Corydalis Bungeanae Herba

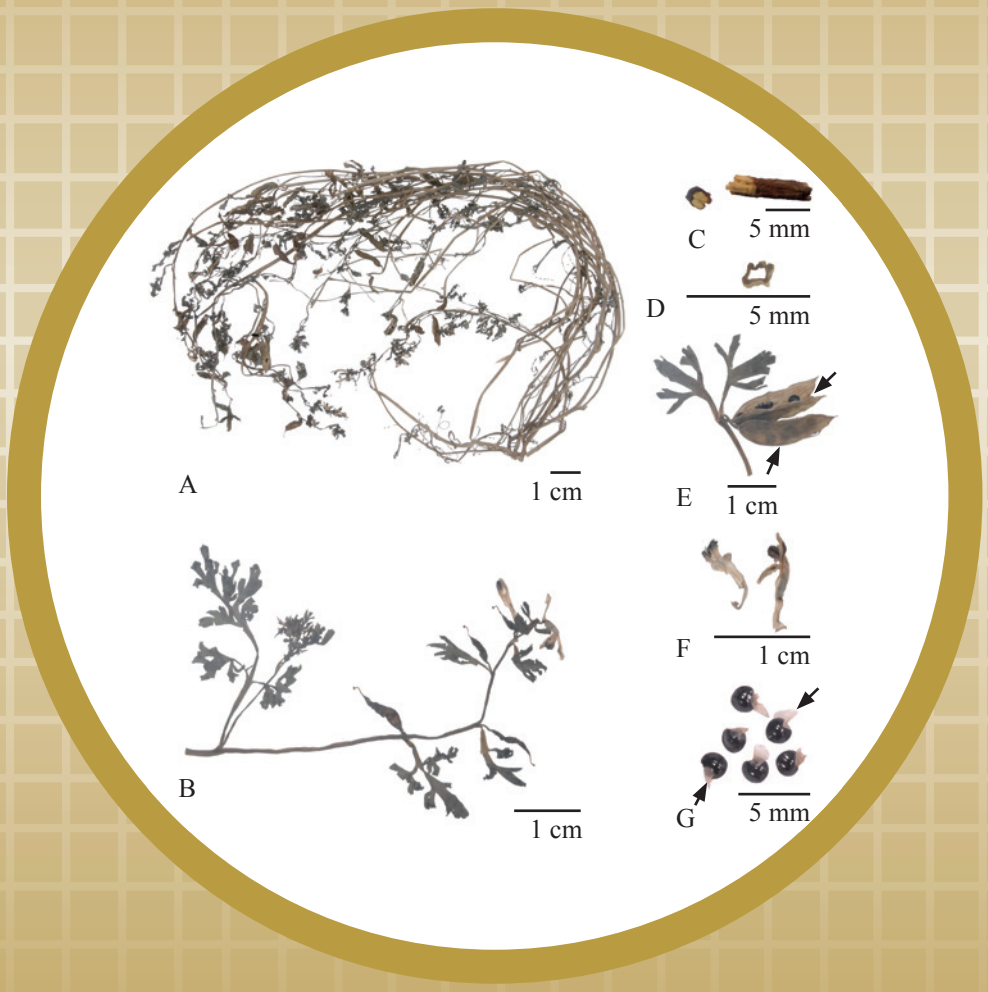

Figure 1 A photograph of Corydalis Bungeanae Herba

A. Corydalis Bungeanae Herba B. Magnified image of aerial part

C. Magnified image of root and fracture of root

D. Magnified image of transverse section of stem

E. Magnified image of leaves and fruits (fruits ⟶)

F. Magnified image of flowers G. Magnified image of seeds (caruncle ⟶)

1. NAMES

Official Name: Corydalis Bungeanae Herba

Chinese Name: 苦地丁

Chinese Phonetic Name: Kudiding

2. SOURCE

Corydalis Bungeanae Herba is the dried whole plant of *Corydalis bungeana* Turcz. (Papaveraceae). The whole plant is collected in summer when fruiting begins, foreign matter removed, then dried under the sun to obtain Corydalis Bungeanae Herba.

3. DESCRIPTION

Crumpled into masses, length varies up to about 30 cm. Small root pieces occasionally found, externally brown. Stems slender, frequently branched, externally yellowish-green to greyish-green, with 5 longitudinal ridges. Texture soft, fracture hollowed. Leaves mostly crumpled and crumbled, greyish-green to dark green; when whole, lamina bi- or tripinnatisect. Flowers rarely found, usually fallen off; if found the corolla bilabiate, lilac. Capsules flat, oblong to long-elliptic, legume-like; seeds flat-cordate, black, lustrous, often with pale yellowish-white membranous caruncle. Odour slight, taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of 1-5 layers of cells, mostly wrinkled or fallen off. Cortex parenchymatous cells tangentially elongated. Phloem parenchymatous cells tangentially elongated, mostly wrinkled. Xylem vessels numerous, mostly several in groups, xylem ray distinct [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of cells, cells tangentially elongated. Cortex parenchymatous cells irregular in shape. Collenchyma consists of 7-11 layers of cells, present in the ridges. Pericycle fibres arranged in a ring. Fibres in the ridges arranged in crescent. Vascular bundles collateral, found in the ridges; phloem narrow; xylem composed of vessels, fibres and parenchymatous cells. Pith relatively large, hollow in the centre [Fig. 2 (ii)].

Leaf: Leaf base consists of 4-7 collateral vascular bundles. Upper and lower epidermis consists of 1 layer of cells. Cortex cells irregular in shape. Vascular bundle collateral; phloem narrow, cells mostly wrinkled; vessels lignified [Fig. 2 (iii)].

Pericarp: Exocarp consists of 1 layer of epidermal cells, cells mostly shrunken, cell walls slightly thickened. Sclerenchymatous cells located on the inner sides of exocarp and endocarp, walls slightly thickened. Mesocarp consists of several layers of parenchymatous cells, cells subrounded or irregular in shape. Vascular bundles collateral; phloem cells small; vessels lignified. Endocarp consists of 1 layer of subrectangular cells, cell walls thickened, non-lignified or slightly lignified [Fig. 2 (iv)].

Seed: Outermost layer of testa blackish-brown, palisade in shape, radially elongated, cell walls thickened, with fine and dense pits; beneath it are several layers of stone cells, cells oblong to subrounded, with thickened walls and distinct pits and pit canals. Decadent cells of testa dark brown. Inner layer of cells of testa oblong, tangentially elongated. Endosperm cells irregular in shape, containing oil droplets and aleurone grains [Fig. 2 (v)].

Powder

Colour brownish-green to dark green. Lower epidermal cells with sinuous walls, with more stomata in lower epidermis; stomata anomocytic, subsidiary cells 3-6. Endocarp cell wall sinuous or beaded-thickened. Stone cells of inner layer of testa brown, oblong, polygonal to subrounded in surface view, with distinct pits and pit canals. Vessels mainly spiral. Endosperm cells irregular in shape, containing fatty oil droplets and aleurone grains. Outermost layer of testa reddish-brown to blackish-brown, cells palisade-like in lateral view, walls with reticulate pattern. Pericycle fibres mostly in bundles, walls thickened. Seed caruncle cells elongated-fusiform, walls thin (Fig. 3).

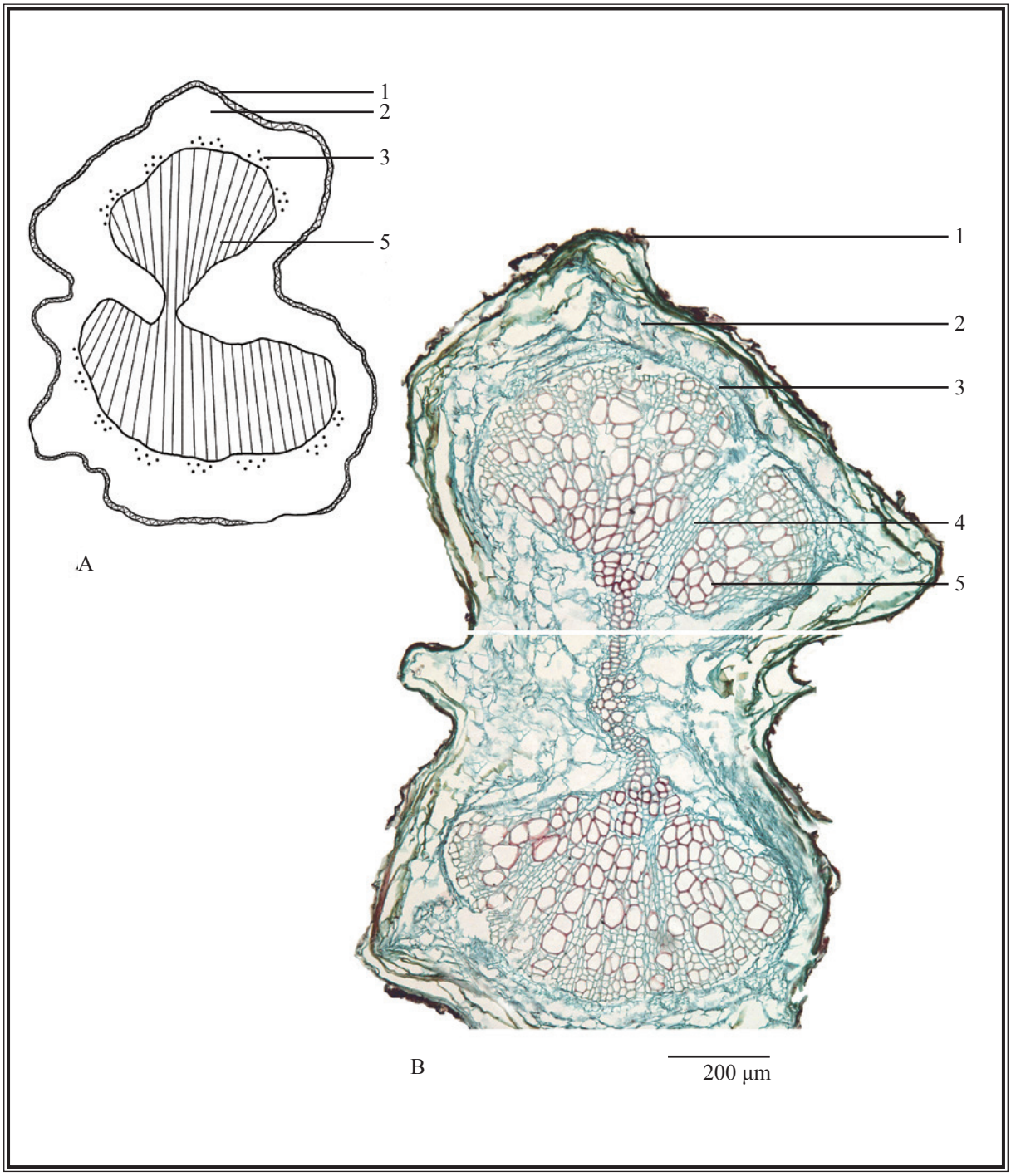

Figure 2 (i) Microscopic features of transverse section of root of Corydalis Bungeanae Herba

A. Sketch B. Section illustration

1. Cork 2. Cortex 3. Phloem 4. Xylem ray 5. Xylem

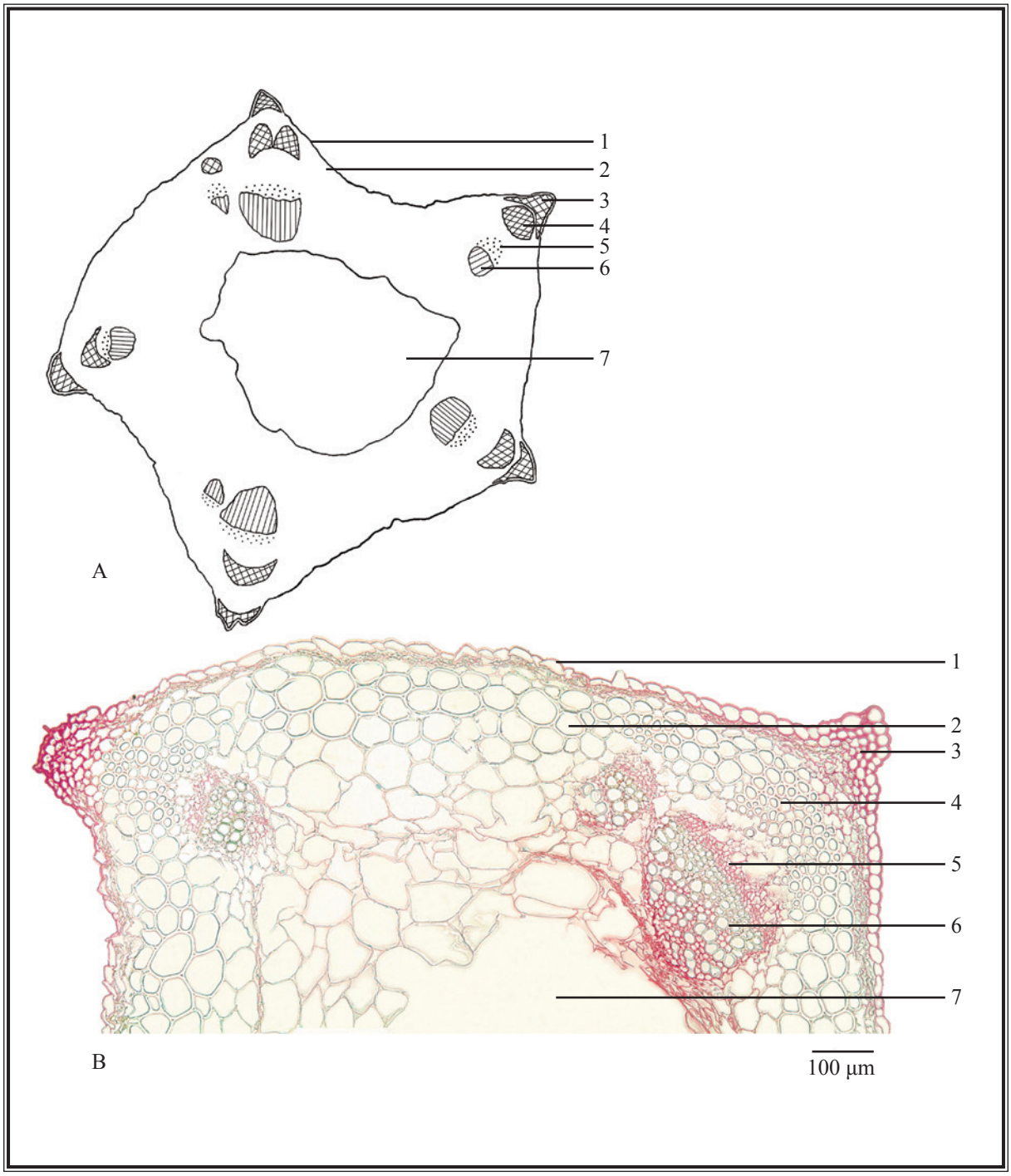

Figure 2 (ii) Microscopic features of transverse section of stem of Corydalis Bungeanae Herba

A. Sketch B. Section illustration

1. Epidermis 2. Cortex 3. Collenchyma 4. Pericycle fibre 5. Phloem 6. Xylem 7. Pith

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

A. Sketch B. Section illustration of the leaf base

1. Upper epidermis 2. Cortex 3. Xylem 4. Phloem 5. Lower epidermis

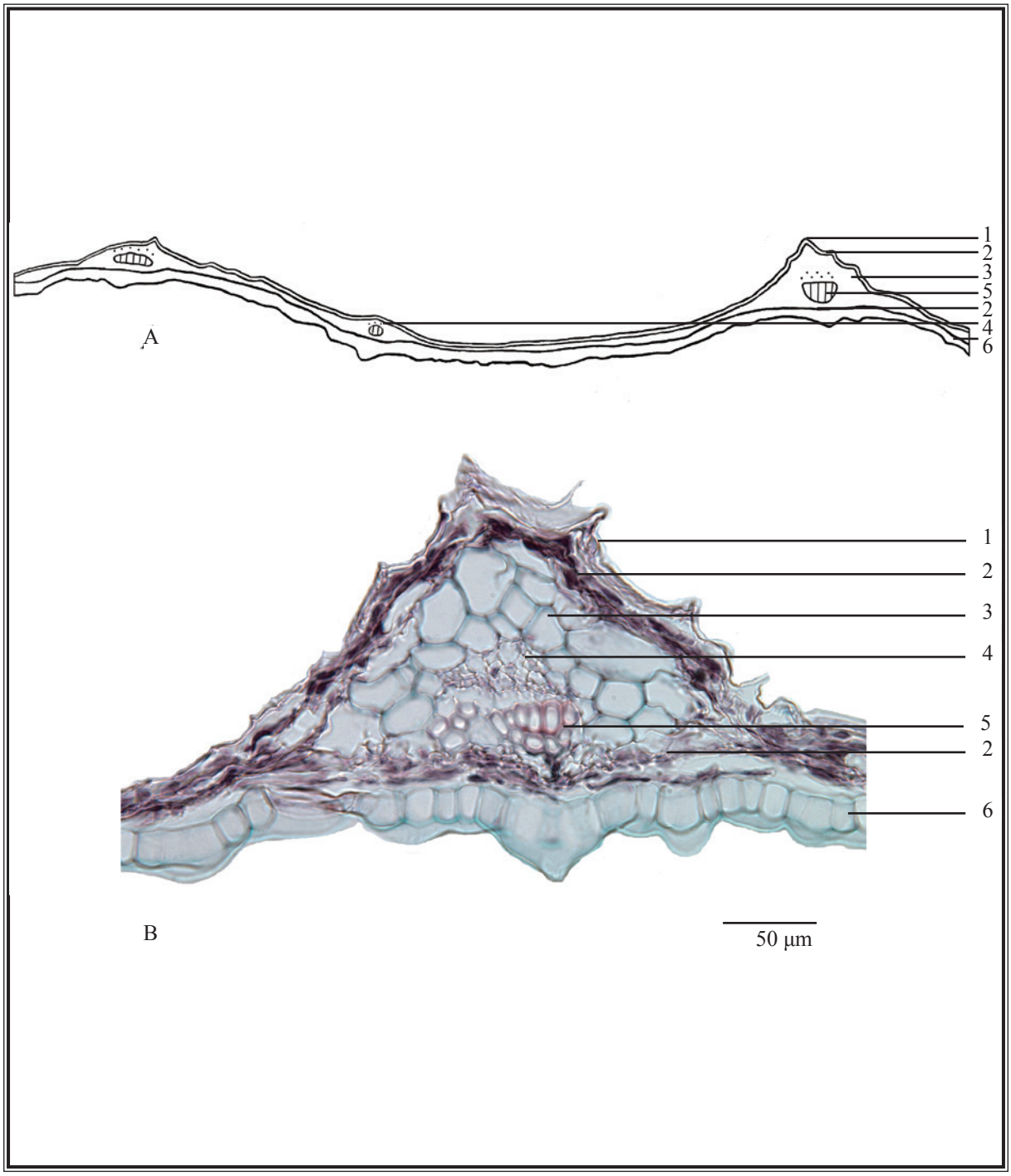

Figure 2 (iv) Microscopic features of transverse section of pericarp of Corydalis Bungeanae Herba

A. Sketch B. Section illustration

1. Exocarp 2. Sclerenchyma 3. Mesocarp 4. Phloem 5. Xylem 6. Endocarp

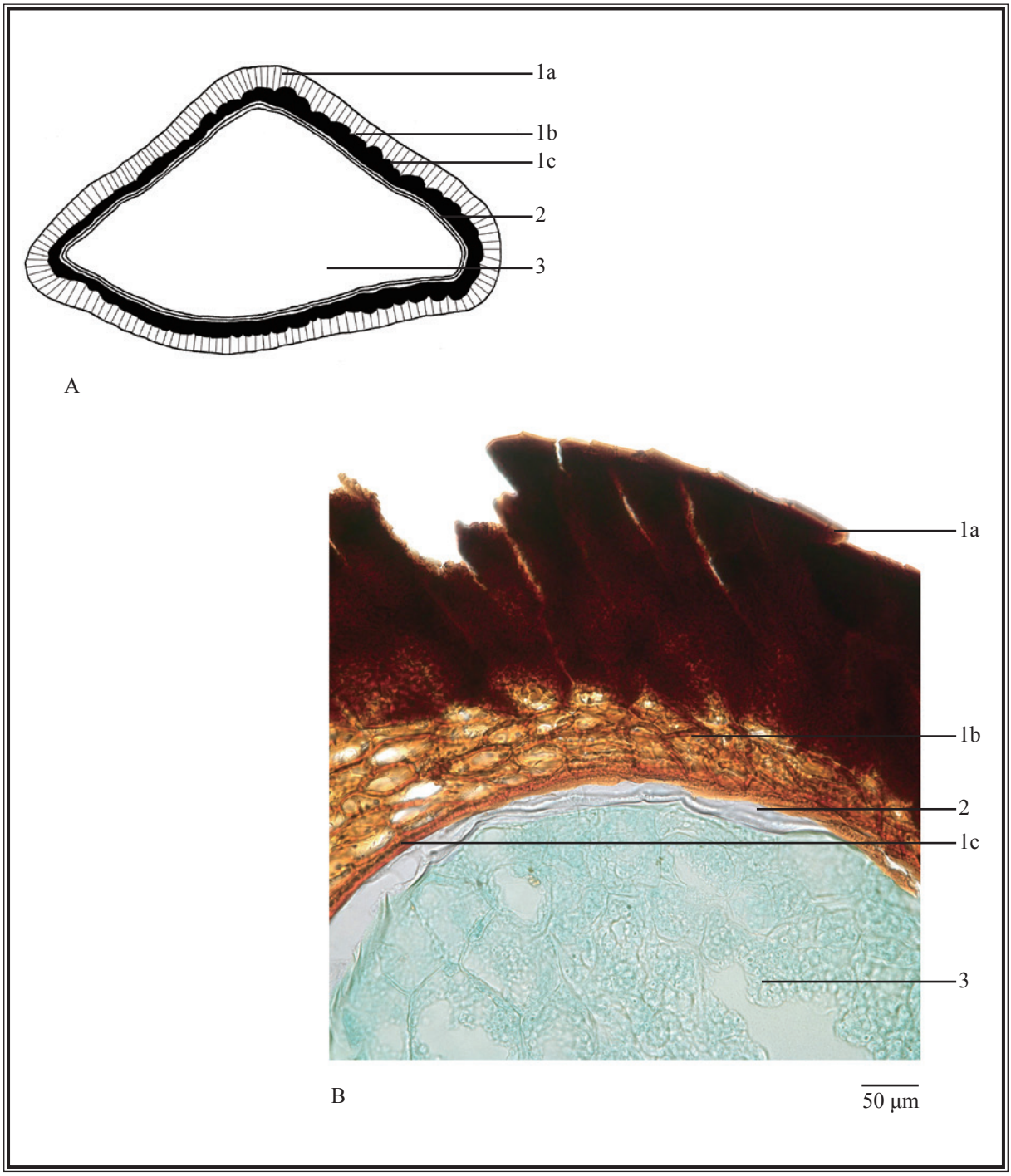

Figure 2 (v) Microscopic features of transverse section of seed of Corydalis Bungeanae Herba

A. Sketch B. Section illustration

1. Testa (1a outermost layer, 1b inner layer with several layers of stone cells, 1c decadent cell layer)

2. Inner epidermis of testa 3. Endosperm

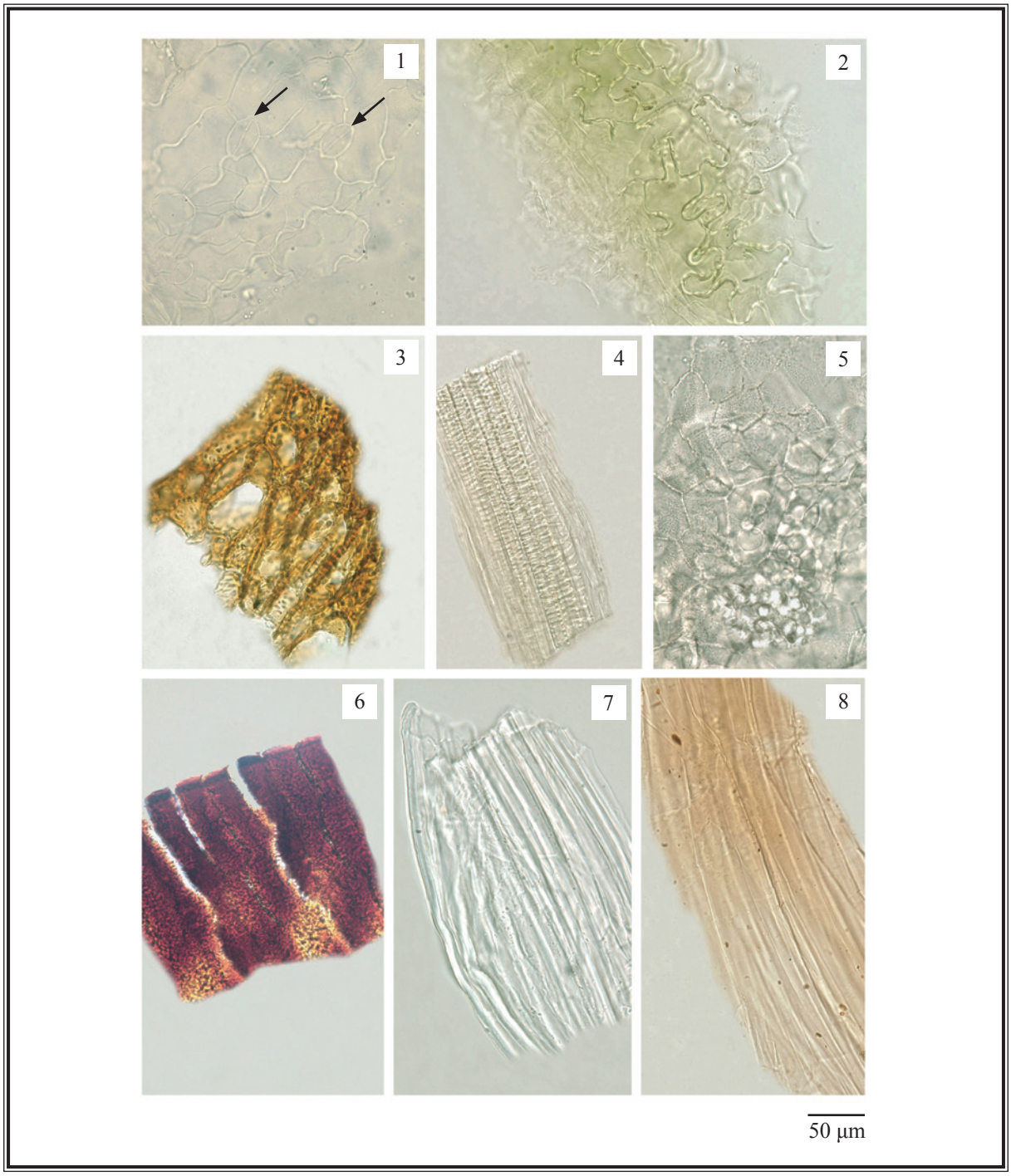

Figure 3 Microscopic features of powder of Corydalis Bungeanae Herba (under the light microscope)

1. Lower epidermal cells with stomata (⟶) 2. Endocarp cells in surface view
3. Stone cells of inner layer of testa 4. Spiral vessels 5. Endosperm cells
6. Outermost layer of testa in lateral view 7. Pericycle fibres
8. Seed caruncle cells elongated fusiform

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

Standard solution

Corynoline standard solution

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

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate and methanol (7:2.5:0.5, v/v).

Spray reagent

Solution A

Weigh 0.85 g of bismuth oxynitrate and dissolve in a mixture of 10 mL of glacial acetic acid and 40 mL of water.

Solution B

Weigh 4 g of potassium iodide and dissolve in 10 mL of water.

Spray reagent

Transfer 5 mL of Solution A, 5 mL of Solution B and 20 mL of glacial acetic acid into a 100-mL volumetric flask and make up to the mark with water. Freshly prepare the reagent.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

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 corynoline standard solution (1 μL) and the test solution (4 μL) to the plate. Develop over a path of about 7 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 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).

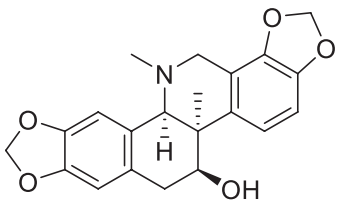

Figure 4 Chemical structure of corynoline

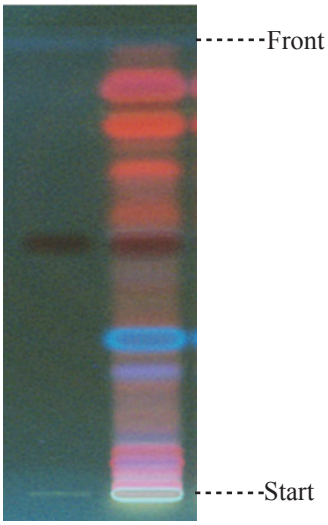

Figure 5 A reference HPTLC chromatogram of Corydalis Bungeanae Herba extract observed under UV light (366 nm) after staining

1. Corynoline 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 corynoline (Fig. 5).

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

Standard solution

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

Weigh 2.5 mg of corynoline CRS and dissolve in 100 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL 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 RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (288 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)	0.2% Triethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 25	60	40	isocratic
25 – 50	60 → 10	40 → 90	linear gradient

System suitability requirements

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

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

by comparing its retention time with that in the chromatogram of corynoline Std-FP. The retention times of corynoline 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 Corydalis Bungeanae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, corynoline)	1.00	-
2	1.16	± 0.04
3	1.25	± 0.05

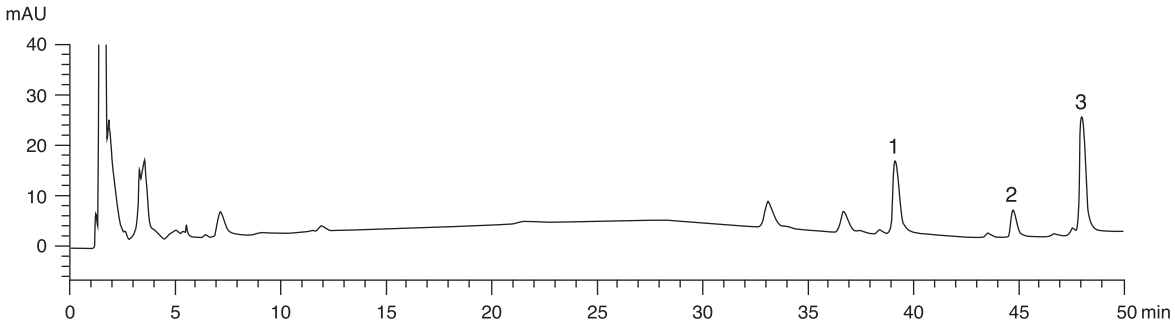

Figure 6 A reference fingerprint chromatogram of Corydalis Bungeanae Herba extract

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 – 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 18.0%.
Acid-insoluble ash: not more than 6.5%.

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 23.0%.
Ethanol-soluble extractives (cold extraction method): not less than 18.0%.

7. ASSAY

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

Standard solution

Corynoline standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of corynoline CRS and dissolve in 10 mL of methanol.

Corynoline standard solution for assay, Std-AS

Measure accurately the volume of the corynoline Std-Stock, dilute with methanol to produce a series of solutions of 5, 12.5, 25, 50, 100 mg/L for corynoline.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL 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 RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (288 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)	0.2% Triethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 25	60	40	isocratic
25 – 50	60 → 10	40 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of corynoline Std-AS (25 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of corynoline should not be more than 5.0%; the RSD of the retention time of corynoline peak should not be more than 2.0%; the column efficiency determined from corynoline peak should not be less than 60000 theoretical plates.

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

Limits

The sample contains not less than 0.14% of corynoline ($C_{21}H_{21}NO_5$), calculated with reference to the dried substance.

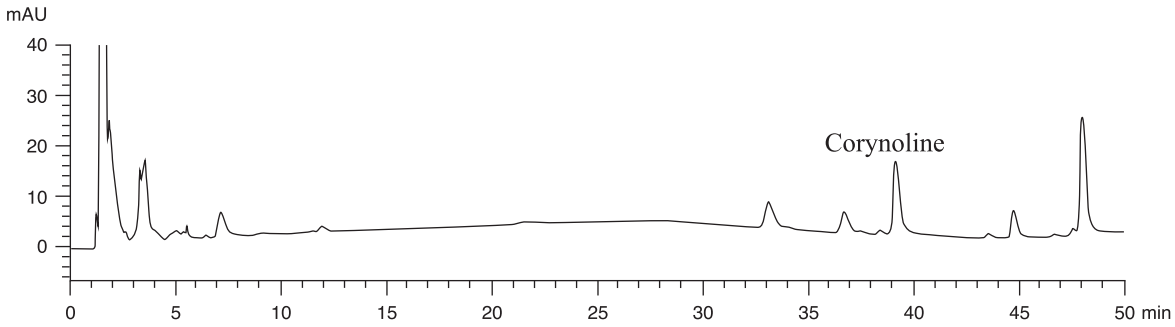

Figure 7 A reference assay chromatogram of Corydalis Bungeanae Herba extract