Fritillariae Cirrhosae Bulbus

Figure 1 (i) A photograph of dried bulb of *Fritillaria cirrhosa* D. Don

A. Bulbs B. Magnified image of bulbs

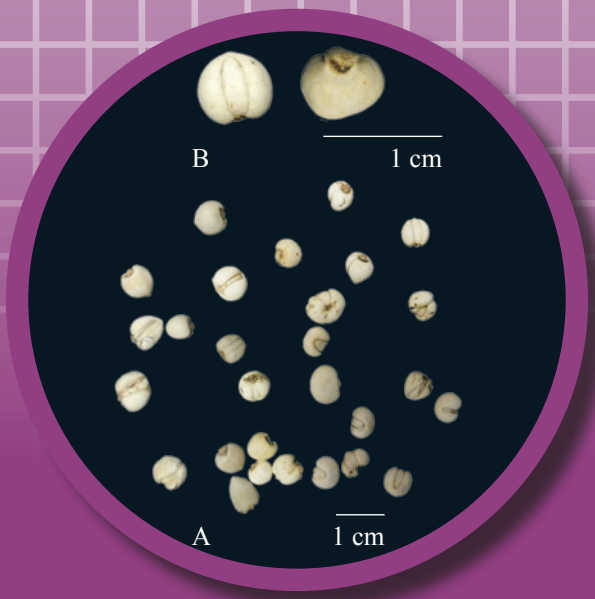

Figure 1 (ii) A photograph of dried bulb of *Fritillaria unibracteata* Hsiao et K. C. Hsia

A. Bulbs B. Magnified image of bulbs

1. NAMES

Official Name: Fritillariae Cirrhosae Bulbus

Chinese Name: 川貝母

Chinese Phonetic Name: Chuanbeimu

2. SOURCE

Fritillariae Cirrhosae Bulbus is the dried bulb of *Fritillaria cirrhosa* D. Don, *Fritillaria unibracteata* Hsiao et K. C. Hsia (Liliaceae). The bulb is collected in summer and autumn, fibrous root, outer coarse bark and soil removed, then dried under the sun or at a lower temperature (40ºC-50ºC) to obtain Fritillariae Cirrhosae Bulbus.

3. DESCRIPTION

***Fritillaria cirrhosa* D. Don:** Subconical or subspherical, some nearly oblate, 0.4-1.4 cm high, 4-16 mm in diameter. Externally whitish. Outer scale leaves 2, varying considerably in size, with the large scale leaf closely embracing the small one, the uncovered part appearing crescent-shaped, commonly known as "Huaizhong Baoyue" (holding the moon in the arms). Apex closed, with subcylindrical and slightly tapered buds and l-2 small scale leaves inside; the apex of the buds obtuse to slightly acute, the base even and slightly concave, with a greyish-brown disk at the central part, with remnants of fibrous roots occasionally attached. Texture hard and fragile. Fracture white, starchy. Odour slight; taste slightly bitter [Fig. 1 (i)].

***Fritillaria unibracteata* Hsiao et K. C. Hsia:** Smaller in size, 0.3-0.8 cm high, 3-9 mm in diameter [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The outer epidermis and inner epidermis of scale leaves consist of 1 layer of cells, the cells subsquared or subrectangular. Parenchymatous cells subrounded, filled with starch granules. Vessels small, scattered in the parenchyma [Fig. 2 (i) and (ii)].

Powder

Colour whitish to pale yellow. Starch granules fairly abundant, broadly ovoid, long spheroidal or irregularly spheroidal, some with uneven or slightly branch-like edges, 5-64 μm in diameter, hilum short slit-shaped, pointed, V-shaped or U-shaped, with faint striations visible; black and cruciate-shaped under the polarized microscope. Epidermal cells subrectangular, anticlinal walls sinuous, anomocytic stomata occasionally found, rounded to oblate. Spiral vessels 5-46 μm in diameter [Fig. 3 (i) and (ii)].

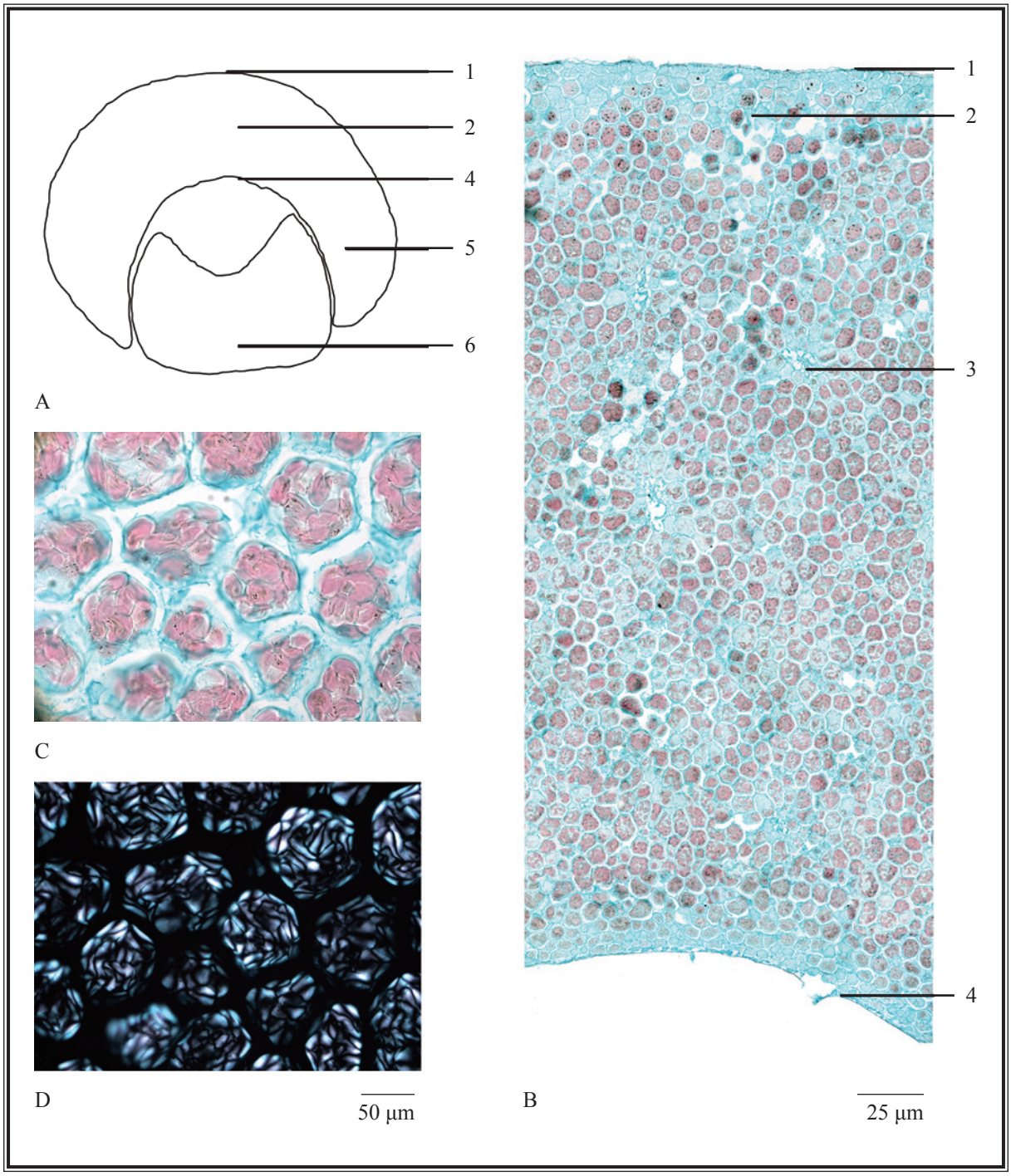

Figure 2 (i) Microscopic features of transverse section of dried bulb of *Fritillaria cirrhosa* D. Don

A. Sketch B. Section illustration of scale leaf C. Starch granules (under the light microscope)
D. Starch granules (under the polarized microscope)

1. Outer epidermis 2. Parenchyma 3. Vessels 4. Inner epidermis 5. Big scale leaf 6. Small scale leaf

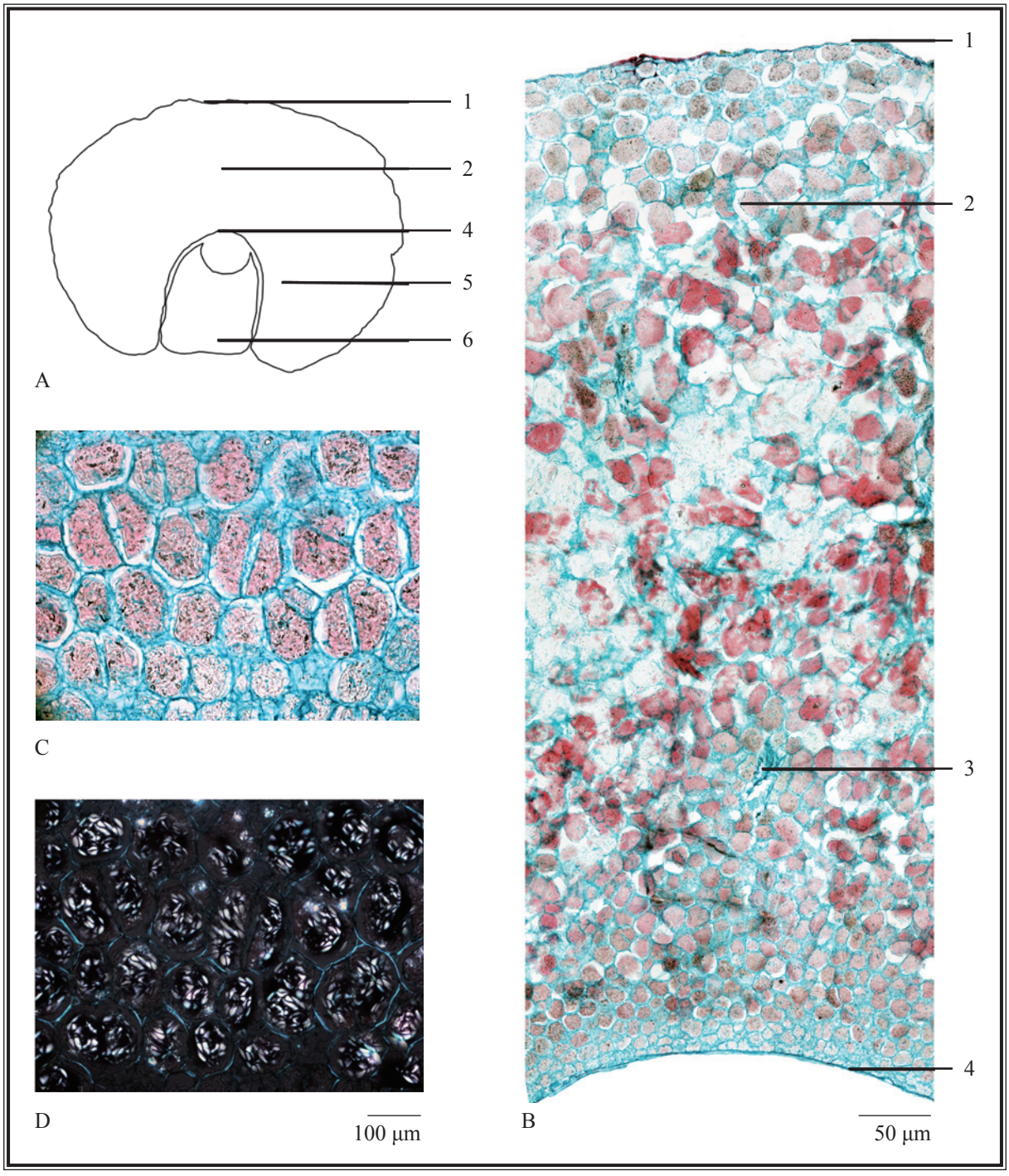

Figure 2 (ii) Microscopic features of transverse section of dried bulb of *Fritillaria unibracteata* Hsiao et K. C. Hsia

A. Sketch B. Section illustration of scale leaf C. Starch granules (under the light microscope)
D. Starch granules (under the polarized microscope)

1. Outer epidermis 2. Parenchyma 3. Vessels 4. Inner epidermis 5. Big scale leaf 6. Small scale leaf

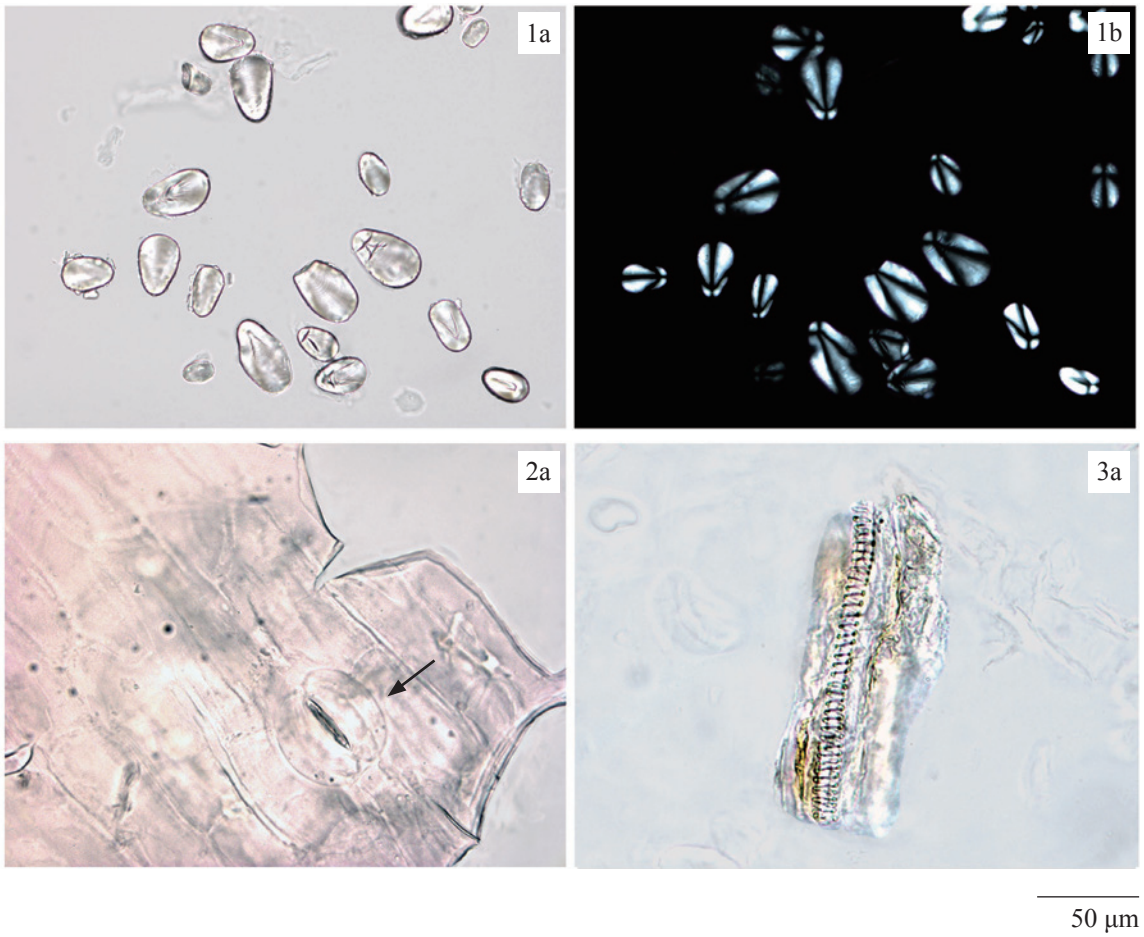

Figure 3 (i) Microscopic features of powder of dried bulb of *Fritillaria cirrhosa* D. Don

1. Starch granules 2. Epidermal cells with stomata (⟶) 3. Spiral vessel

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

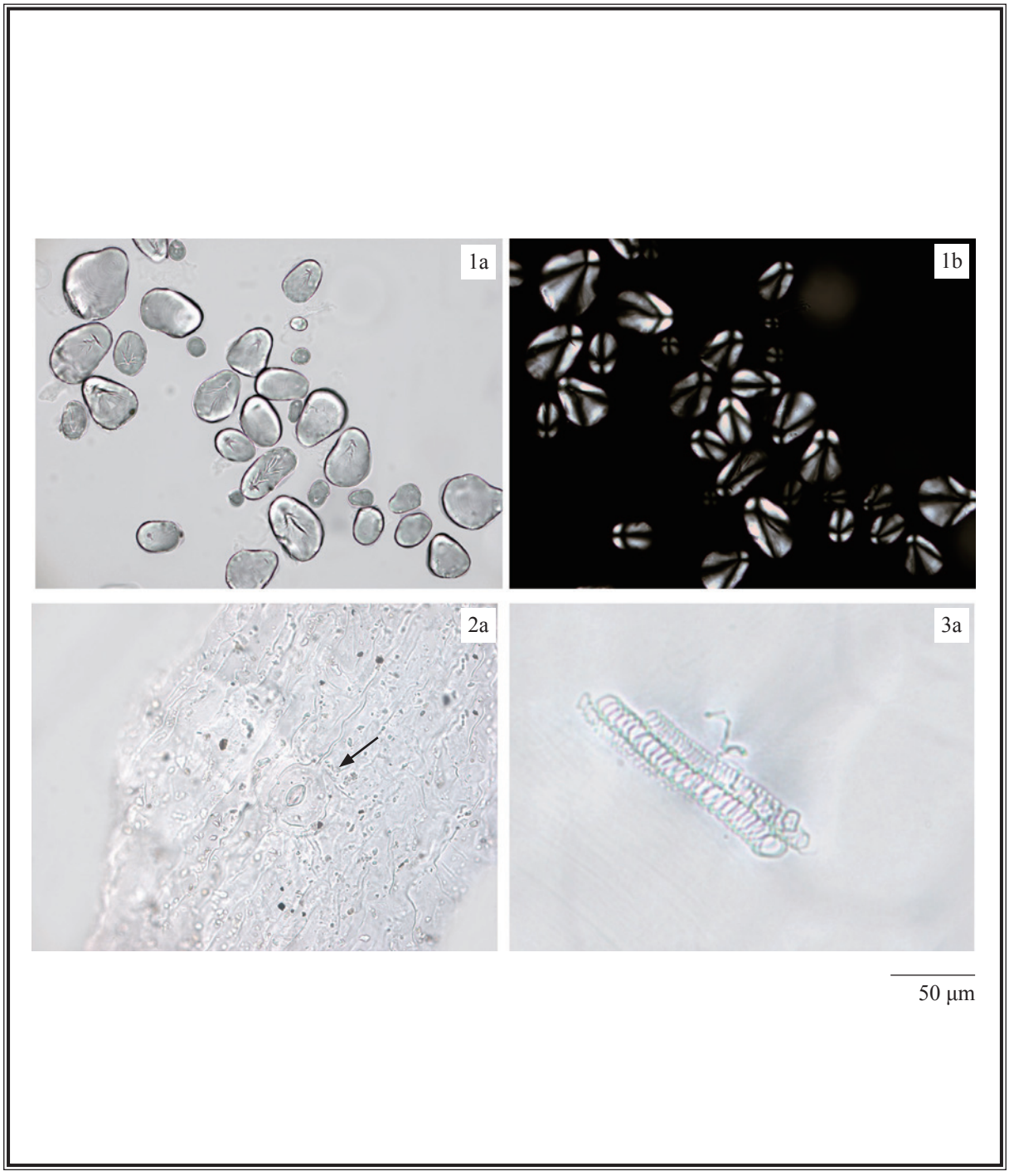

Figure 3 (ii) Microscopic features of powder of dried bulb of *Fritillaria unibracteata* Hsiao et K. C. Hsia

1. Starch granules 2. Epidermal cells with stomata (⟶) 3. Spiral vessels

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

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

Standard solution

Peimisine standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, methanol, ammonium hydroxide solution (25%, v/v) and water (18:2:1:0.1, v/v).

Spray reagent

Weigh 1 g of vanillin and dissolve in 100 mL of sulphuric acid.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of ammonium hydroxide solution (25%, v/v) and 30 mL of dichloromethane. Sonicate (100 W) the mixture for 1 h. 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 TLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately peimisine standard solution (2 µL) and the test solution (12 µL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC plate for development. 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).

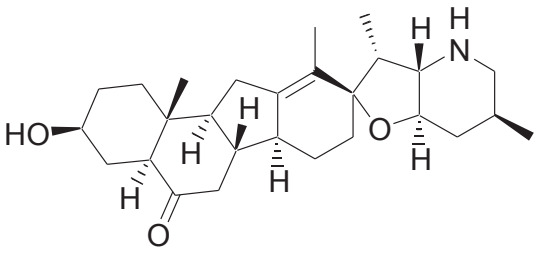

Figure 4 Chemical structure of peimisine

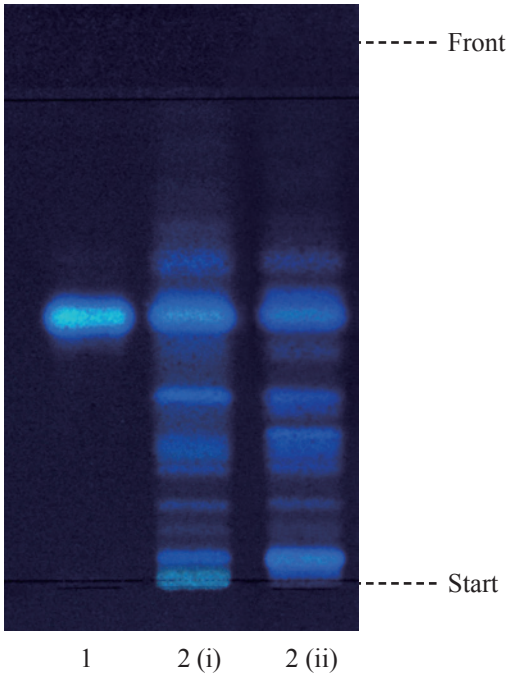

Figure 5 A reference TLC chromatogram of Fritillariae Cirrhosae Bulbus extract observed under UV light (366 nm) after staining

1. Peimisine standard solution
2. Test solution of
(i) dried bulb of *Fritillaria cirrhosa* D. Don
(ii) dried bulb of *Fritillaria unibracteata* Hsiao et K. C. Hsia

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 peimisine (Fig. 5).

4.3 DNA Identification by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) *(Appendix XV)*

Carry out the method as directed in Appendix XV.

Primer sequences for PCR-RFLP

ITS-P1: 5'-CGT AAC AAG GTT TCC GTA GGT GAA-3'

ITS-P3: 5'-GCT ACG TTC TTC ATC GAT-3'

DNA Extraction

Weigh 20 mg of the powdered sample and place it in a 1.5-mL microcentrifuge tube. Extract DNA from plant tissue according to the manufacturer's manual of DNeasy Plant Mini Kit. Briefly, after re-suspension, add the lysis buffer with detergent or surfactant remove lipid on cell surface and to lyse the cells; RNAse A is then added to remove RNA; Afterwards, add ethanol to precipitate DNA; the isolated DNA is ready to use after re-dissolved in TE buffer or UltraPure water.

Procedure

Perform PCR for the method blank, samples (extracted DNA) and sample duplicate. Identify the PCR products by electrophoresis analysis. Measure the fragment length of the DNA fragments by referring to the DNA size marker. Carry out restriction analysis with the PCR products using *Sma I* restriction enzyme (total reaction volume 20 µL). Perform electrophoresis and detection for the method blank and the RFLP products. Measure the fragment length of all polymorphic DNA fragments by referring to the DNA size marker.

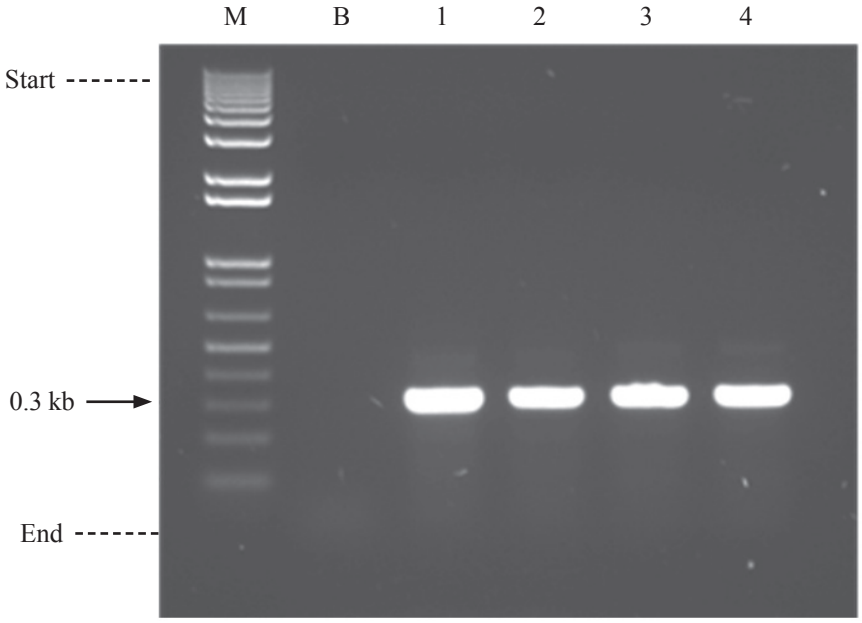

Figure 6 PCR products generated by primers (ITS-P1 and ITS-P3) flanking the nrDNA ITS1 region using template DNA from 4 batches of Fritillariae Cirrhosae Bulbus (Lane 1-4) and blank sample (B)

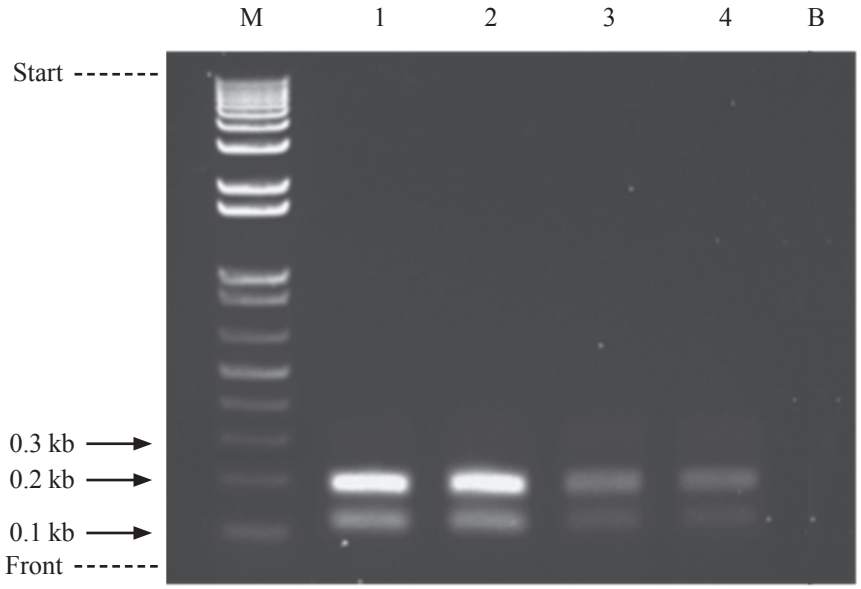

Figure 7 A reference PCR-RFLP patterns of ITS1 region digested with *Sma I*. M: 1 kb DNA ladder; B: Blank; 1-2: *Fritillaria cirrhosa* D. Don; 3-4: *Fritillaria unibracteata* Hsiao et K. C. Hsia

For positive identification, the PCR products of sample and sample duplicate must give a band at 0.3 kb in the electrophoresis detection (Fig. 6). The correct PCR products of sample and sample duplicate are then proceeding to restriction analysis. The RFLP products of sample and sample duplicate must give two bands at ~ 0.1 and 0.2 kb in the electrophoresis detection (Fig. 7).

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 2.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 8.0%.
Ethanol-soluble extractives (hot extraction method): not less than 9.0%.

7. ASSAY

Carry out the method as directed in Appendix XIV.

Reagents

0.2 M Sodium hydroxide solution

Weigh 0.4 g of sodium hydroxide and dissolve in 50 mL of water.

Bromocresol green solution

Weigh 0.05 g of bromocresol green and dissolve in 6 mL of 0.2 M sodium hydroxide solution. Transfer the solution to a 100-mL volumetric flask. Add 1 g of monobasic potassium phosphate and make up to the mark with water.

Standard solution

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

Weigh accurately 5.0 mg of peimisine CRS and dissolve in 10 mL of dichloromethane.

Peimisine standard solution for assay, Std-AS

Measure accurately the volume of the peimisine Std-Stock. Transfer the solution to a 50-mL round-bottomed flask and evaporate to dryness at reduced pressure in a rotary evaporator. Pipette 10 mL of dichloromethane to dissolve the residue and produce a series of solutions of 2.5, 5, 12.5, 25, 37.5 mg/L for peimisine.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 3 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 1 h. Add 40 mL of a mixture of dichloromethane and methanol (4:1, v/v). Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue for two times each with 2 mL of a mixture of dichloromethane and methanol (4:1, v/v). Combine the solutions and make up to the mark with a mixture of dichloromethane and methanol (4:1, v/v). Pipette 8 mL of the solution into a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Pipette 10 mL of dichloromethane to dissolve the residue.

Blank solution

Pipette 10 mL of dichloromethane into a 50-mL round-bottomed flask.

Ultraviolet/ Visible spectrophotometric system

The spectrophotometer is set at 415 nm.

Colourimetric method

Pipette 5 mL of water and 2 mL of bromocresol green solution to the 50-mL round-bottomed flask containing the standard solution, test solution or blank solution. Shake vigorously for 2 min. Transfer the mixture to a 50-mL separating funnel. Allow to stand for 30 min. Collect the dichloromethane layer. Proceed to UV/ Visible analysis at 415 nm.

System suitability requirements

Perform at least five replicates of absorbance measurements, each using 0.25 mL of peimisine Std-AS (12.5 mg/L) by colourmetric method. The requirement of the system suitability parameter is as follows: the RSD of the absorbance of peimisine should not be more than 5.0%.

Calibration curve

Determine a series of peimisine Std-AS (0.25 mL each) in the ultraviolet/ visible spectrophotometric system and record the absorbance by colourimetric method. Plot the absorbances of peimisine against the corresponding concentrations of peimisine Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Measure the absorbance and calculate the concentration (in milligram per litre) of peimisine in the test solution, and calculate the percentage content of peimisine in the sample by using the equations indicated in Appendix XIV.

Limits

The sample contains not less than 0.030% of the total alkaloids [calculated as peimisine ($C_{21}H_{41}NO_3$)], calculated with reference to the dried substance.