Abri Herba

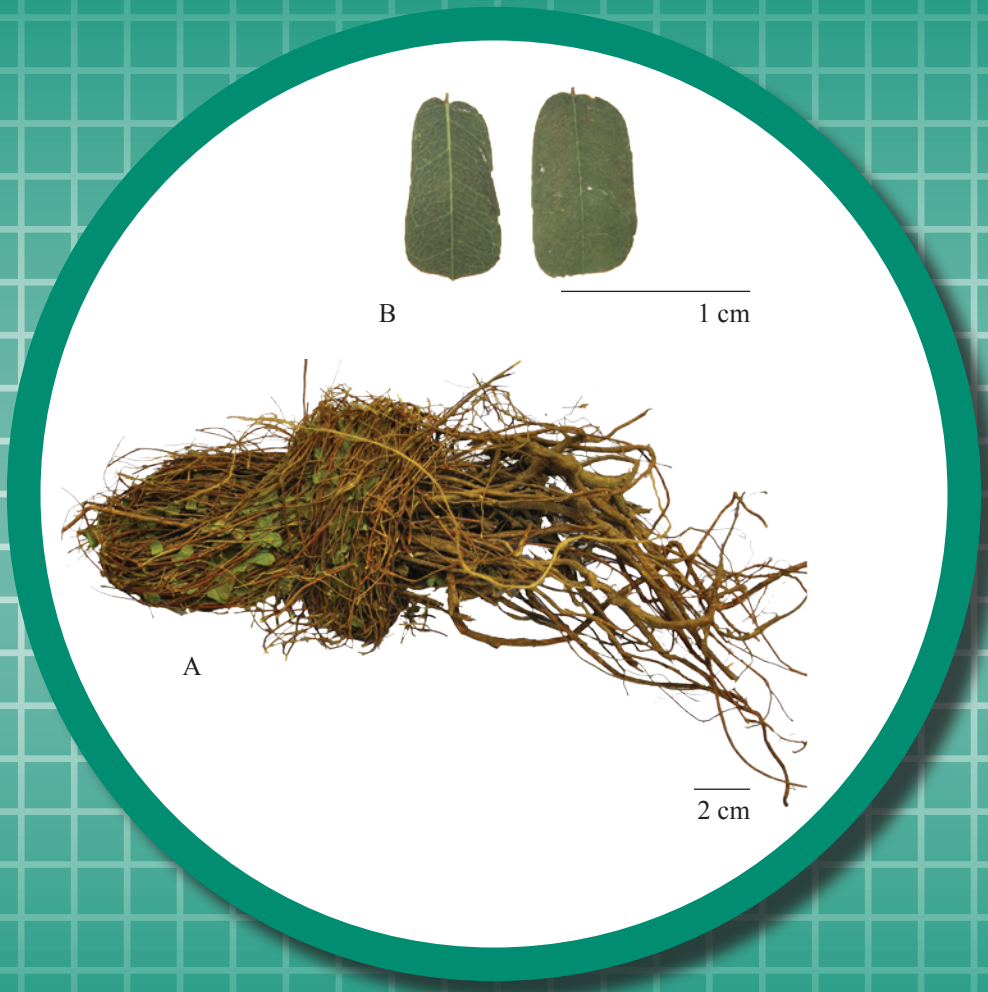

Figure 1 A photograph of Abri Herba

A. Abri Herba B. Leaves

1. NAMES

Official Name: Abri Herba

Chinese Name: 雞骨草

Chinese Phonetic Name: Jigucao

2. SOURCE

Abri Herba is the dried whole plant of *Abrus cantoniensis* Hance (Fabaceae). The whole plant is collected all year round, foreign matter removed, then dried under the sun to obtain Abri Herba.

3. DESCRIPTION

Root externally greyish-brown to purplish-brown, rough, with fine longitudinal wrinkles, tapering to the end, usually branched and varying in size. Stem caespitose, extremely slender, greyish-brown to purplish-brown, with sparse and short pubescences. Leaves pinnately compound, alternate; leaflets almost fallen off, petiole short; lamina subrectangular, 0.5-1.5 cm long and 0.3-0.6 cm wide, apex truncate and mucronulate, margins entire. Odour slightly aromatic; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of several layers of cells, cells subsquare or rectangular, reddish-brown. Cortex narrow, stone cells and prisms of calcium oxalate arranged in an interrupted ring. Phloem relatively thin. Cambium in a ring. Xylem vessels scattered, arranged radially; ray distinct, 2 to several rows of cells wide [Fig. 2 (i)].

Stem: Cork consists of several layers of brown cells, cells subsquare or rectangular, arranged in order. Cortex relatively thin, prisms of calcium oxalate and stone cells visible. Phloem fibres numerous, arranged in a ring. Phloem narrow. Xylem relatively broad, vessels radially arranged; ray distinct. Pith broad, parenchymatous cells subrounded, mostly broken, hollow in centre [Fig. 2 (ii)].

Leaf: Upper epidermis consists of 1 layer of cells, cells subsquare or rectangular, prisms of calcium oxalate occasionally found under the epidermis. Palisade tissue consists of 2 layers of palisade cells. Spongy tissue cells subrounded, arranged loosely. Vascular bundles collateral, xylem vessels arranged radially. Numerous fibres visible around the vascular bundles. Lower epidermis consists of 1 layer of cells, irregular in shape [Fig. 2 (iii)].

Powder

Colour greyish-green. Cork cells brown, rectangular, elliptical or irregular. Prisms of calcium oxalate numerous, 6-43 μm in diameter; bright white or polychromatic under the polarized microscope. Non-glandular hairs unicellular, apex gradually acuminate, with fine warty protuberance on the surface. Vessels mainly bordered-pitted, 10-53 μm in diameter. Stone cells elliptical or subsquare, 11-74 μm in diameter, walls slightly thick. Epidermal cells and stomata of leaf occasionally visible, cell walls slightly curved, stomata diacytic. Fibres single or in bundles, 8-36 μm in diameter, walls relative thick, fibre bundles sometimes surrounded by cells contain prisms of calcium oxalate, forming crystal fibres; polychromatic under the polarized microscope (Fig. 3).

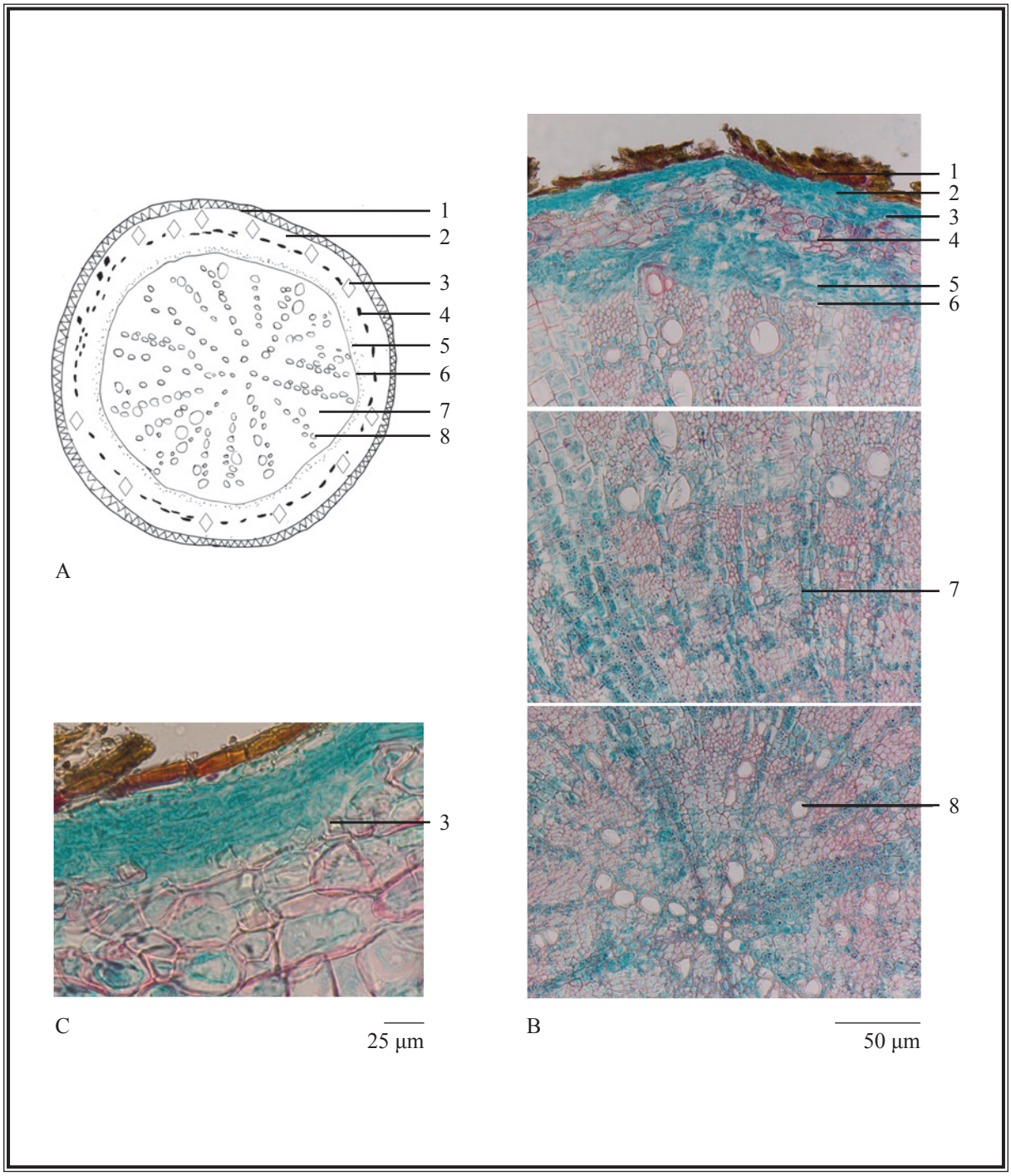

Figure 2 (i) Microscopic features of transverse section of root of Abri Herba

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

1. Cork 2. Cortex 3. Prisms of calcium oxalate 4. Stone cells 5. Phloem
6. Cambium 7. Ray 8. Xylem

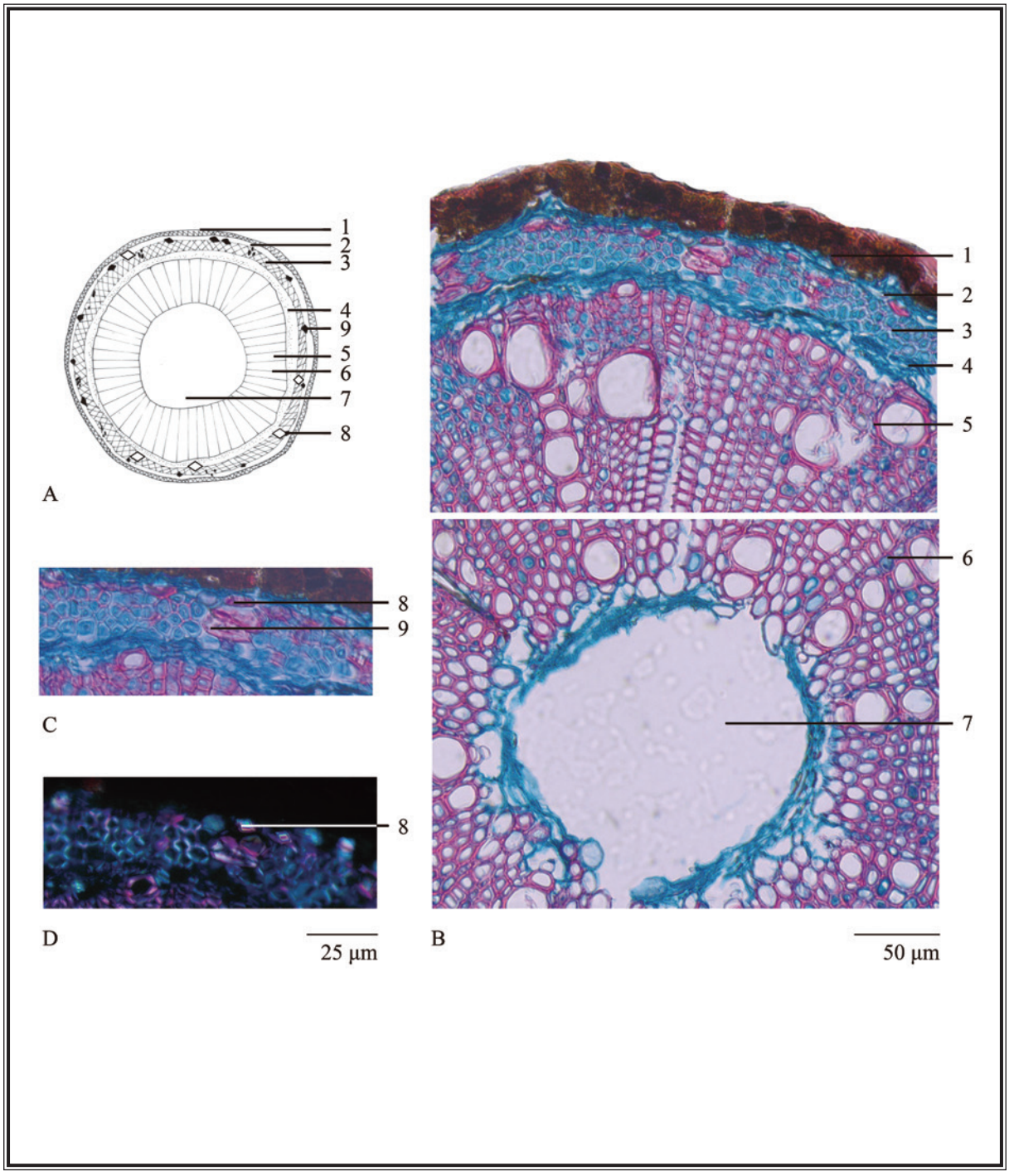

Figure 2 (ii) Microscopic features of transverse section of stem of Abri Herba

A. Sketch B. Sections illustration C. Prisms of calcium oxalate and stone cells
D. Prisms of calcium oxalate (under the polarized microscope)

1. Cork 2. Cortex 3. Phloem fibres 4. Phloem 5. Xylem 6. Ray 7. Pith
8. Prisms of calcium oxalate 9. Stone cells

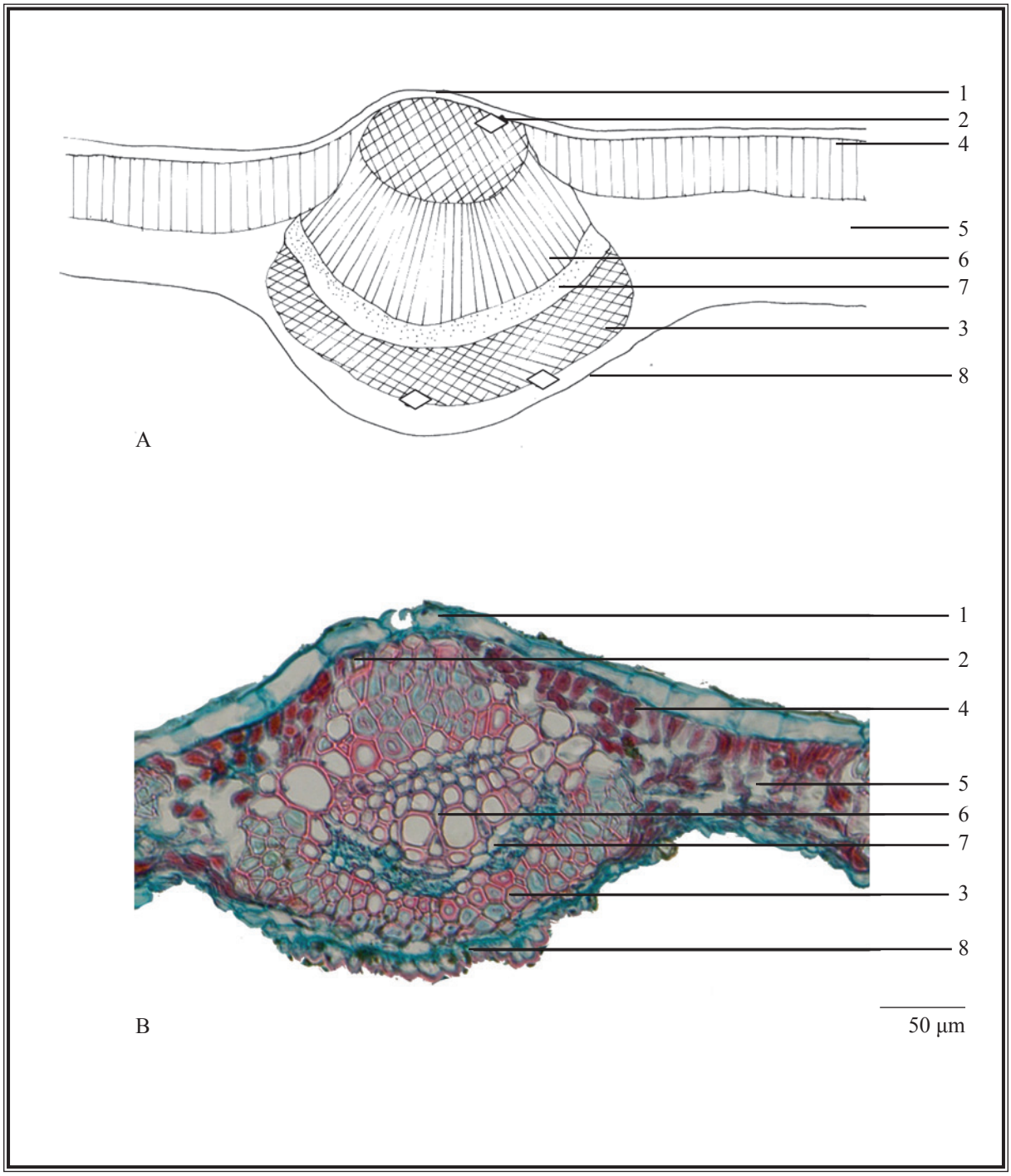

Figure 2 (iii) Microscopic features of transverse section of leaf of Abri Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Prisms of calcium oxalate 3. Fibres 4. Palisade tissue
5. Spongy tissue 6. Xylem 7. Phloem 8. Lower epidermis

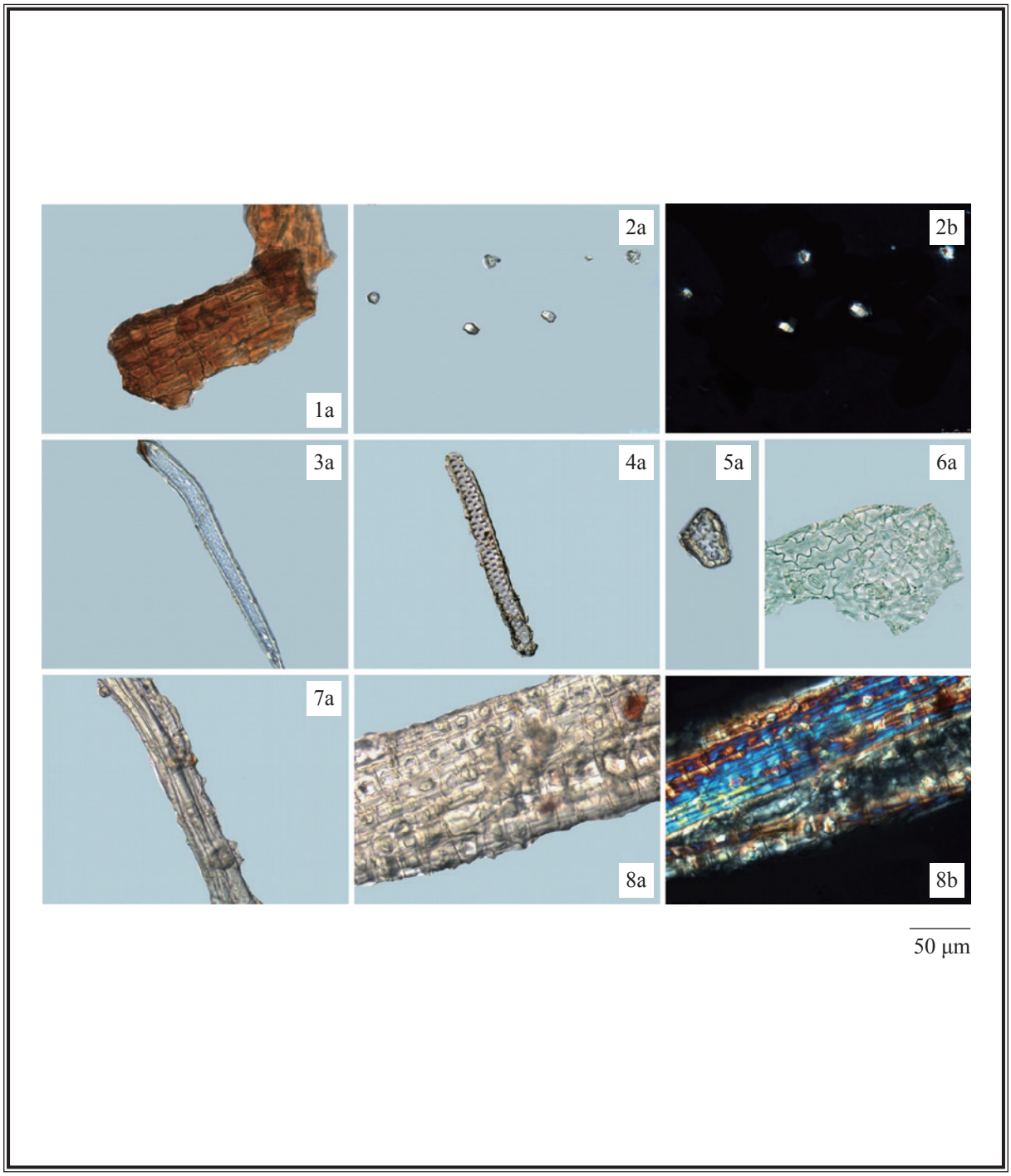

Figure 3 Microscopic features of powder of Abri Herba

1. Cork cells 2. Prisms of calcium oxalate 3. Non-glandular hair
4. Bordered-pitted vessel 5. Stone cell 6. Epidermal cells of leaf 7. Fibre bundle 8. Crystal Fibres

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

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

Standard solution

Abrine (N-Methyl-L-tryptophan) standard solution

Weigh 0.5 mg of abrine CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of water, *n*-butanol and glacial acetic acid (5:4:1, v/v).

Spray reagent

Weigh 2 g of ninhydrin and dissolve in 100 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 100-mL conical flask, then add 20 mL of methanol. Sonicate (350 W) the mixture for 30 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 and then 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 abrine standard solution and the test solution (3 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 5 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 7.5 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 4 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

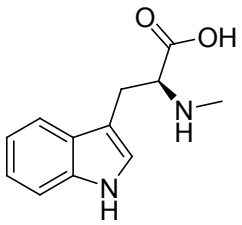

Figure 4 Chemical structure of abrine (*N*-methyl-L-tryptophan)

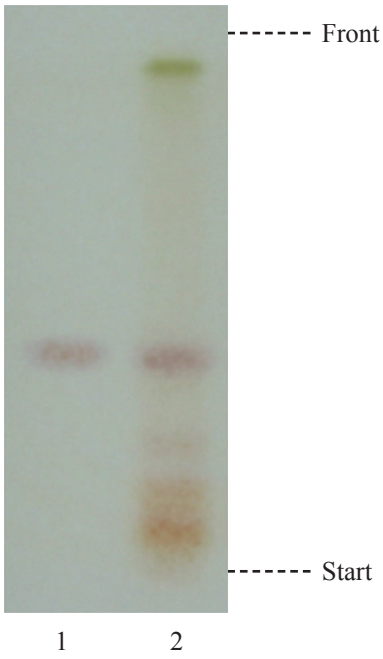

Figure 5 A reference HPTLC chromatogram of Abri Herba extract observed under visible light after staining

1. Abrine 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 abrine (Fig. 5).

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

Standard solution

Abrine (N-Methyl-L-tryptophan) standard solution for fingerprinting, Std-FP (13 mg/L)

Weigh 0.13 mg of abrine CRS and dissolve in 10 mL of ethanol (30%)

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (30%). Sonicate (350 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC 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)	0.2% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	95 → 87	5 → 13	linear gradient
20 – 45	87 → 81	13 → 19	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of abrine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of abrine should not be more than 5.0%; the RSD of the retention time of abrine peak should not be more than 2.0%; the column efficiency determined from abrine peak should not be less than 30000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject abrine Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of abrine peak in the chromatogram of abrine Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify abrine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of abrine Std-FP. The retention

times of abrine 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 three characteristic peaks of Abri Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Abri Herba extract

Peak No.	RRT	Acceptable Range
1	0.56	± 0.03
2	0.61	± 0.03
3 (marker, abrine)	1.00	-

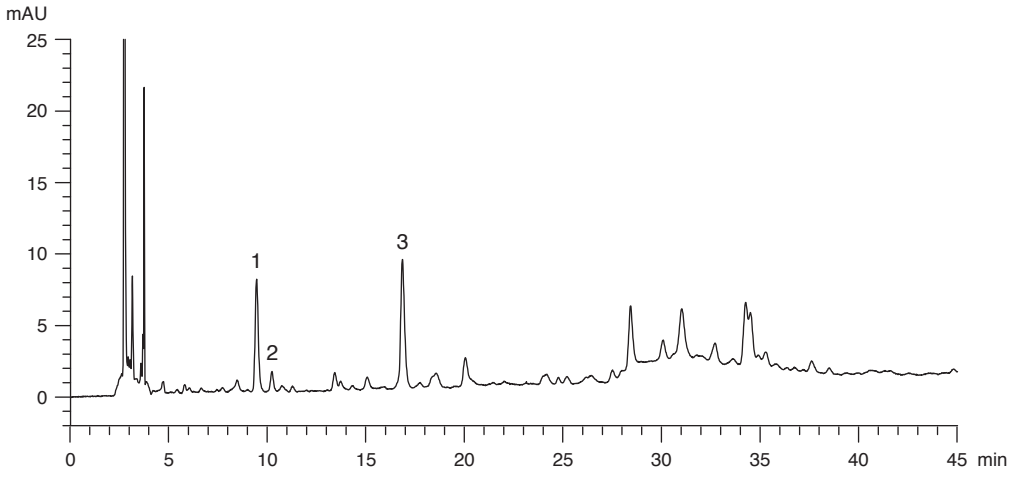

Figure 6 A reference fingerprint chromatogram of Abri Herba extract

For positive identification, the sample must give the above three 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)*: 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.5%.
Acid-insoluble ash: not more than 3.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

Abrine (N-Methyl-L-tryptophan) standard stock solution, Std-Stock (100 mg/L)
Weigh accurately 1.0 mg of abrine CRS and dissolve in 10 mL of ethanol (30%).
Abrine standard solution for assay, Std-AS
Measure accurately the volume of the abrine Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 2.5, 7.5, 15, 30, 60 mg/L for abrine.

Test solution

Weigh accurately 0.8 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (30%). Sonicate (350 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (30%). Combine the solutions and make up to the mark with ethanol (30%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (278 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during

the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	95 → 87	5 → 13	linear gradient
20 – 25	87 → 85.5	13 → 14.5	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of abrine Std-AS (15 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of abrine should not be more than 5.0%; the RSD of the retention time of abrine peak should not be more than 2.0%; the column efficiency determined from abrine peak should not be less than 30000 theoretical plates.

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

Limits

The sample contains not less than 0.025% of abrine ($C_{12}H_{14}N_2O_2$), calculated with reference to the dried substance.