Hedyotidis Diffusae Herba

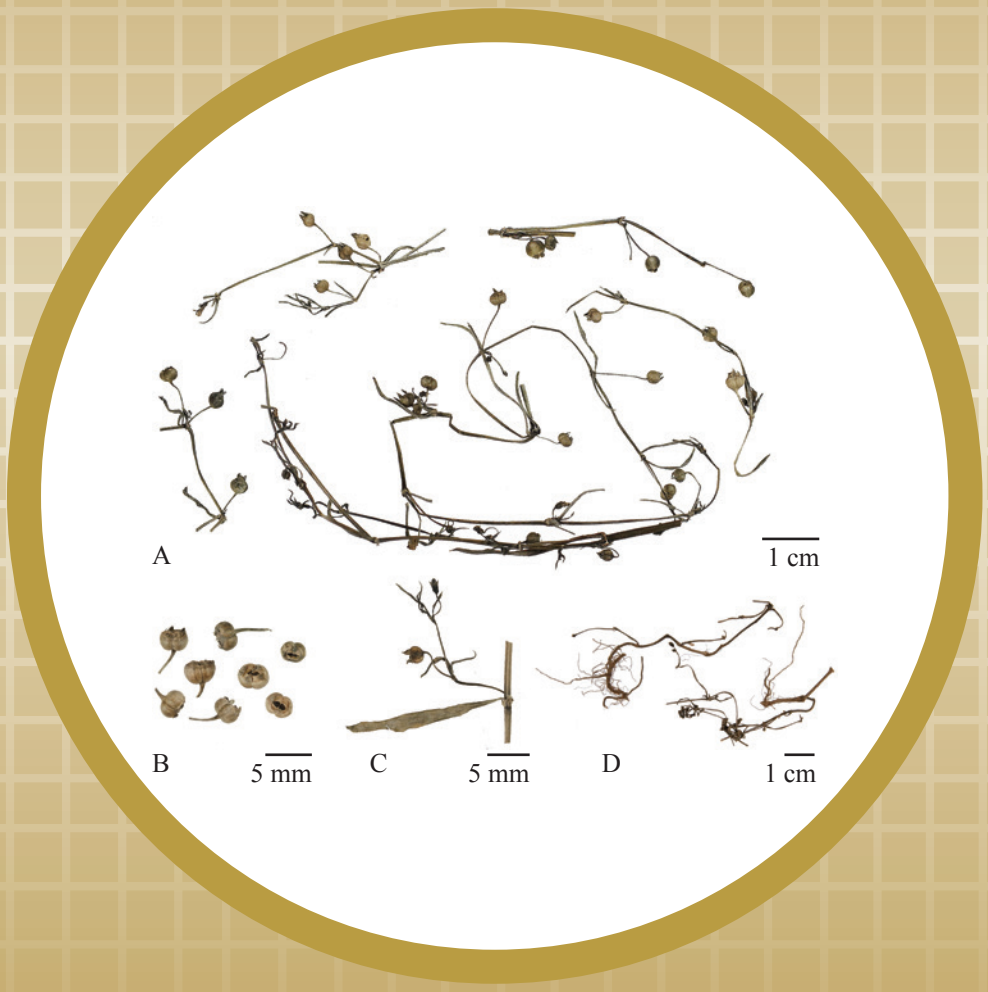

Figure 1 A photograph of Hedyotidis Diffusae Herba

A. Hedyotidis Diffusae Herba B. Magnified image of capsules
C. A leaf branch with flower D. Root

1. NAMES

Official Name: Hedyotidis Diffusae Herba

Chinese Name: 白花蛇舌草

Chinese Phonetic Name: Baihuasheshecao

2. SOURCE

Hedyotidis Diffusae Herba is the dried whole plant of *Hedyotis diffusa* Willd. (Rubiaceae). The whole plant is collected in summer and autumn, foreign matter removed, washed clean, then dried under the sun to obtain Hedyotidis Diffusae Herba.

3. DESCRIPTION

Usually tangled into masses or cut into sections, varying in length, externally greyish-yellow to greyish-green. Main root slightly curved, bearing numerous rootlets. Stems slender, slightly compressed, many branched at the base. Leaves simple opposite, sessile, mostly crumpled and broken, linear to linear-lanceolate when intact flattened out; apex acute, margin slightly curved inwards. Flowers solitary or forming a paired inflorescences funnelform, frequently fallen off. Capsules solitary or opposite in leaf axis, compressed globose, 1-4 mm in diameter, loculicidal. Calyx persistent, 4-lobed at the upper part. Texture fragile, easily broken. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of several layers of subrounded to subrectangular cells, arranged densely. Cortex scattered with numerous raphides of calcium oxalate which sometimes present in phloem. Phloem narrow. Cambium in a ring. Xylem broad; vessels arranged radially [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of subsquare to ovate cells. Cortex narrow, scattered with raphides and clusters of calcium oxalate. Endodermis consists of 1 layer of subrounded to irregular cells. Phloem narrow. Cambium in a ring. Xylem broad, arranged in a ring. Pith broad, mostly occupied 1/2 portion of the diameter of stem, containing numerous starch granules [Fig. 2 (ii)].

Leaf: Upper epidermis consists of 1 layer of large subsquare cells. Palisade tissue consists of 1 layer of irregularly shaped cells, scattered with clusters of calcium oxalate. Spongy tissue consists of loosely arranged cells, scattered with raphides of calcium oxalate. Vascular bundle of midrib amphicribrals; xylem vessels arranged radially. Collenchyma located in the inner side of lower epidermis at the midrib. Lower epidermis consists of 1 layer of relatively small cells [Fig. 2 (iii)].

Powder

Colour greyish-yellow to greyish-green. Raphides of calcium oxalate abundant, slender, 21-189 μm long; polychromatic under the polarized microscope. Pollen grains subspherical, 19-35 μm in diameter, with distinct reticulate sculptures on the surface. Lower epidermal cells polygonal or irregular in shape; stomata paracytic, arranged densely. Fibres of pericarp long fusiform or strip-shaped, walls slightly thickened and lignified, always in group and arranged in a crisscross pattern; bright white under the polarized microscope. Clusters of calcium oxalate small, 4-16 μm in diameter, usually in rosette aggregate; polychromatic under the polarized microscope. Vessels mostly spiral, reticulate vessels sometimes visible. Starch granules abundant, mainly simple, broadly ovoid, long spheroidal or irregularly spheroidal, 4-16 μm in diameter; striations mostly indistinct; black and cruciate-shaped under the polarized microscope (Fig. 3).

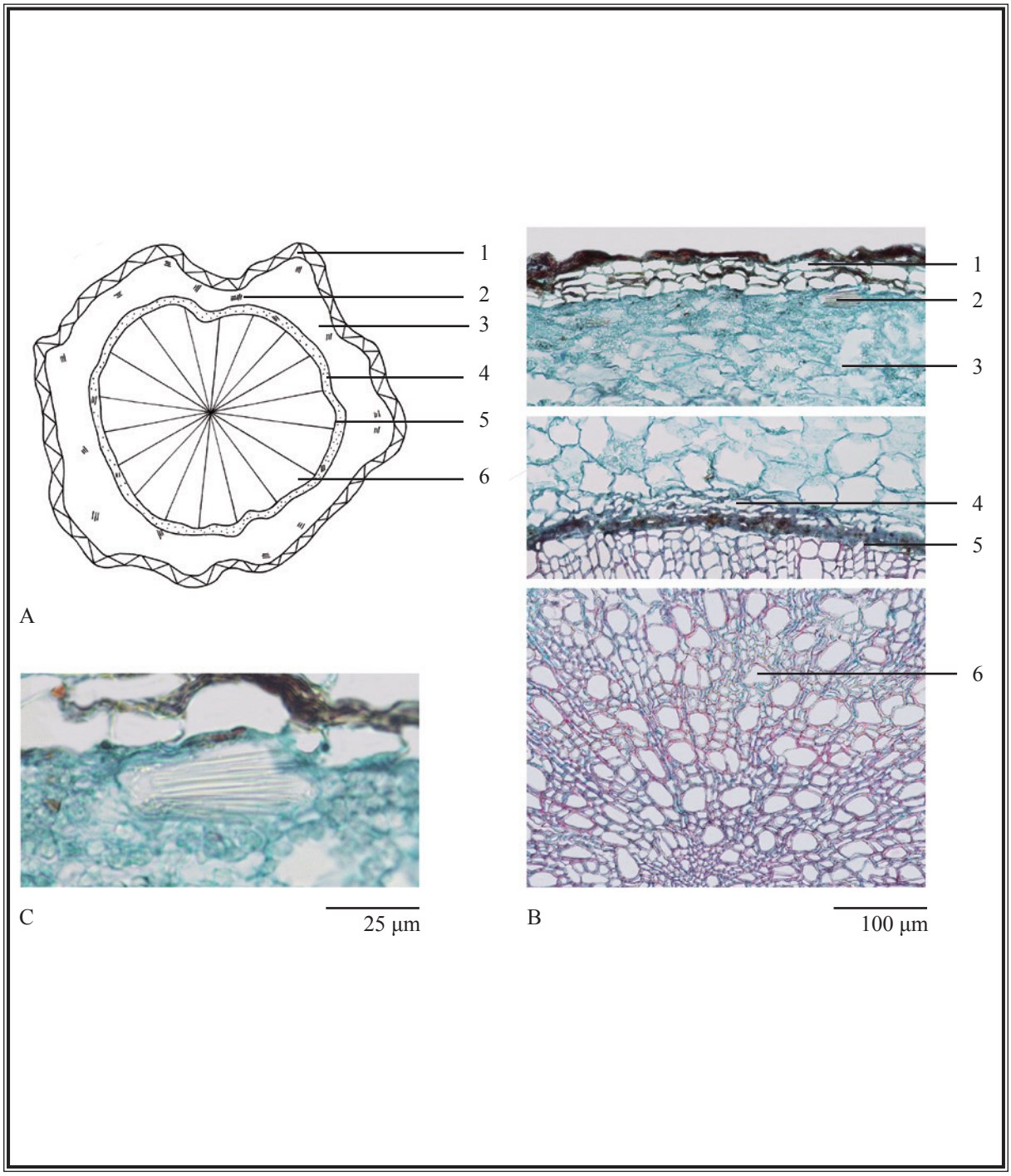

Figure 2 (i) Microscopic features of transverse section of root of Hedyotidis Diffusae Herba

A. Sketch B. Section illustration C. Raphides of calcium oxalate

1. Cork 2. Raphides of calcium oxalate 3. Cortex 4. Phloem 5. Cambium 6. Xylem

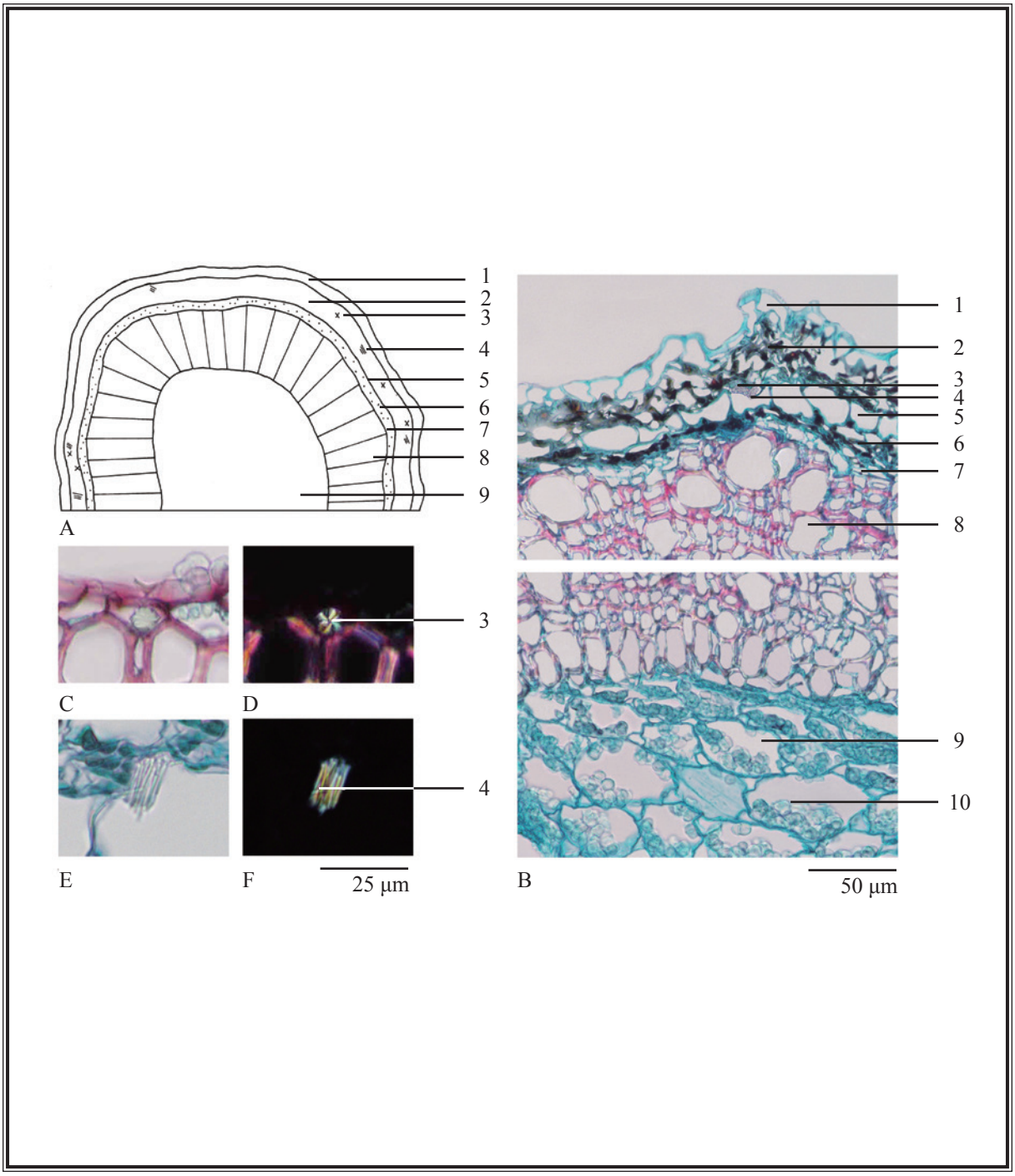

Figure 2 (ii) Microscopic features of transverse section of stem of Hedyotidis Diffusae Herba

A. Sketch B. Section illustration C. Cluster of calcium oxalate (under the light microscope)
D. Cluster of calcium oxalate (under the polarized microscope) E. Raphides of calcium oxalate (under the light microscope)
F. Raphides of calcium oxalate (under the polarized microscope)

1. Epidermis 2. Cortex 3. Cluster of calcium oxalate 4. Raphides of calcium oxalate
5. Endodermis 6. Phloem 7. Cambium 8. Xylem 9. Pith 10. Starch granules

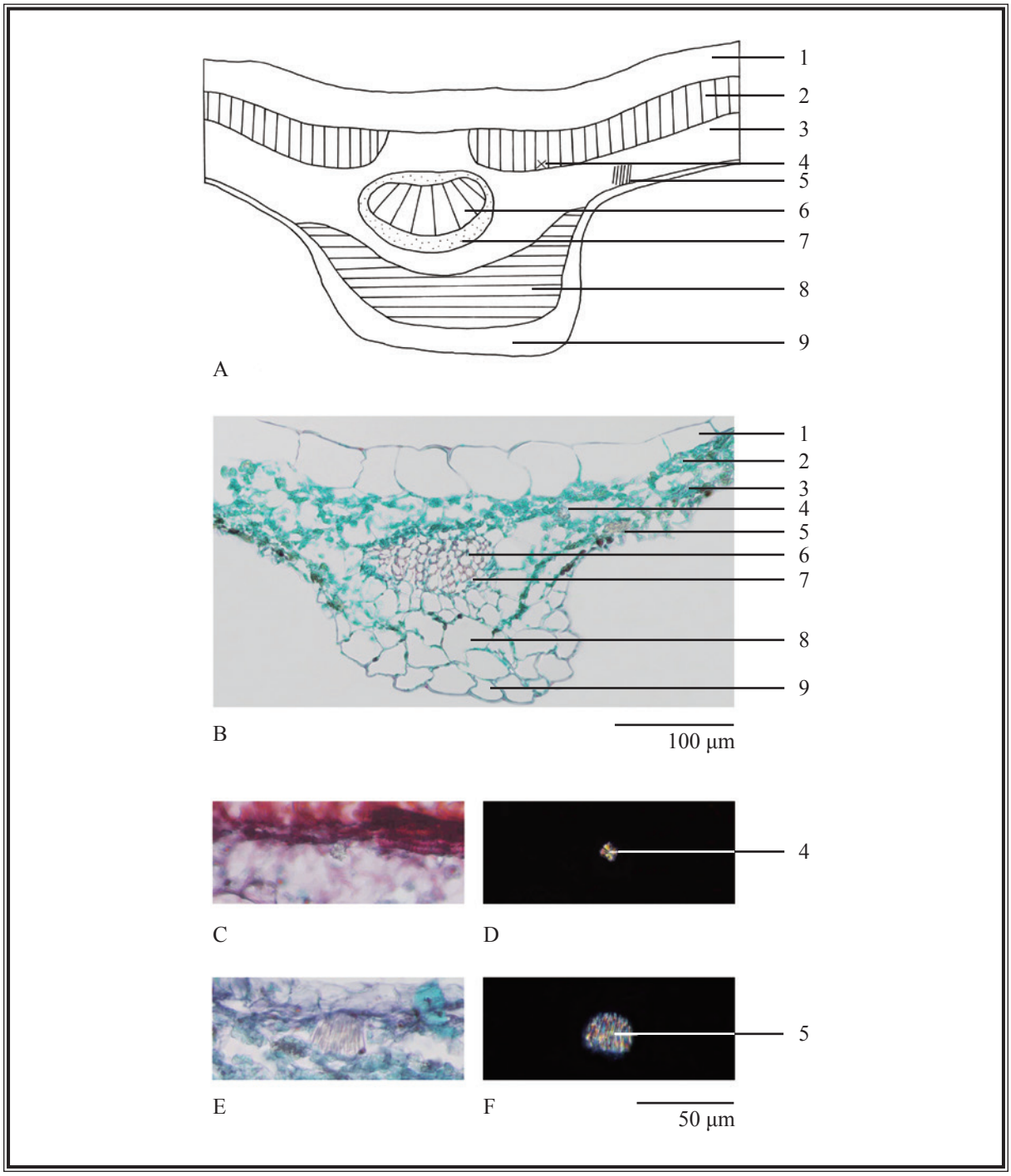

Figure 2 (iii) Microscopic features of transverse section of leaf of Hedyotidis Diffusae Herba

A. Sketch B. Section illustration C. Cluster of calcium oxalate (under the light microscope)
D. Cluster of calcium oxalate (under the polarized microscope) E. Raphides of calcium oxalate (under the light microscope)
F. Raphides of calcium oxalate (under the polarized microscope)

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Cluster of calcium oxalate
5. Raphides of calcium oxalate 6. Xylem 7. Phloem 8. Collenchyma 9. Lower epidermis

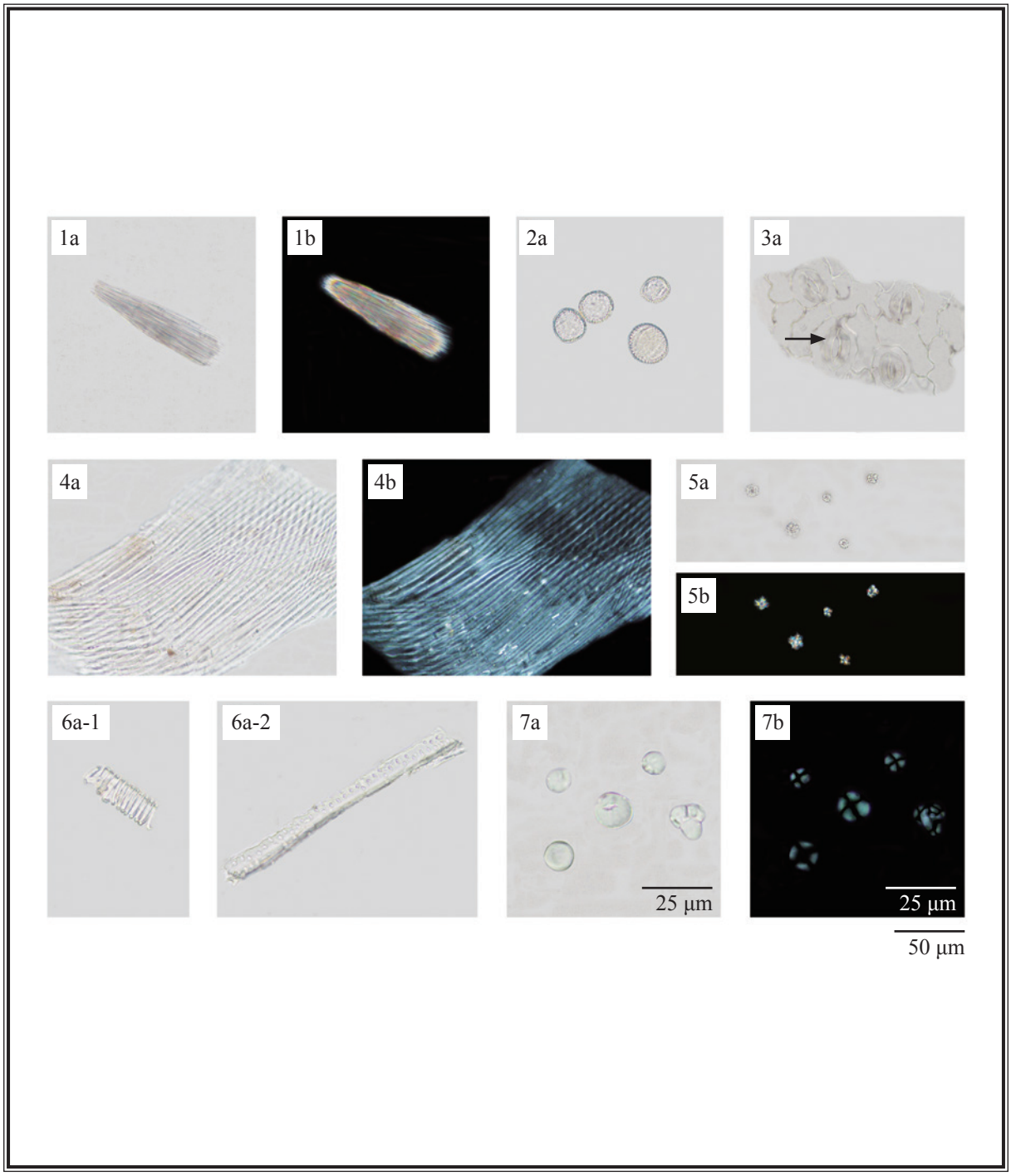

Figure 3 Microscopic features of powder of Hedyotidis Diffusae Herba

1. Raphides of calcium oxalate 2. Pollen grains
3. Lower epidermal cells with paracytic stomata (→)
4. Fibres of pericarp 5. Clusters of calcium oxalate
6. Vessels (6-1 spiral vessel, 6-2 reticulate vessel) 7. 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

Asperuloside standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, methanol and water (8:2:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (400 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

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 asperuloside standard solution (2 μL) and the test solution (5 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. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 7 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

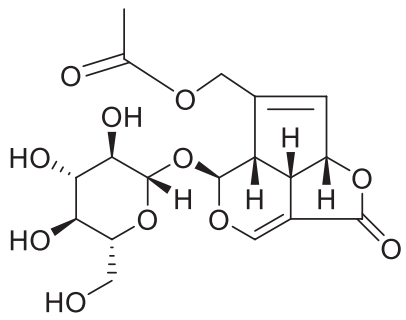

Figure 4 Chemical structure of asperuloside

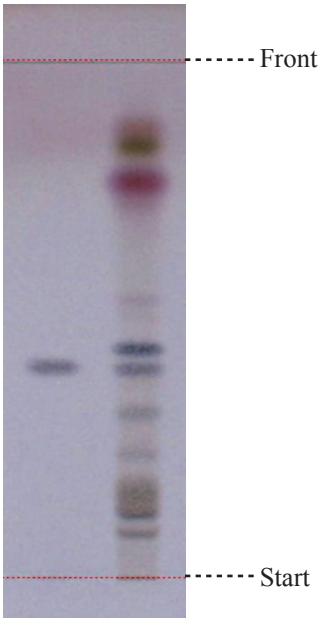

Figure 5 A reference HPTLC chromatogram of Hedyotidis Diffusae Herba extract observed under visible light after staining

1. Asperuloside 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 asperuloside (Fig. 5).

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

Standard solution

Asperuloside standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of asperuloside CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 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 (70%). 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 column temperature is maintained at 35ºC during the separation. The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	95	5	isocratic
10 – 20	95 → 90	5 → 10	linear gradient
20 – 45	90 → 85	10 → 15	linear gradient
45 – 60	85 → 82	15 → 18	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of asperuloside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of asperuloside should not be more than 5.0%; the RSD of the retention time of asperuloside peak should not be more than 2.0%; the column efficiency determined from asperuloside peak should not be less than 60000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Hedyotidis Diffusae Herba extract

Peak No.	RRT	Acceptable Range
1	0.30	± 0.03
2	0.80	± 0.03
3	0.83	± 0.03
4 (marker, asperuloside)	1.00	-
5	1.53	± 0.03

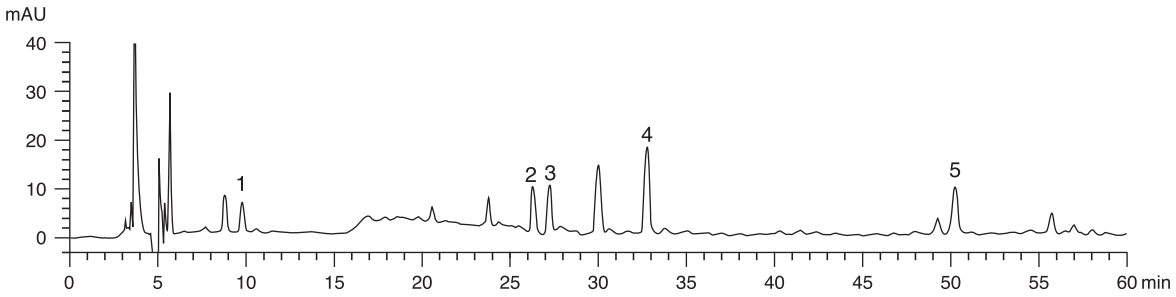

Figure 6 A reference fingerprint chromatogram of Hedyotidis Diffusae Herba extract

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. 6).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: The CMM shall meet the requirements for arsenic, lead and mercury as specified in Appendix V. For cadmium, Hedyotidis Diffusae Herba should meet the specified limit of not more than 5.0 mg/kg, when the CMM will be processed as a decoction in the final consumption form; otherwise, the limit for cadmium specified in Appendix V shall be applied.

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 10.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.5%.
Acid-insoluble ash: not more than 3.0%.

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 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution

Asperuloside standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 5.0 mg of asperuloside CRS and dissolve in 25 mL of methanol (70%).

Asperuloside standard solution for assay, Std-AS

Measure accurately the volume of the asperuloside Std-Stock, dilute with methanol (70%) to produce a series of solutions of 3, 6, 12, 24, 48 mg/L for asperuloside.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 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 (70%). 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 column temperature is maintained at 35ºC during the separation. The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	95	5	isocratic
10 – 20	95 → 90	5 → 10	linear gradient
20 – 45	90 → 85	10 → 15	linear gradient
45 – 60	85 → 82	15 → 18	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of asperuloside Std-AS (12 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of asperuloside should not be more than 5.0%; the RSD of the retention time of asperuloside peak should not be more than 2.0%; the column efficiency determined from asperuloside peak should not be less than 60000 theoretical plates.

The *R* value between asperuloside peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of asperuloside Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of asperuloside against the corresponding concentrations of asperuloside 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 asperuloside peak (Fig.7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of asperuloside Std-AS. The retention times of asperuloside 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 asperuloside in the test solution, and calculate the percentage content of asperuloside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.090% of asperuloside ($C_{18}H_{22}O_{11}$), calculated with reference to the dried substance.

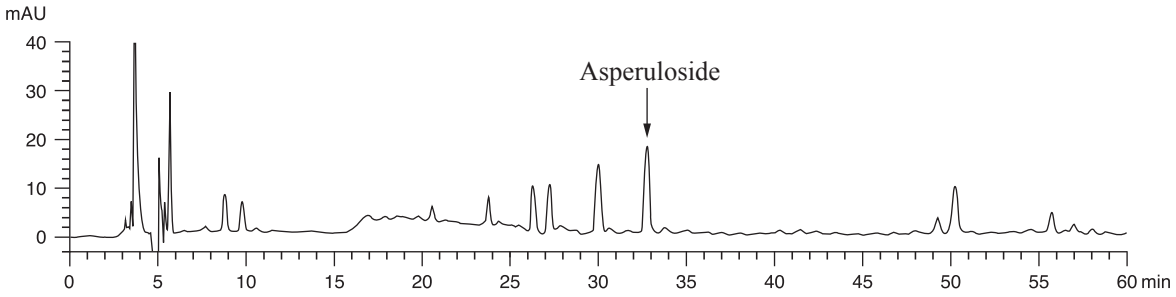

Figure 7 A reference assay chromatogram of Hedyotidis Diffusae Herba extract

8. CAUTION

This CMM should be used after proper processing (such as decoction).