Rhizoma Coptidis

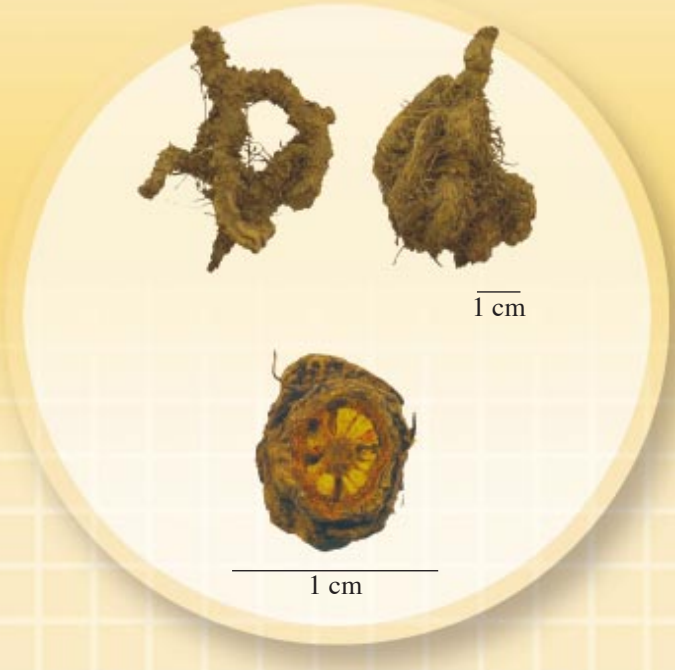


Figure 1(i) A photograph of dried rhizome of *Coptis chinensis* Franch.

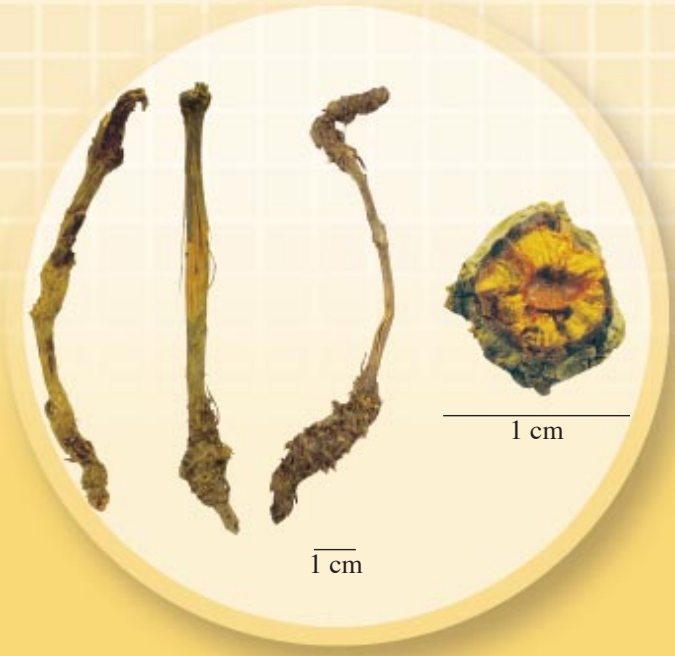


Figure 1(ii) A photograph of dried rhizome of *Coptis deltoidea* C. Y. Cheng et Hsiao

1. NAMES

Official Name: Rhizoma Coptidis

Chinese Name: 黃連

Chinese Phonetic Name: Huanglian

2. SOURCE

Rhizoma Coptidis is the dried rhizome of *Coptis chinensis* Franch. or *Coptis deltoidea* C. Y. Cheng et Hsiao (Ranunculaceae), commonly known as "Wei-lian" or "Ya-lian", respectively. The rhizome is collected in the autumn, the rootlets and soil removed, then dried to obtain Rhizoma Coptidis.

3. DESCRIPTION

***Coptis chinensis* Franch. :** Mostly gathered as clusters of curved rhizomes, resembling "chicken feet"; single rhizome 3-7 cm long, 3-9 mm in diameter. Externally greyish-yellow or yellowish-brown, rough, bearing irregular nodular protrusions, rootlets, and remains of rootlets; some internodes smooth as the aerial stem. The upper part bearing remains of brown scale leaves, the apex often bearing remains of stems or petioles. Texture hard; fracture uneven; bark orange-red or dark brown, wood bright yellow or orange-yellow, radially arranged; pith sometimes hollowed. Odour slight; taste very bitter [Fig. 1(i)].

***Coptis deltoidea* C. Y. Cheng et Hsiao :** Mostly single rhizomes; somewhat cylindrical, slightly curved, 3-9 cm long, 3-10 mm in diameter. Internodes smooth and relatively long. Apex with some remains of stems [Fig. 1(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The cork consisting of several layers of cells; sometimes scale leaf epidermis outside of the cork is observed. Stone cells scattered singly or in groups in the cortex. Phloem fibres occur in bundles

and may be accompanied by a few stone cells. Xylem lignified. Pith consists of parenchyma cells, sometimes with stone cells [Fig. 2(i) and (ii)].

Powder

Colour yellowish-brown to brown. Stone cells yellow or yellowish-brown, subsquare, subrectangular, subround or polygonal in shape, 13-90 μm in diameter; the wall fairly thickened and has pits, pit canals and striations; polychrome can be observed under the polarized microscope. Xylem fibres slightly thick-walled, 8-25 μm in diameter. Phloem fibres occur in bundles, the wall fairly thickened and has cleft or pointed pits. Cork cells yellowish-brown, subsquare, subrectangular or polygonal in shape. Scale leaf epidermal cells greenish-yellow or yellowish-brown, the wall sinuous or with beaded-thickening. Vessels occasionally found [Fig. 3(i) and (ii)].

4.2 Physicochemical Identification

Reagent

Potassium iodobismuthate solution R_2

Dissolve 0.4 g of bismuth subnitrate in 5 mL of acetic acid and 20 mL of water, then add 25 mL of aqueous potassium iodide solution (40%, w/v).

Procedure

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of ethanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000×*g* for 5 min. Transfer 1 mL of the supernatant to a test tube. Add 2 drops of potassium iodobismuthate solution R_2 and allow the precipitate to settle. Reddish-orange precipitate is observed.

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

Standard solutions

Berberine chloride standard solution

Weigh 1.0 mg of berberine chloride CRS (Fig. 4) and dissolve in 2 mL of methanol.

Palmatine chloride standard solution

Weigh 1.0 mg of palmatine chloride CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of toluene, ethyl acetate, methanol, 2-propanol and ammonia solution (12:6:3:3:1, v/v).

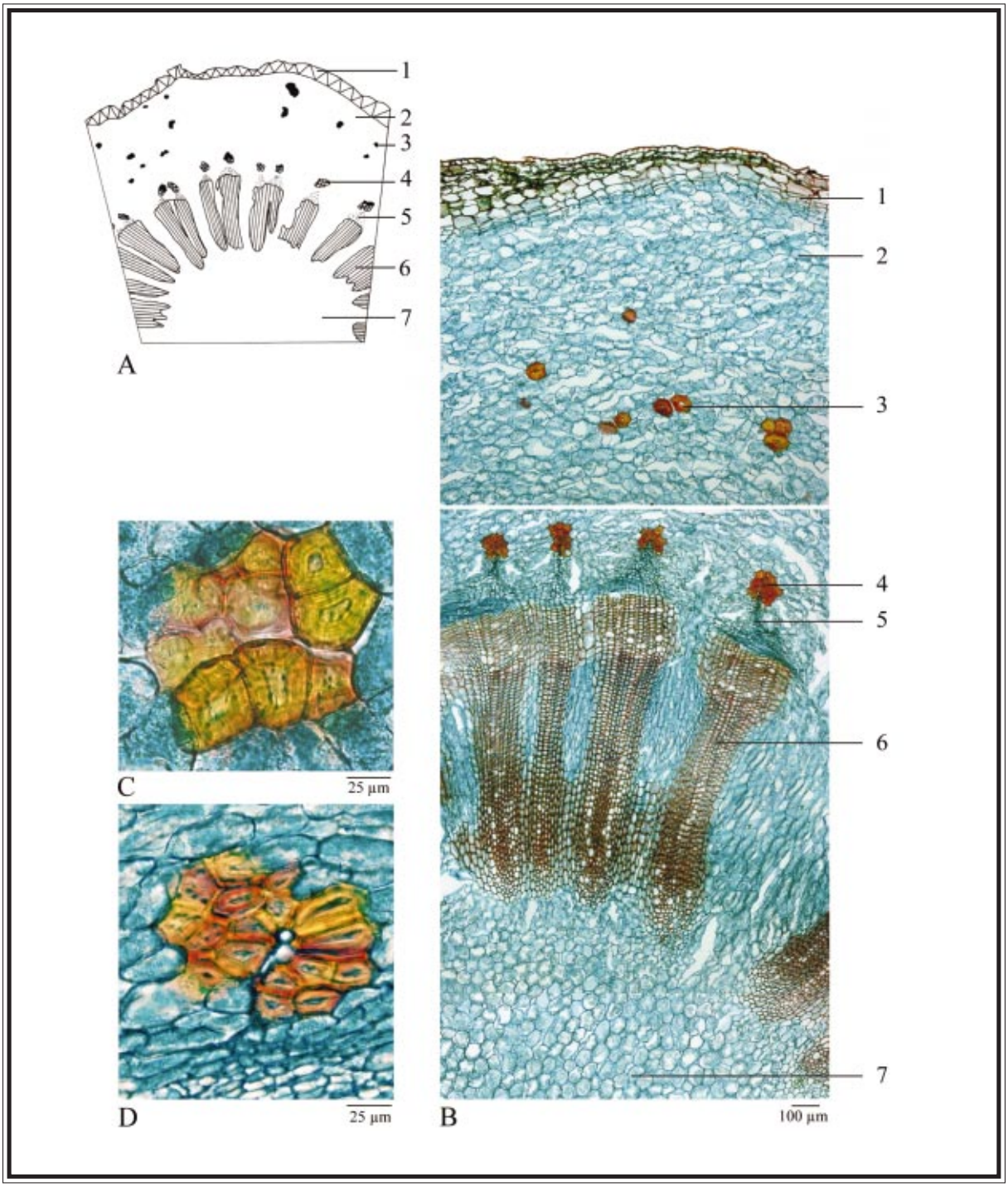


Figure 2(i) Microscopic features of transverse section of dried rhizome of *Coptis chinensis* Franch.

A. Sketch B. Section illustration C. Stone cells D. Phloem fibres

1. Cork 2. Cortex 3. Stone cells 4. Phloem fibres 5. Phloem 6. Xylem 7. Pith

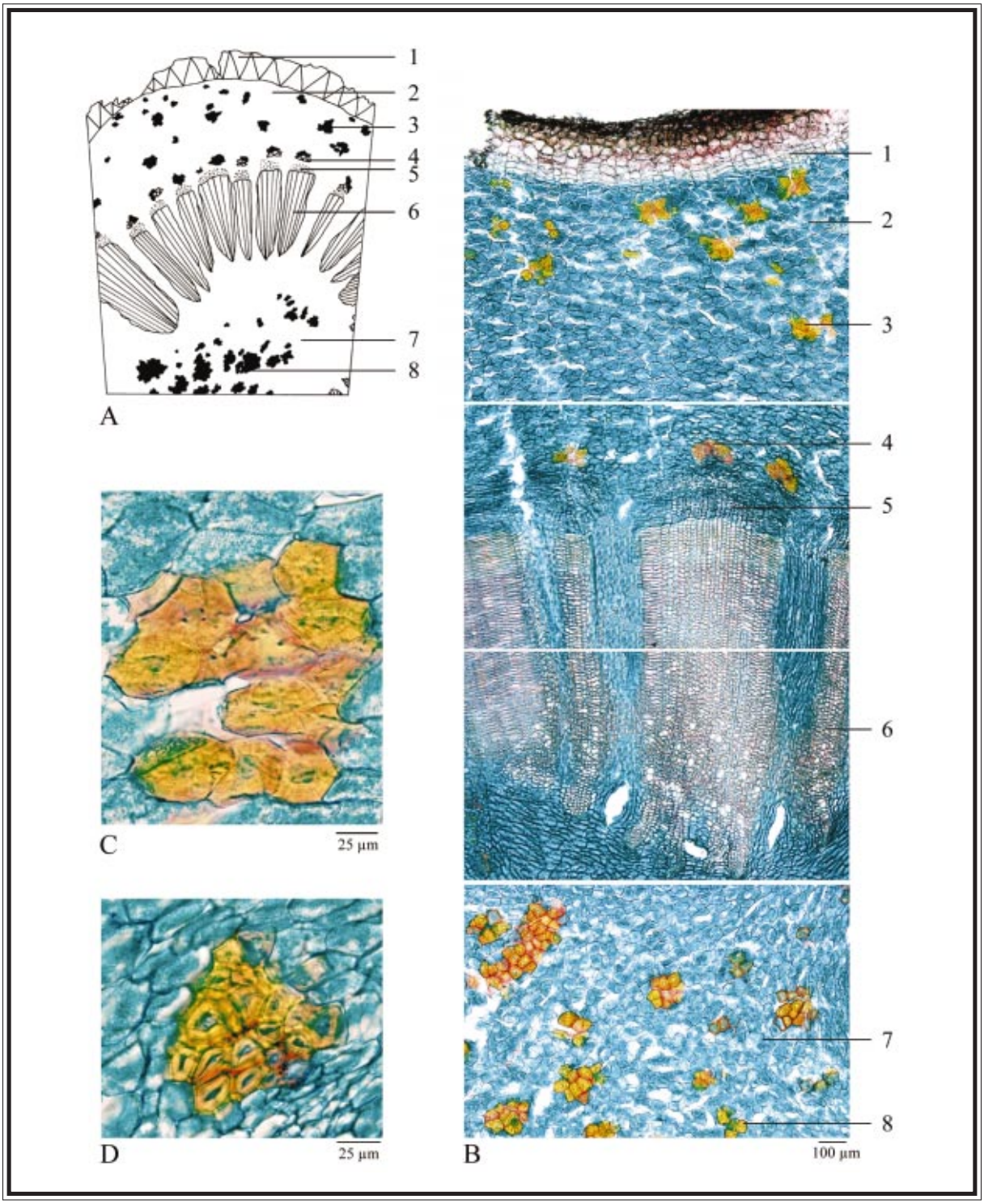


Figure 2(ii) Microscopic features of transverse section of dried rhizome of *Coptis deltoidea* C. Y. Cheng et Hsiao

A. Sketch B. Section illustration C. Stone cells D. Phloem fibres

1. Cork 2. Cortex 3. Stone cells 4. Phloem fibres 5. Phloem 6. Xylem 7. Pith 8. Stone cells in pith

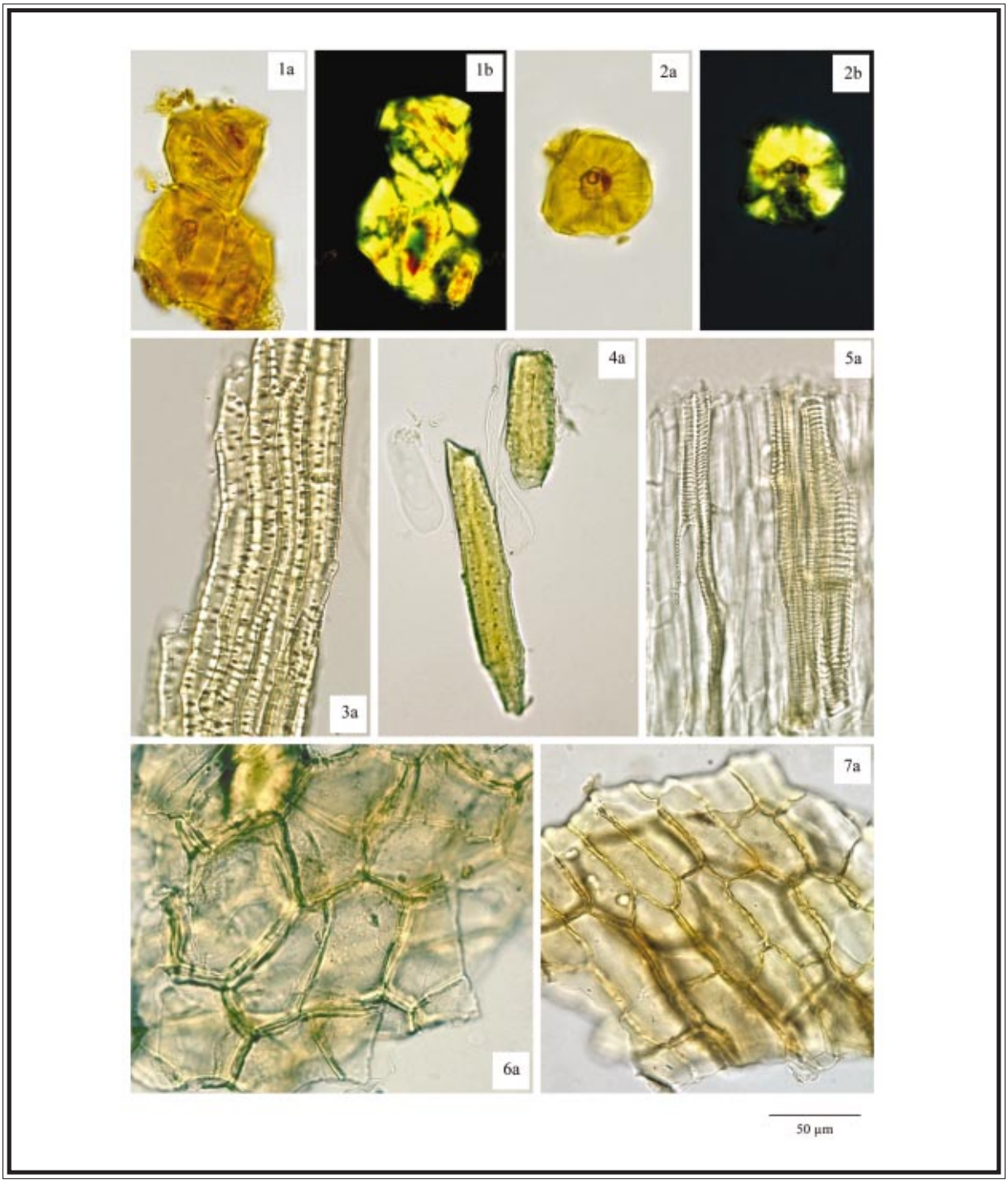


Figure 3(i) Microscopic features of powder of dried rhizome of *Coptis chinensis* Franch.

1. Stone cells in a group 2. Stone cell 3. Xylem fibres 4. Phloem fibres 5. Vessels 6. Cork cells
7. Scale leaf epidermal cells

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

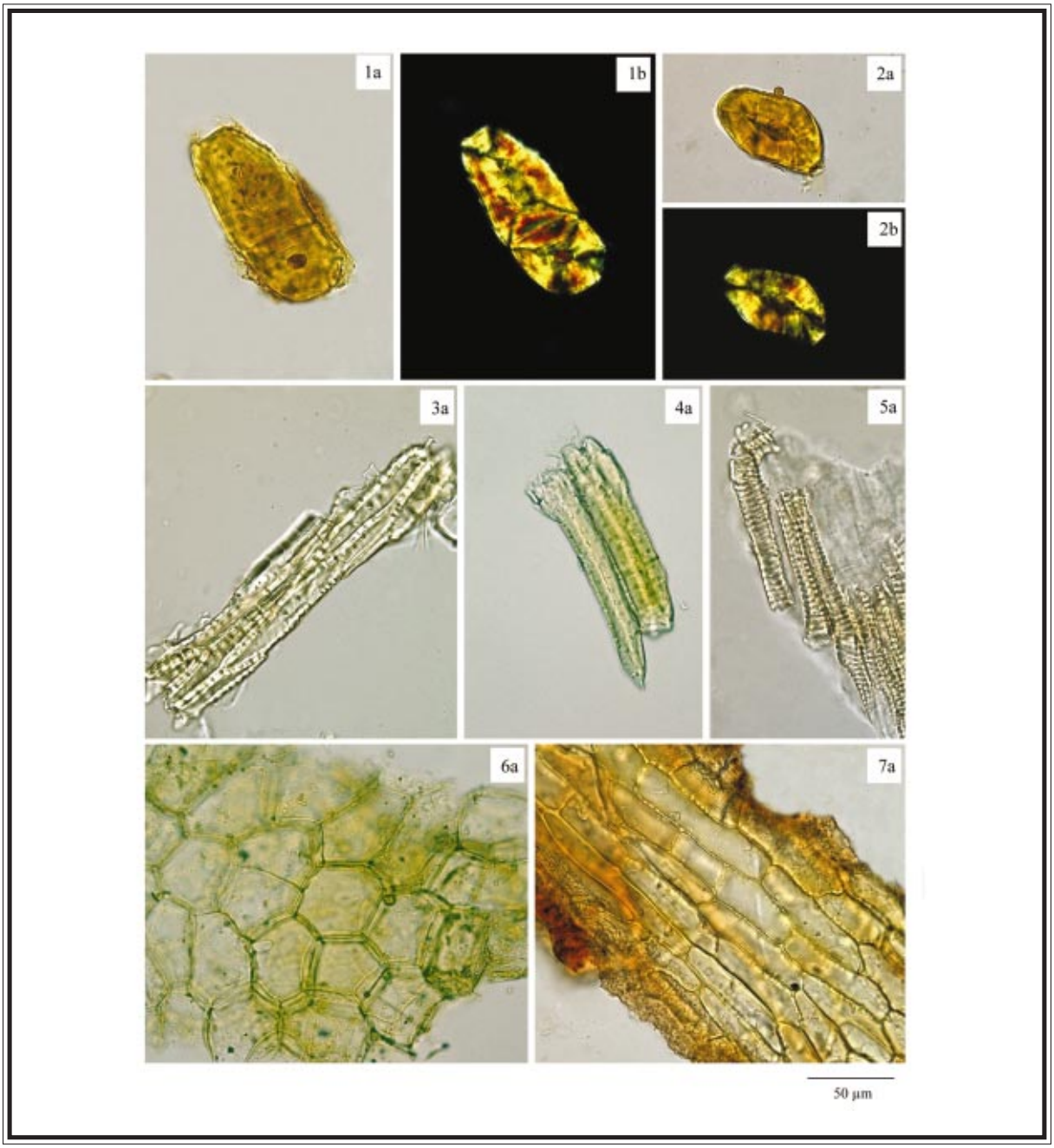


Figure 3(ii) Microscopic features of powder of dried rhizome of *Coptis deltoidea* C. Y. Cheng et Hsiao

1. Stone cells in a group 2. Stone cell 3. Xylem fibres 4. Phloem fibres 5. Vessels 6. Cork cells 7. Scale leaf epidermal cells

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

Test solution

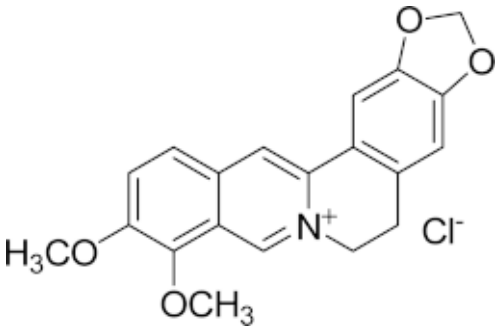
Weigh 0.5 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 4 mL of methanol.

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. Before the development, add the developing solvent to one of the troughs of the chamber and add the same volume of ammonia solution to the other trough. Cover the chamber with a lid and leave for 15 min for equilibrium. Apply separately berberine chloride standard solution, palmatine chloride standard solution and the test solution (1 µL each) to the plate. Place the plate into the trough of the chamber containing the developing solvent. 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 (365 nm). Calculate the R_f values by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of berberine chloride and palmatine chloride.

(i)



(ii)

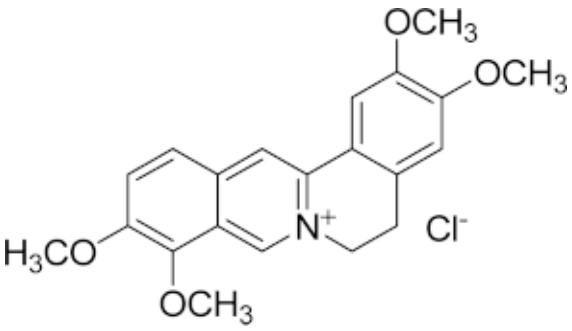


Figure 4 Chemical structures of (i) berberine chloride and (ii) palmatine chloride

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

Standard solution

Berberine chloride standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of berberine chloride CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (346 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 –

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 48	100 → 50	0 → 50	linear gradient
48 – 55	50 → 0	50 → 100	linear gradient
55 – 60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 5 μL of berberine chloride Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of berberine chloride should not be more than 5.0%; the RSD of the retention time of berberine chloride peak should not be more than 2.0%; the column efficiency determined from berberine chloride peak should not be less than 20000 theoretical plates.

The *R* value between peak 3 and peak 4 in the chromatogram of the test solution should not be less than 1.5 [Fig. 5(i) or (ii)].

Procedure

Separately inject berberine chloride Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of berberine chloride peak in the chromatogram of berberine chloride Std-FP and the retention times of the seven characteristic peaks [Fig. 5(i) or (ii)] in the chromatogram of the test solution. Under the same HPLC conditions,

identify berberine chloride peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of berberine chloride Std-FP. The retention times of berberine chloride 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 seven characteristic peaks of Rhizoma Coptidis extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the seven characteristic peaks of Rhizoma Coptidis extract

Peak No.	RRT	Acceptable Range
1	0.81	±0.03
2	0.88	±0.03
3 (jatrorrhizine chloride)	0.89	±0.03
4	0.90	±0.03
5 (coptisine chloride)	0.92	±0.03
6 (palmatine chloride)	0.98	±0.03
7 (marker, berberine chloride)	1.00	-

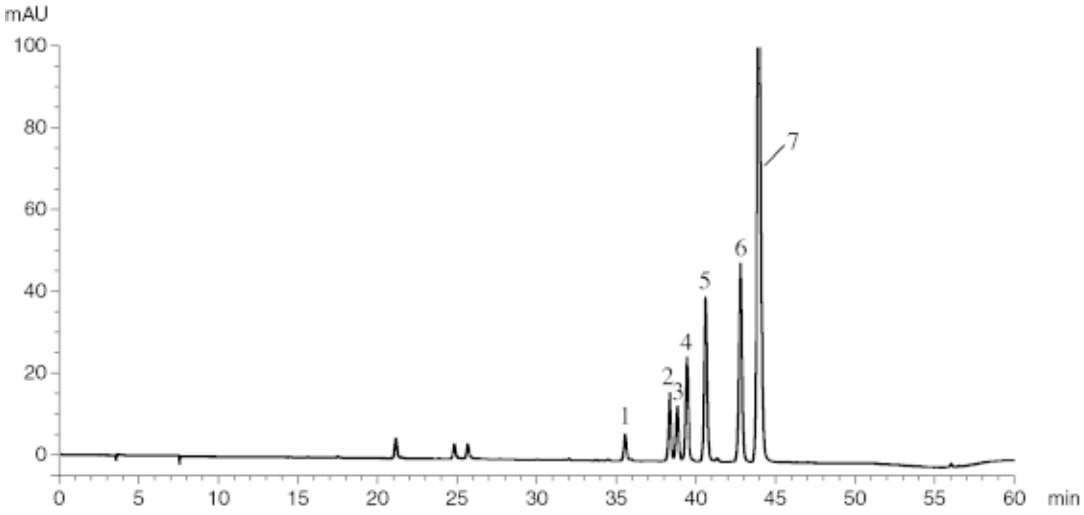


Figure 5(i) A reference fingerprint chromatogram of dried rhizome of *Coptis chinensis* Franch. extract

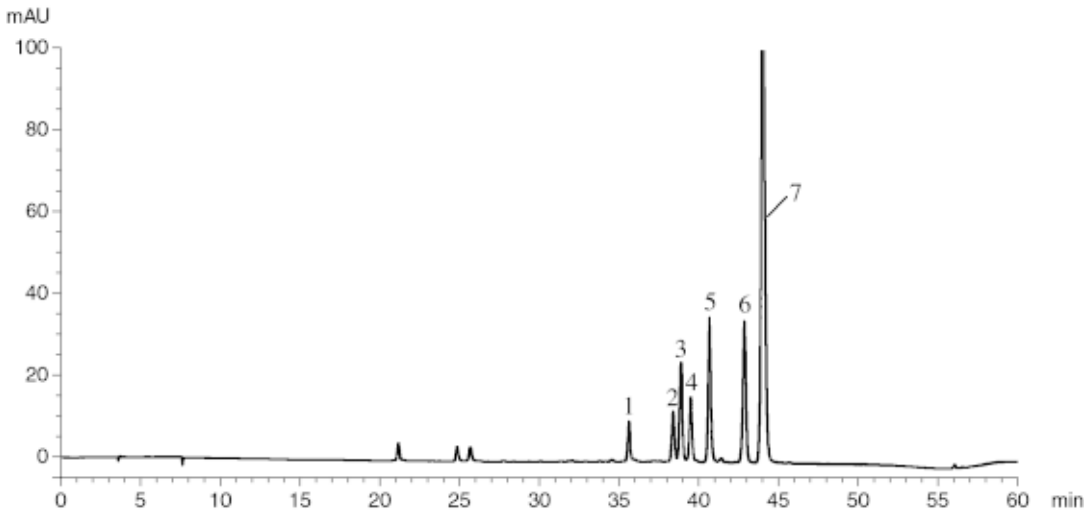


Figure 5(ii) A reference fingerprint chromatogram of dried rhizome of *Coptis deltoidea* C. Y. Cheng et Hsiao extract

For positive identification, the sample must give the above seven characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram [Fig. 5(i) or (ii)].

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 5.0%.
Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 17.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution

Mixed berberine chloride and palmatine chloride standard stock solution, Std-Stock (1000 mg/L each)
Weigh accurately 10.0 mg of berberine chloride CRS and 10.0 mg of palmatine chloride CRS and dissolve in 10 mL of methanol.
Mixed berberine chloride and palmatine chloride standard solution for assay, Std-AS
Measure accurately the volume of the mixed berberine chloride and palmatine chloride Std-Stock, dilute with methanol to produce a series of solutions of 10, 50, 100, 200, 400 mg/L for both berberine chloride and palmatine chloride.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter. Repeat the extraction twice. Combine the filtrate. Transfer the solution to a 50-mL volumetric flask 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 detector (346 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	90 → 10	10 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections each with 5 μL of the mixed berberine chloride and palmatine

chloride Std-AS (50 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of berberine chloride and palmatine chloride should not be more than 5.0%; the RSD of the retention times of berberine chloride peak and palmatine chloride peak should not be more than 2.0%; the column efficiencies determined from berberine chloride peak and palmatine chloride peak should not be less than 80000 theoretical plates.

The *R* value between berberine chloride peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of mixed berberine chloride and palmatine chloride Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of berberine chloride and palmatine chloride against the corresponding concentrations of mixed berberine chloride and palmatine chloride Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify berberine chloride peak and palmatine chloride peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed berberine chloride and palmatine chloride Std-AS. The retention times of berberine chloride peaks and palmatine chloride peaks from the two chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of berberine chloride and palmatine chloride in the test solution, and calculate the percentage contents of berberine chloride and palmatine chloride in the sample by using the equations indicated in Appendix IV(B).

Limits

The dried rhizome of *Coptis chinensis* Franch. contains not less than 4.1% of berberine [calculated as berberine chloride ($C_{20}H_{18}NO_4Cl$)], and not less than 0.30% of palmatine [calculated as palmatine chloride ($C_{21}H_{22}NO_4Cl$)], calculated with reference to the dried substance.

The dried rhizome of *Coptis deltoidea* C. Y. Cheng et Hsiao contains not less than 2.3% of berberine [calculated as berberine chloride ($C_{20}H_{18}NO_4Cl$)], and not less than 0.30% of palmatine [calculated as palmatine chloride ($C_{21}H_{22}NO_4Cl$)], calculated with reference to the dried substance.