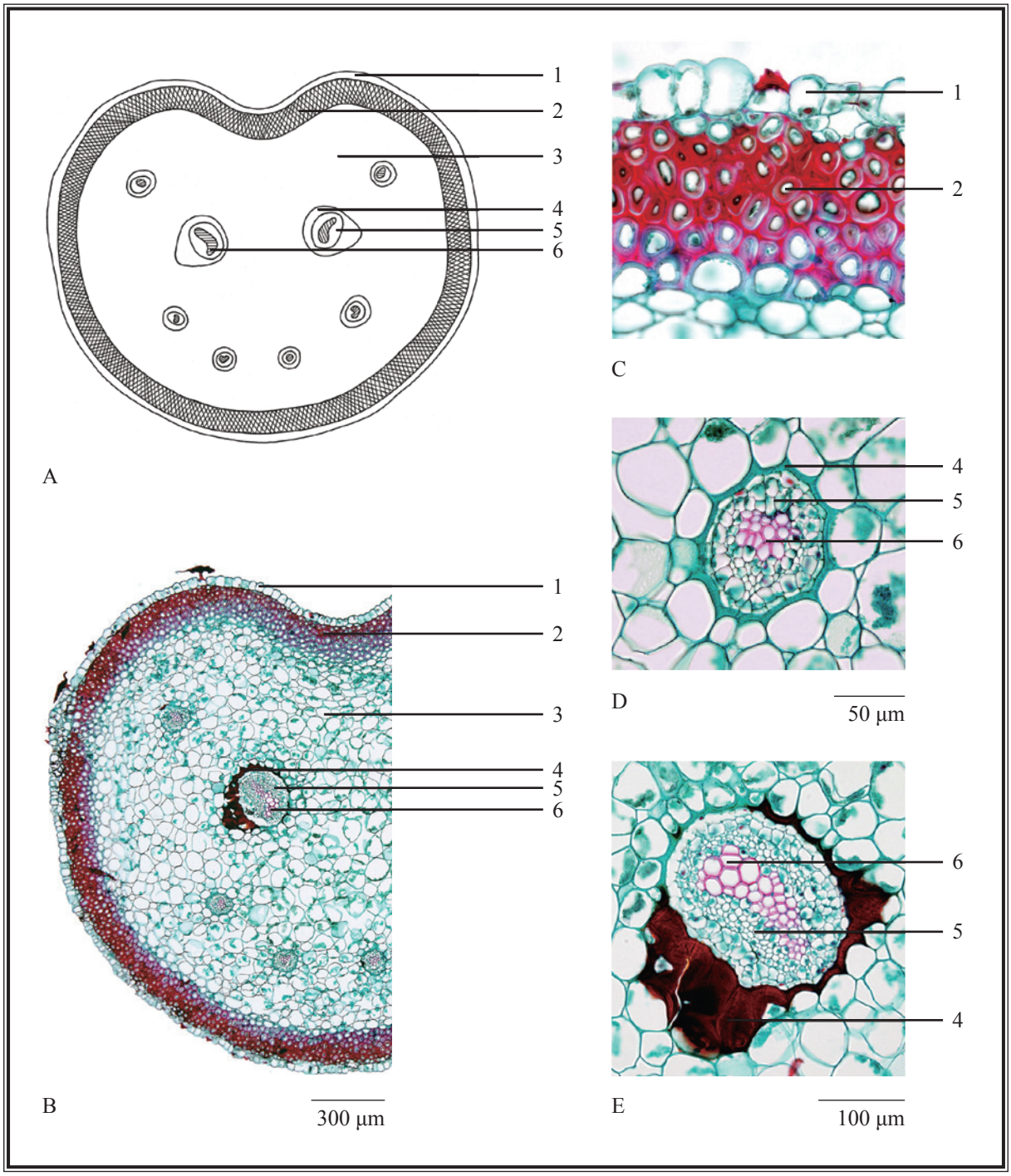

Figure 3 (ii) Microscopic features of transverse section of stipe of *Pyrrosia lingua* (Thunb.) Farwell

A. Sketch B. Section illustration C. Epidermis D, E. Vascular bundle

1. Epidermis 2. Sclerenchyma 3. Cortex 4. Endodermis 5. Phloem 6. Xylem

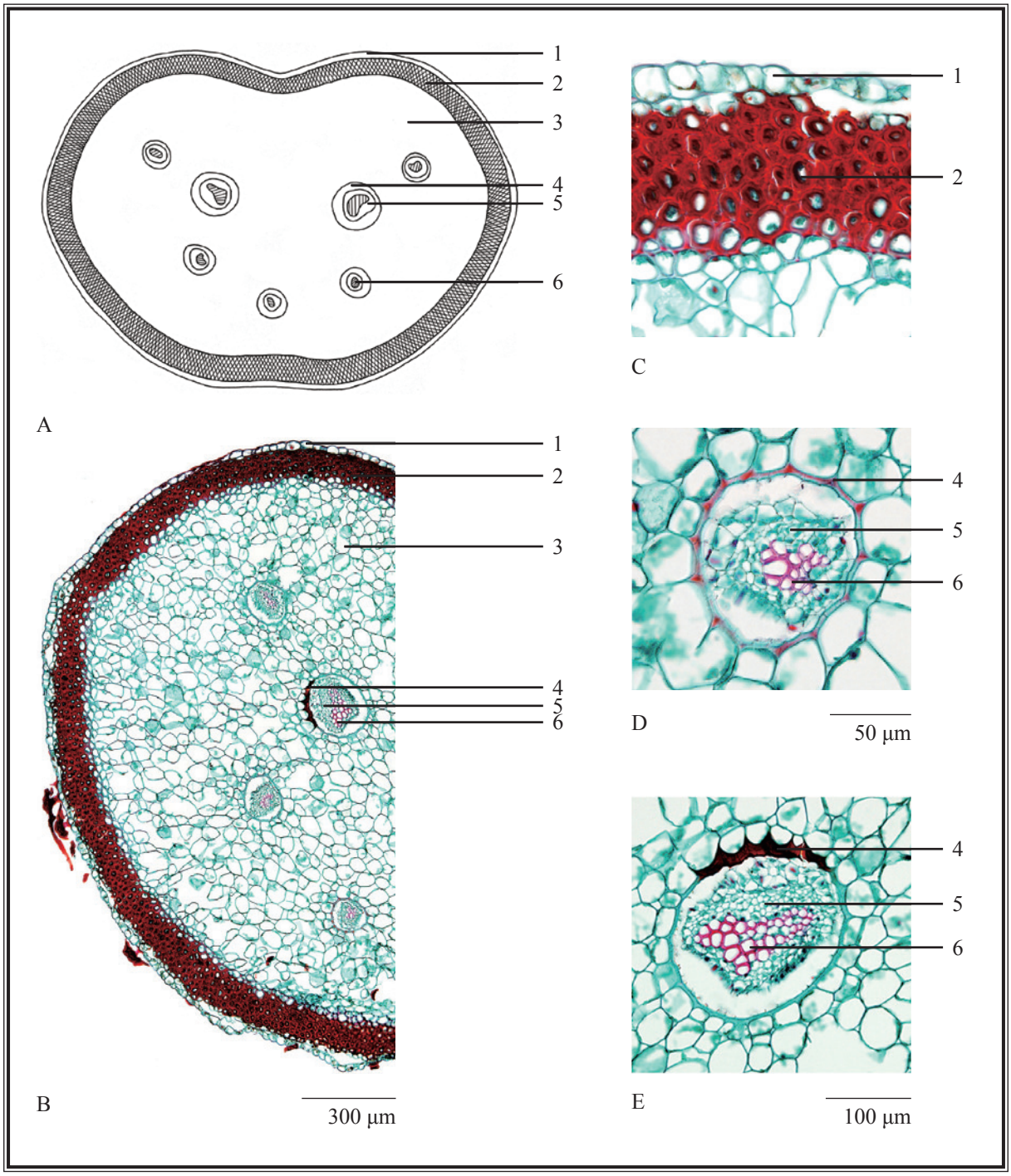

Figure 3 (iii) Microscopic features of transverse section of stipe of *Pyrrosia petiolosa* (Christ) Ching

A. Sketch B. Section illustration C. Epidermis D, E. Vascular bundle

1. Epidermis 2. Sclerenchyma 3. Cortex 4. Endodermis 5. Phloem 6. Xylem

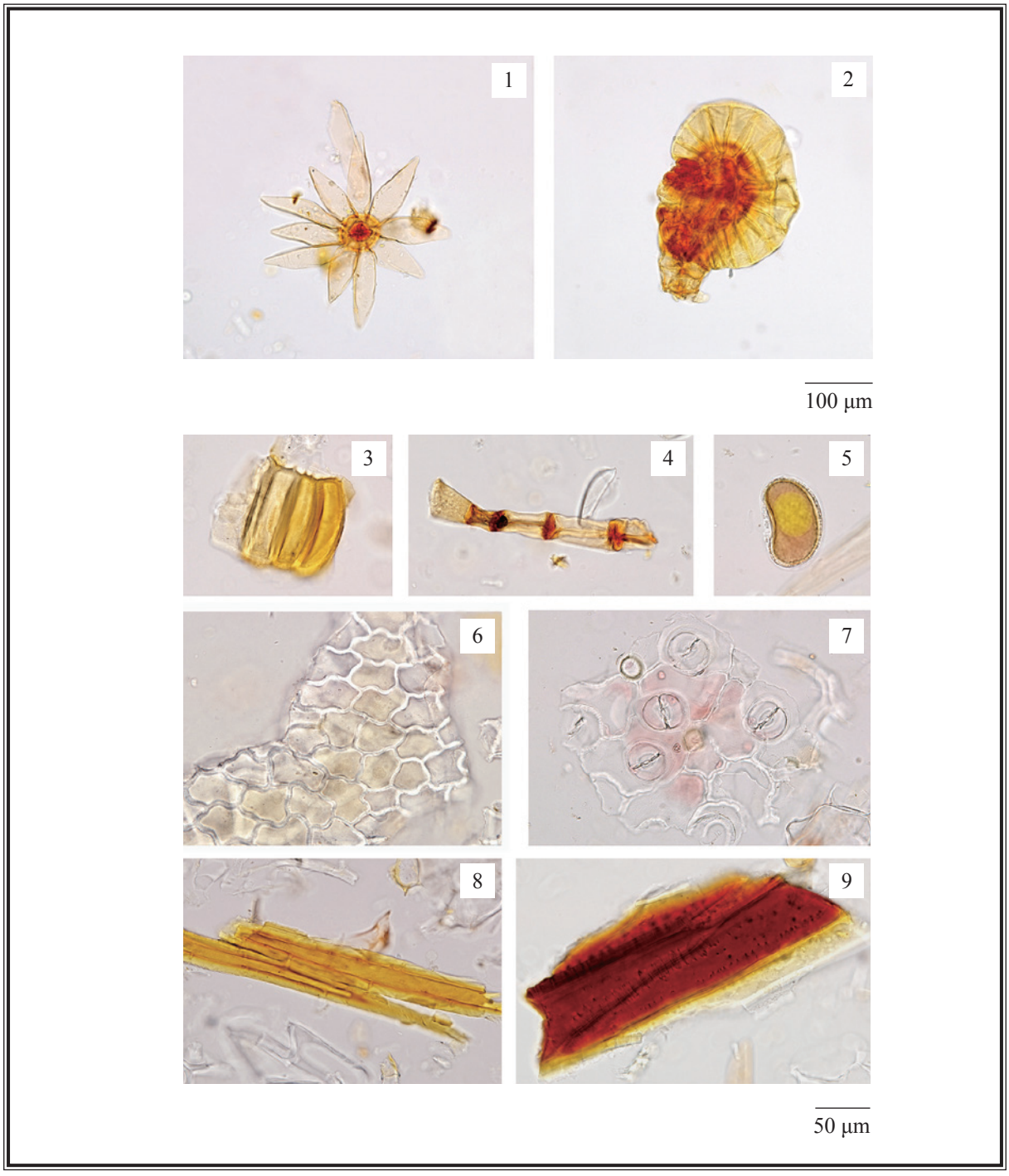

Figure 4 (i) Microscopic features of powder of dried leaf of *Pyrrosia sheareri* (Bak.) Ching (under the light microscope)

1. Stellate hair 2. Sporangium 3. Fragment of sporangium (in surface view)
4. Stalk of stellate hair 5. Spore 6. Upper epidermal cells
7. Lower epidermal cells with stomata 8. Fibres 9. Endodermal cells

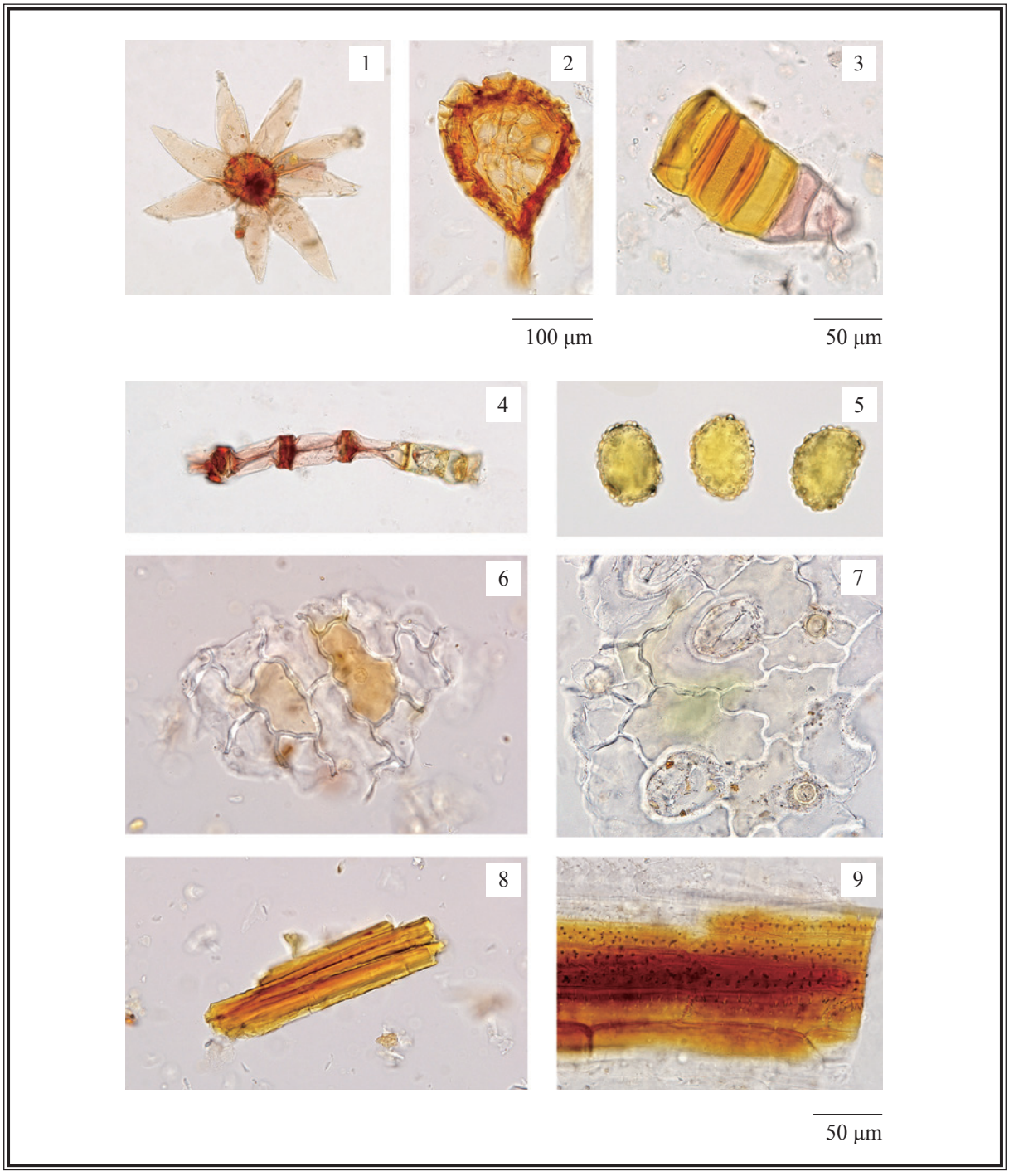

Figure 4 (ii) Microscopic features of powder of dried leaf of *Pyrrosia lingua* (Thunb.) Farwell (under the light microscope)

1. Stellate hair 2. Sporangium 3. Fragment of sporangium (in surface view)
4. Stalk of stellate hair 5. Spores 6. Upper epidermal cells
7. Lower epidermal cells with stomata 8. Fibres 9. Endodermal cells

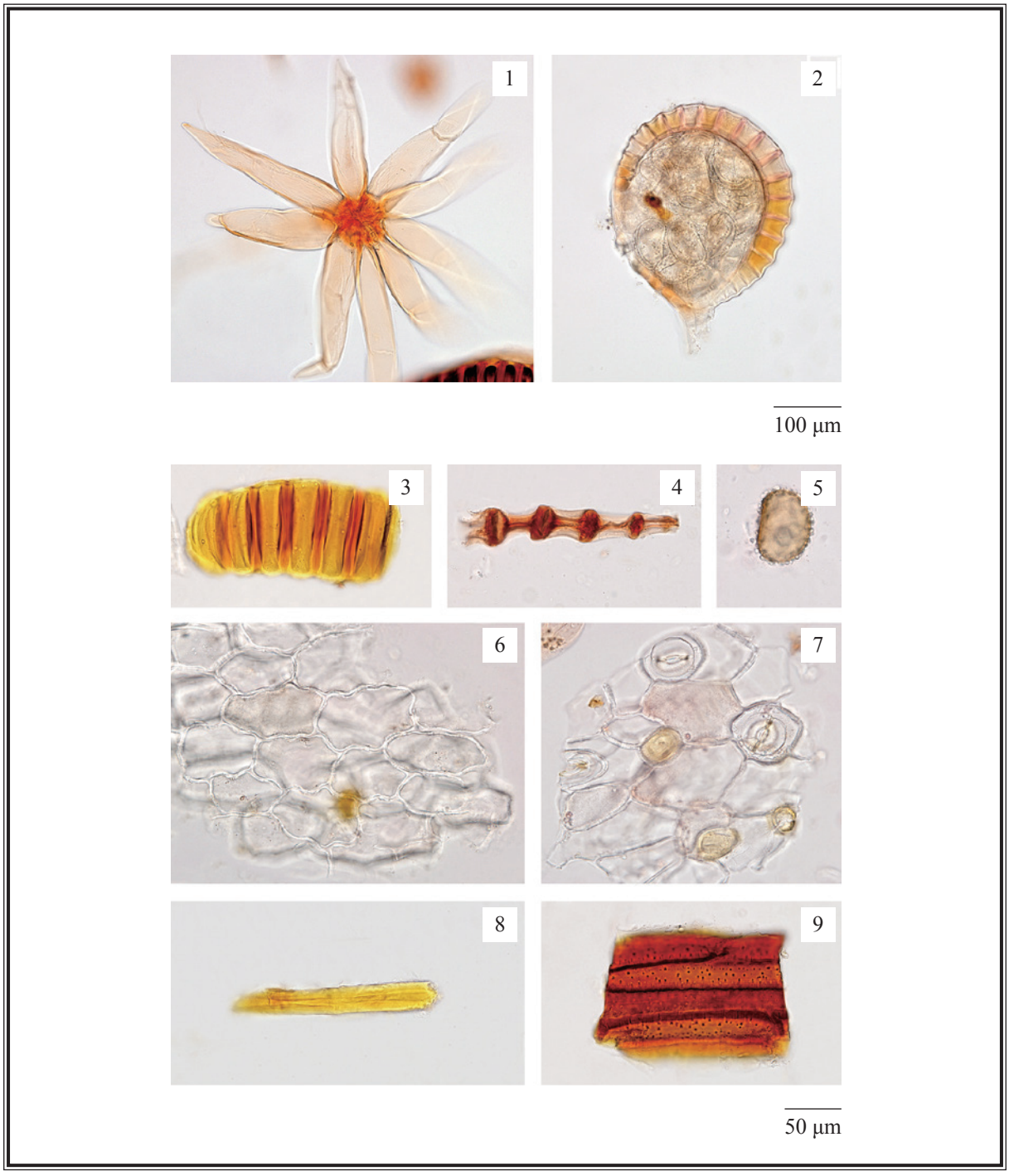

Figure 4 (iii) Microscopic features of powder of dried leaf of *Pyrrosia petiolosa* (Christ) Ching (under the light microscope)

1. Stellate hair 2. Sporangium 3. Fragment of sporangium (in surface view)
4. Stalk of stellate hair 5. Spore 6. Upper epidermal cells
7. Lower epidermal cells with stomata 8. Fibre 9. Endodermal cells

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

Standard solution

Chlorogenic acid standard solution

Weigh 1.0 mg of chlorogenic acid CRS (Fig. 5) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, formic acid and water (20:3:1.5:1.5, v/v).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol (50%). Sonicate (220 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} (2-10 μm) plate and a freshly prepared developing solvent system as described above. Apply separately chlorogenic acid standard solution (3 μL) and the test solution (4 μL) 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 (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

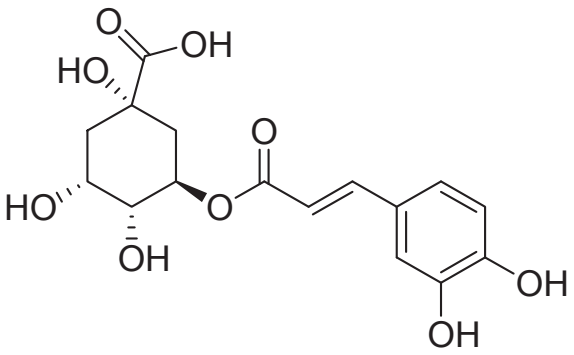

Figure 5 Chemical structure of chlorogenic acid

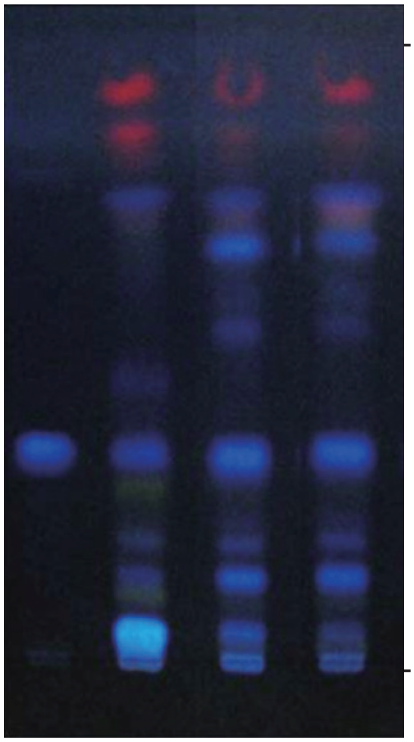

Figure 6 A reference HPTLC chromatogram of Pyrrosiae Folium extract observed under UV light (366 nm)

1. Chlorogenic acid standard solution
2. Test solution of
(i) dried leaf of *Pyrrosia sheareri* (Bak.) Ching
(ii) dried leaf of *Pyrrosia lingua* (Thunb.) Farwell
(iii) dried leaf of *Pyrrosia petiolosa* (Christ) Ching

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 chlorogenic acid (Fig. 6).

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

Standard solution

Chlorogenic acid standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol (50%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol (50%). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. 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 (326 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.9 mL/min. The mobile phase is a mixture of 0.5% phosphoric acid and acetonitrile (91:9, v/v). The elution time is about 30 min.

System suitability requirements

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

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 7 (i), (ii) or (iii)].

Procedure

Separately inject chlorogenic acid Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of chlorogenic acid peak in the chromatogram of chlorogenic acid Std-FP and the retention times of the four characteristic peaks [Fig. 7 (i), (ii) or (iii)] in the chromatogram of the test solution. Identify chlorogenic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of chlorogenic acid Std-FP. The retention times of chlorogenic acid 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 Pyrrosiae Folium extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Pyrrosiae Folium extract

Peak No.	RRT	Acceptable Range
1	0.45	± 0.03
2	0.50	± 0.03
3 (marker, chlorogenic acid)	1.00	-
4	1.06	± 0.03

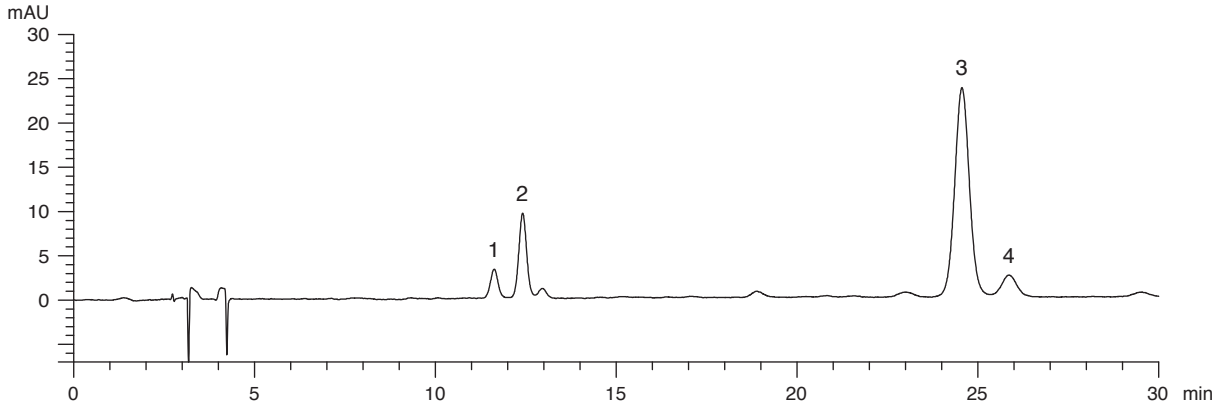

Figure 7 (i) A reference fingerprint chromatogram of dried leaf of *Pyrrosia sheareri* (Bak.) Ching extract

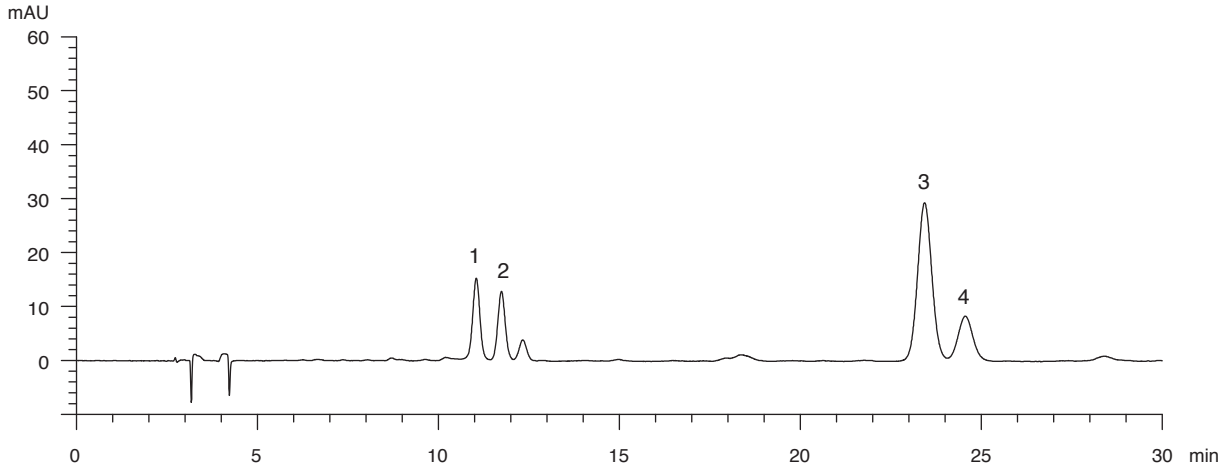

Figure 7 (ii) A reference fingerprint chromatogram of dried leaf of *Pyrrosia lingua* (Thunb.) Farwell extract

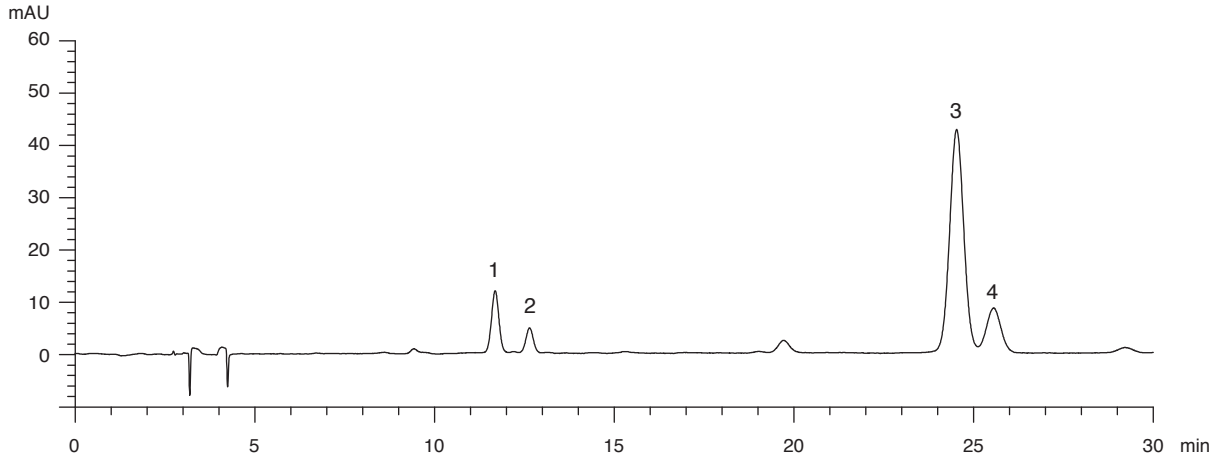

Figure 7 (iii) A reference fingerprint chromatogram of dried leaf of *Pyrrosia petiolosa* (Christ) Ching extract

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. 7 (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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 1.5%.

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 18.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

Chlorogenic acid standard stock solution, Std-Stock (50 mg/L)

Weigh accurately 0.5 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol (50%).

Chlorogenic acid standard solution for assay, Std-AS

Measure accurately the volume of the chlorogenic acid Std-Stock, dilute with methanol (50%) to produce a series of solutions of 1, 5, 10, 20, 30 mg/L for chlorogenic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 15 mL of methanol (50%). Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the filtrates 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 (326 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 0.5% phosphoric acid and acetonitrile (90:10, v/v). The elution time is about 30 min.

System suitability requirements

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

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

Limits

The sample contains not less than 0.21% of chlorogenic acid ($C_{16}H_{18}O_9$), calculated with reference to the dried substance.