Cistanches Herba

Figure 1 (i) A photograph of dried fleshy stem, with scales, of *Cistanche deserticola* Y. C. Ma

Figure 1 (ii) A photograph of dried fleshy stem, with scales, of *Cistanche tubulosa* (Schrenk) Wight

1. NAMES

Official Name: Cistanches Herba

Chinese Name: 肉蓯蓉

Chinese Phonetic Name: Roucongrong

2. SOURCE

Cistanches Herba is the dried fleshy stem, with scales, of *Cistanche deserticola* Y. C. Ma or *Cistanche tubulosa* (Schrenk) Wight (Orobanchaceae). The herb is collected in spring before or at the time the stem emerges from the ground; the inflorescences and foreign matter removed, then cut into sections and dried under the sun to obtain Cistanches Herba.

3. DESCRIPTION

***Cistanche deserticola* Y. C. Ma:** Flattened-cylindrical, slightly curved, 3-33 cm long, 20-100 mm in diameter. Externally brown or greyish-brown, densely covered with imbricated scales, the apex of scales usually broken. Texture heavy, hard and slightly pliable, uneasily broken; fracture brown, dotted by pale brownish vascular bundles, arranged in sinuous rings. Odour slight; taste sweet and slightly bitter [Fig. 1 (i)].

***Cistanche tubulosa* (Schrenk) Wight:** Subfusiform, flattened-fusiform and flattened-cylindrical, 4-26 cm long, 15-90 mm in diameter. Externally dark brown. Fracture granular, greyish-brown, dotted with scattered vascular bundles [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem and scale leaf of *Cistanche deserticola* Y. C. Ma: Stem epidermis consists of 1 layer of cells. Cortex consists of several layers of parenchymatous cells and scattered with leaf trace vascular bundles. Vascular bundles collateral, arranged in sinuous interrupted rings, and obliterated into a sharp, spine-like tail at the distal end of phloem. Pith is distinct. A scale leaf can be seen in the transverse section; it consists of upper epidermis, parenchyma, 3-7 vascular bundles, and lower epidermis [Fig. 2 (i)].

Stem of *Cistanche tubulosa* (Schrenk) Wight: Epidermis consists of 1 layer of cells, but sometimes fallen off. Metaderm consists of several layers of suberized cells. The cortex is narrow. Collateral vascular bundles ovate, arranged irregularly, with 3-6 bundles grouped in a ring formation, with phloem on the inner and xylem on the outer side. Pith is indistinct [Fig. 2(ii)].

Powder

***Cistanche deserticola* Y. C. Ma:** Colour dark brown. Starch granules simple, subglobular, rectangular-ovoid to ellipsoid, 16-54 µm in diameter, hilum dotted or V-shaped, striations distinct; black and cruciate in shape under the polarized microscope. Reticulate and spiral vessels mainly in group; reticulate vessels 25-65 µm in diameter, spiral vessels 11-32 µm in diameter. Epidermal cells pale yellow, sub-rectangular in shape, wall thickened, pit canals oblique and clear [Fig. 3 (i)].

***Cistanche tubulosa* (Schrenk) Wight:** Colour dark brown. Starch granules simple or compound, compound granules composed of 2 units, subglobular, ovoid to ellipsoid, 5-50 µm in diameter, hilum V-shaped, cleft-like or star-shaped, striations indistinct; black and cruciate in shape under the polarized microscope. Reticulate vessels 21-55 µm in diameter; spiral vessels 11-22 µm in diameter. Epidermal cells pale yellow, polygonal in shape [Fig. 3 (ii)].

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

Standard solutions

Acteoside standard solution

Weigh 1.0 mg of acteoside CRS (Fig. 4) and dissolve in 5 mL of ethanol.

Echinacoside standard solution

Weigh 1.0 mg of echinacoside CRS (Fig. 4) and dissolve in 2 mL of ethanol.

Developing solvent system

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

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of ethanol.

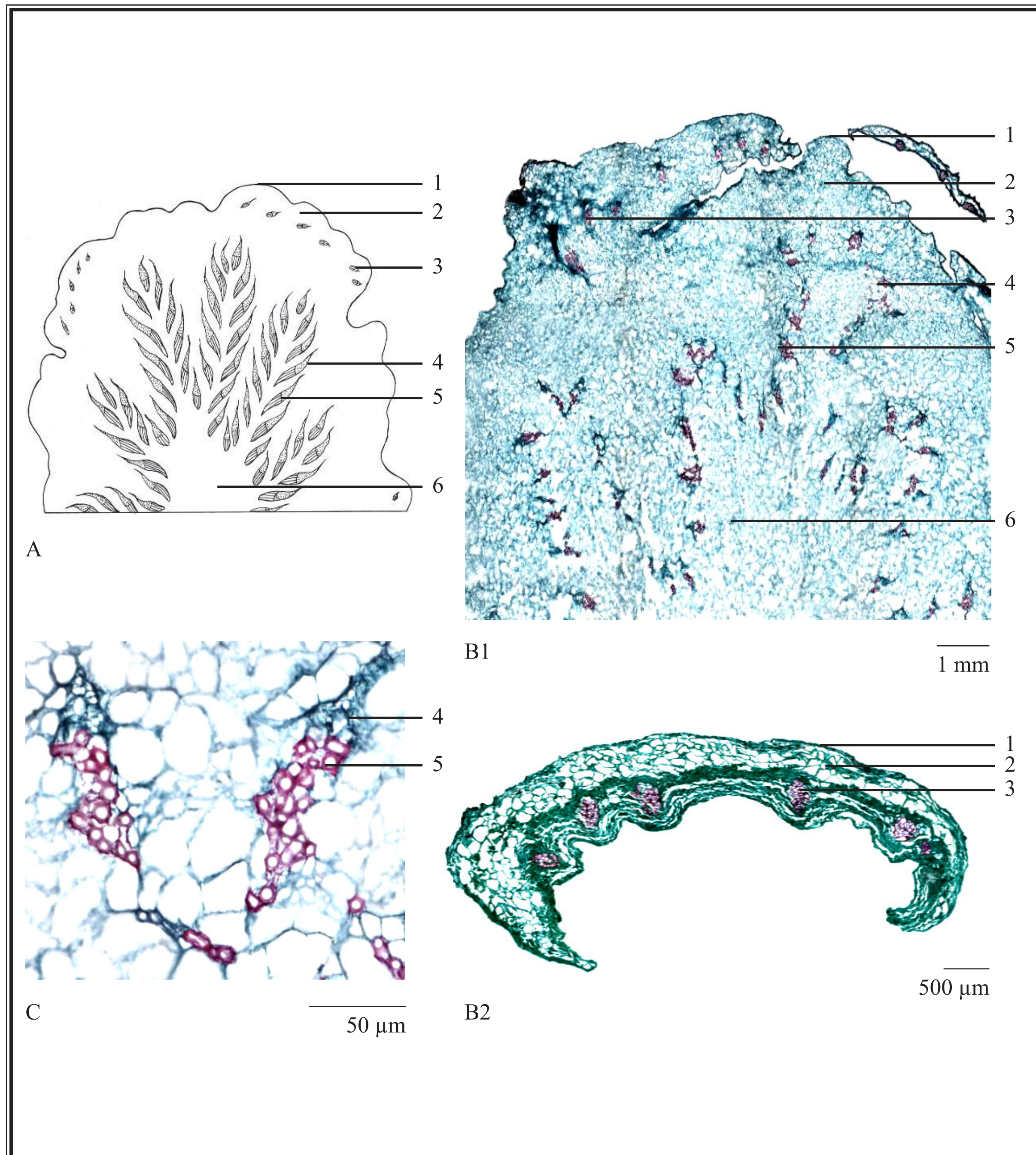

Figure 2 (i) Microscopic features of transverse section of dried fleshy stem, with scales, of *Cistanche deserticola* Y. C. Ma

A. Sketch of stem B1. Section illustration of stem B2. Section illustration of scale leaf
C. Stem cross section showing vascular bundles

1. Epidermis 2. Cortex 3. Leaf trace vascular bundle 4. Phloem 5. Xylem 6. Pith

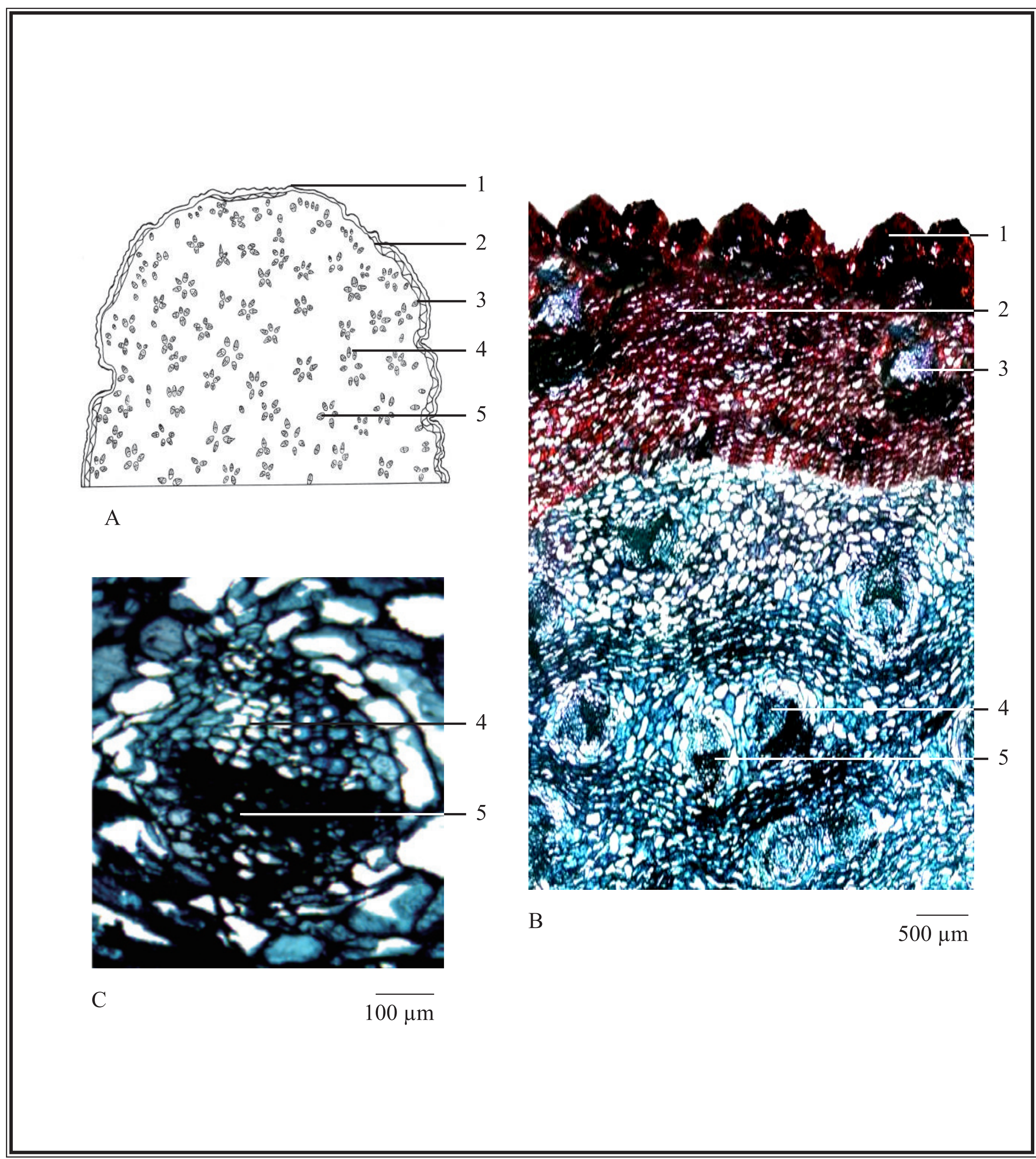

Figure 2 (ii) Microscopic features of transverse section of dried fleshy stem of *Cistanche tubulosa* (Schrenk) Wight

A. Sketch B. Section illustration C. Vascular bundles

1. Epidermis 2. Metaderm 3. Leaf trace vascular bundle 4. Xylem 5. Phloem

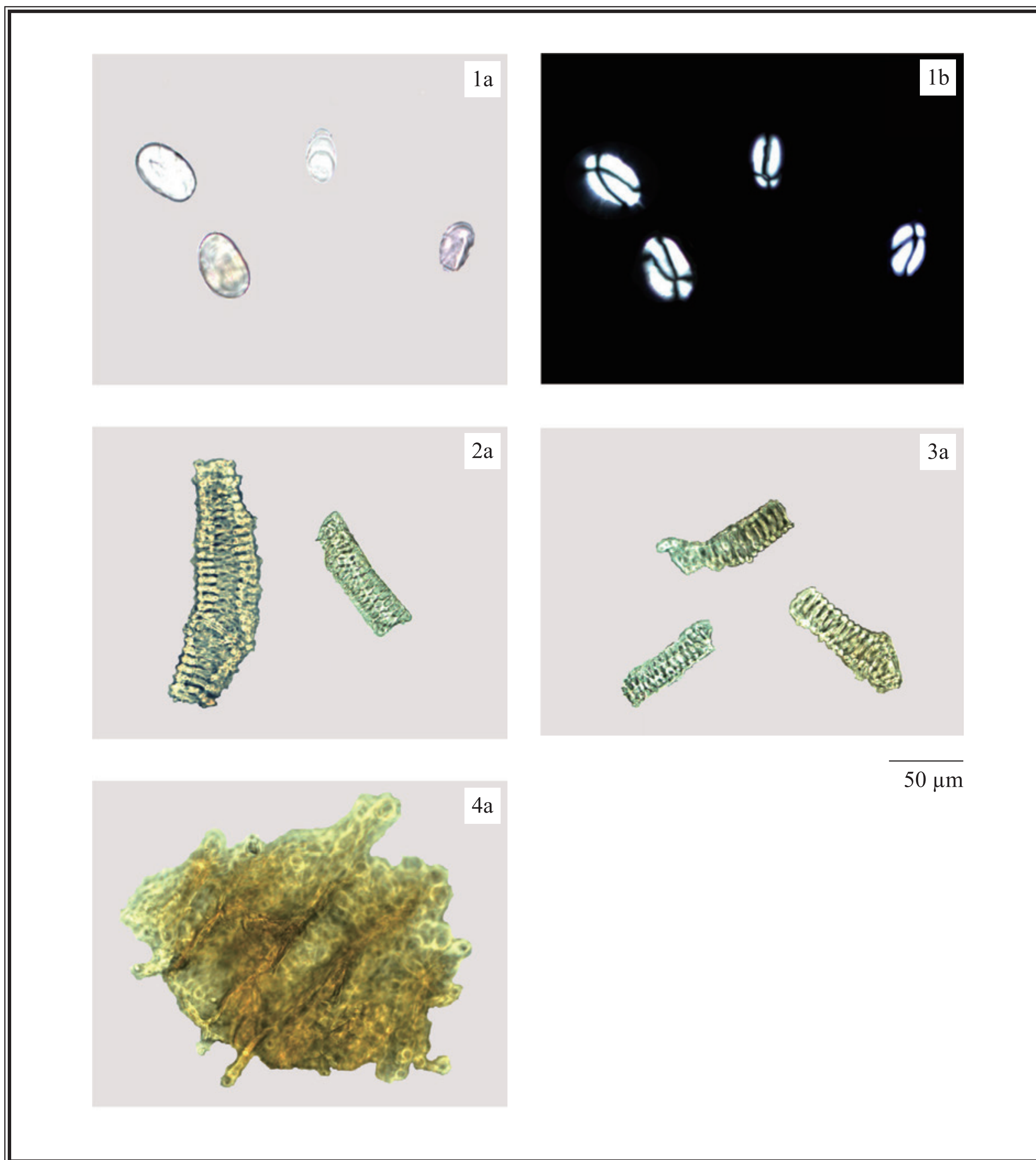

Figure 3 (i) Microscopic features of powder of dried fleshy stem, with scales, of *Cistanche deserticola* Y. C. Ma

1. Starch granules 2. Reticulate vessels 3. Spiral vessels 4. Epidermal cells

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

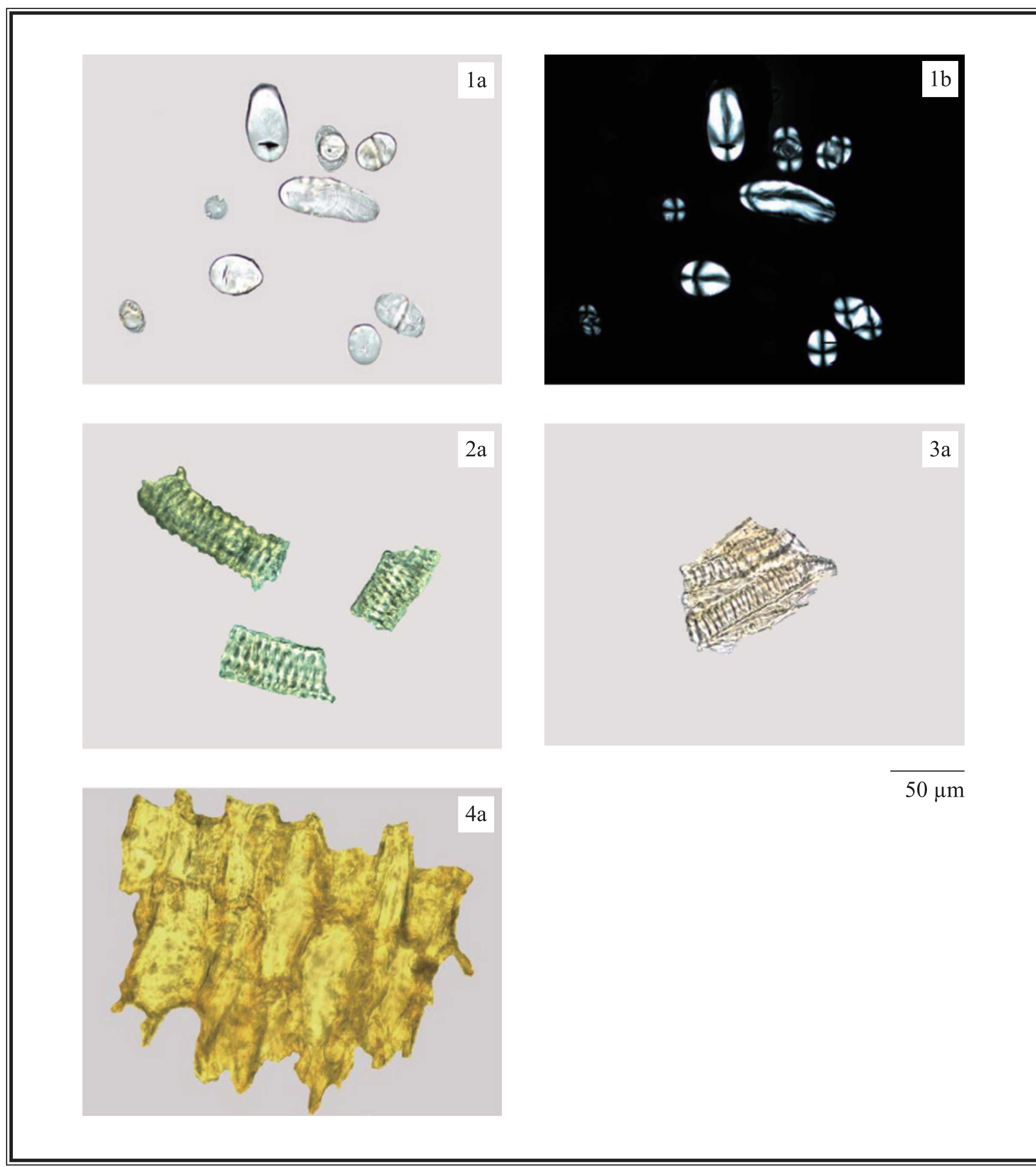

Figure 3 (ii) Microscopic features of powder of dried fleshy stem, with scales, of *Cistanche tubulosa* (Schrenk) Wight

1. Starch granules 2. Reticulate vessels 3. Spiral vessels 4. Epidermal cells

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

Procedure

Carry out the method by using a TLC polyamide plate and a freshly prepared developing solvent system as described above. Apply separately acteoside standard solution (1 μL), echinacoside standard solution (1 μL) and the test solution (2 μL) to the plate. 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. Examine the plate under UV light (366 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 acteoside and echinacoside.

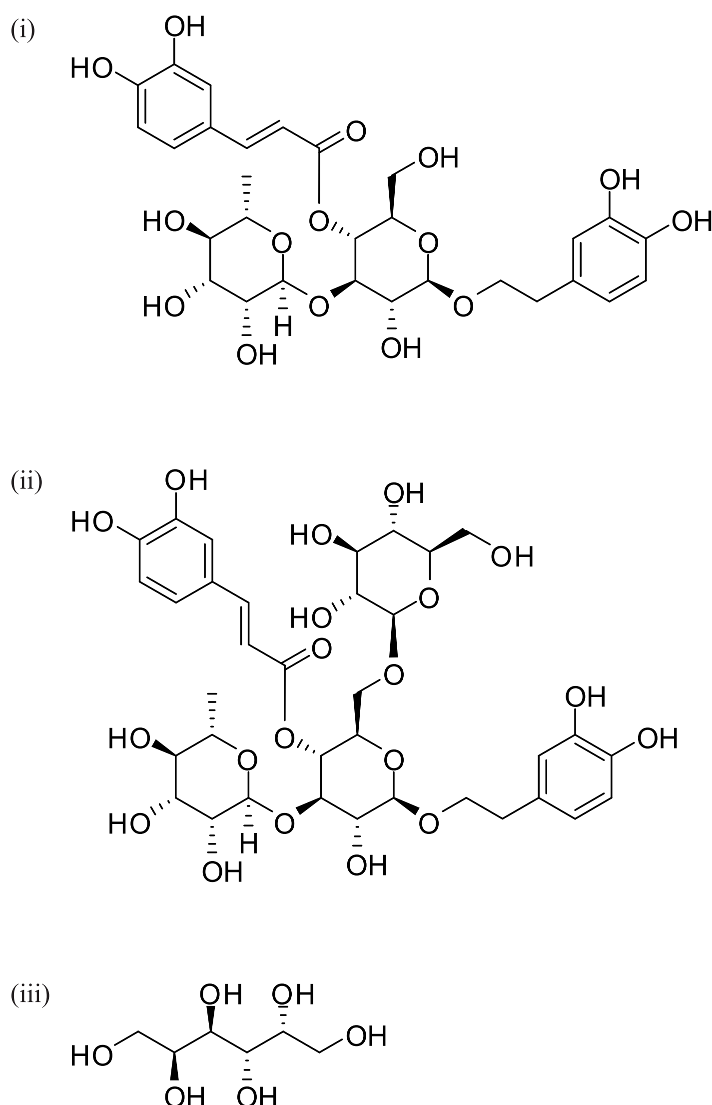

Figure 4 Chemical structures of (i) acteoside (ii) echinacoside and (iii) galactitol

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

Standard solutions

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

Weigh 2.5 mg of acteoside CRS and dissolve in 25 mL of methanol (90%).

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

Weigh 2.5 mg of echinacoside CRS and dissolve in 25 mL of methanol (90%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (90%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (90%). Transfer the solution to a 25-mL volumetric flask and make up to the mark with methanol (90%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	86	14	isocratic
20 – 21	86 → 83	14 → 17	linear gradient
21 – 30	83	17	isocratic
30 – 31	83 → 80	17 → 20	linear gradient
31 – 60	80	20	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of acteoside Std-FP and echinacoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of acteoside and echinacoside should not be more than 5.0%; the RSD of the retention times of acteoside and echinacoside peaks should not be more than 2.0%; the column efficiencies determined from acteoside and echinacoside peaks should not be less than 25000 and 7000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 [Fig. 5 (i) or (ii)].

Procedure

Separately inject acteoside Std-FP, echinacoside Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of acteoside and echinacoside peaks in the chromatograms of acteoside Std-FP, echinacoside Std-FP and the retention times of the three characteristic peaks [Fig. 5 (i) or (ii)] in the chromatogram of the test solution. Identify acteoside and echinacoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of acteoside Std-FP and echinacoside Std-FP. The retention times of acteoside and echinacoside peaks in the chromatograms of the test solution and the corresponding Std-FP 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 Cistanches Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (echinacoside)	0.45	± 0.03
2 (marker, acteoside)	1.00	-
3	1.45	± 0.03

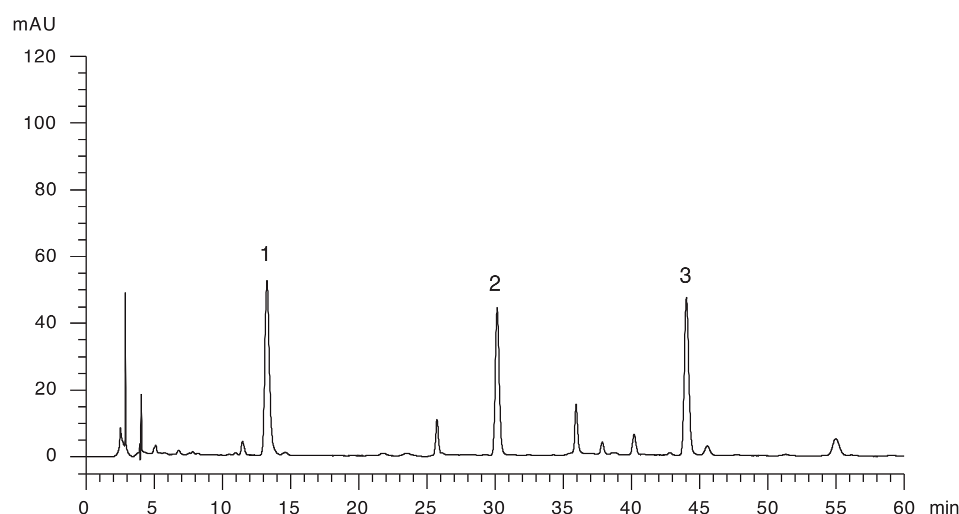

Figure 5 (i) A reference fingerprint chromatogram of dried fleshy stem, with scales, of *Cistanche deserticola* Y.C. Ma extract

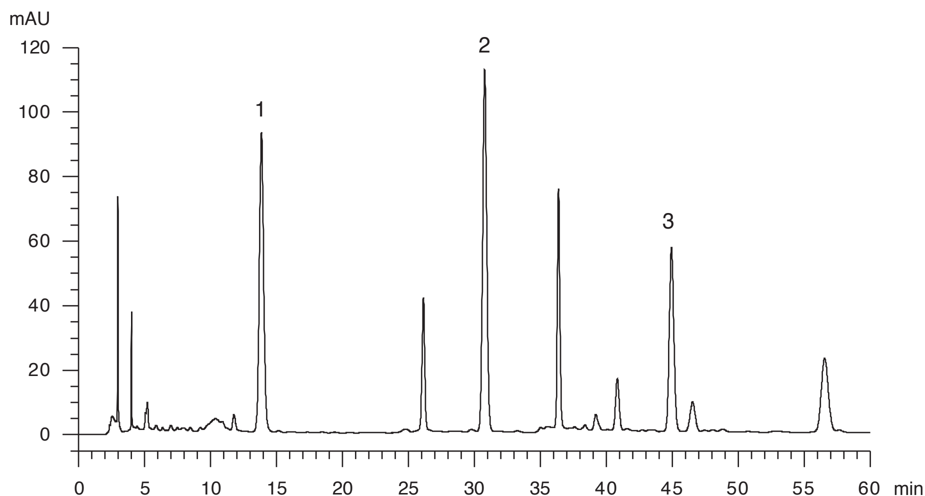

Figure 5 (ii) A reference fingerprint chromatogram of dried fleshy stem, with scales, of *Cistanche tubulosa* (Schrenk) Wight extract

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 respective reference fingerprint chromatograms [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 XVIII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%

6. EXTRACTIVES *(Appendix XI)*

Dried fleshy stem, with scales, of *Cistanche deserticola* Y.C. Ma
Water-soluble extractives (cold extraction method): not less than 28.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

Dried fleshy stem, with scales, of *Cistanche tubulosa* (Schrenk) Wight
Water-soluble extractives (cold extraction method): not less than 18.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

7.1 Assay of the total content of acteoside and echinacoside

Standard solution

Mixed acteoside and echinacoside standard stock solution, Std-Stock (400 mg/L each)

Weigh accurately 20.0 mg of acteoside CRS and 20.0 mg of echinacoside CRS, and dissolve in 50 mL of methanol (90%).

Mixed acteoside and echinacoside standard solution for assay, Std-AS

Measure accurately the volume of the mixed acteoside and echinacoside Std-Stock, dilute with methanol (90%) to produce a series of solutions of 6, 30, 100, 200, 400 mg/L for both acteoside and echinacoside.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (90%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (90%). Transfer the solution to a 25-mL volumetric flask and make up to the mark with methanol (90%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 16	86	14	isocratic
16 – 17	86 → 80	14 → 20	linear gradient
17 – 30	80	20	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed acteoside and echinacoside Std-AS (100 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of acteoside and echinacoside should not be more than 5.0%; the RSD of the retention times of acteoside and echinacoside peaks should not be more than 2.0%; the column efficiencies determined from acteoside and echinacoside peaks should not be less than 80000 and 7500 theoretical plates respectively.

The *R* value between acteoside peak and the closest peak; and the *R* value between echinacoside 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 the mixed acteoside and echinacoside Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of acteoside and echinacoside against the corresponding concentrations of the mixed acteoside and echinacoside Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify acteoside and echinacoside peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed acteoside and echinacoside Std-AS. The retention times of acteoside and echinacoside peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of acteoside and echinacoside in the test solution, and calculate the percentage contents of acteoside and echinacoside in the sample by using the equations indicated in Appendix IV(B).

Limits

The dried fleshy stem, with scales, of *Cistanche deserticola* Y.C. Ma contains not less than 0.30% of the total content of acteoside ($C_{29}H_{36}O_{15}$) and echinacoside ($C_{35}H_{46}O_{20}$), calculated with reference to the dried substance.

The dried fleshy stem, with scales, of *Cistanche tubulosa* (Schrenk) Wight contains not less than 1.5% of the total content of acteoside ($C_{29}H_{36}O_{15}$) and echinacoside ($C_{35}H_{46}O_{20}$), calculated with reference to the dried substance.

7.2 Assay of galactitol

Standard solution

Galactitol standard stock solution, Std-Stock (1120 mg/L)

Weigh accurately 5.6 mg of galactitol CRS (Fig. 4) and dissolve in 5 mL of methanol (50%).

Galactitol standard solution for assay, Std-AS

Measure accurately the volume of the galactitol Std-Stock, dilute with methanol (50%) to produce a series of solutions of 112, 224, 448, 672, 1120 mg/L for galactitol.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 12 mL of methanol (50%). Sonicate (240 W) the mixture for 1 h. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 80°C; nebulizer gas (N_2) flow: 3.0 L/min] and a column (4.6 × 250 mm) packed with polymer bead (5 µm particle size, pore size 300Å). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of acetonitrile and water (80:20, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of galactitol Std-AS (448 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of galactitol should not be more than 5.0%; the RSD of the retention time of galactitol peak should not be more than 2.0%; the column efficiency determined from galactitol peak should not be less than 10000 theoretical plates.

The *R* value between galactitol 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 galactitol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of galactitol against the natural logarithm of the corresponding concentrations of galactitol 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 galactitol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of galactitol Std-AS. The retention times of galactitol peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of galactitol in the test solution by using the following equation –

$$\text{Concentration of galactitol in the test solution} = e^{[\mathrm{Ln(A)}-I]/m}$$

Where A = the peak area of galactitol in the test solution,

I = the y-intercept of the 5-point calibration curve of galactitol,

m = the slope of the 5-point calibration curve of galactitol.

Calculate the percentage content of galactitol in the sample by using the equations indicated in Appendix IV(B).

Limits

The dried fleshy stem, with scales, of *Cistanche deserticola* Y.C. Ma contains not less than 4.6% of galactitol ($C_6H_{14}O_6$), calculated with reference to the dried substance.

The dried fleshy stem, with scales, of *Cistanche tubulosa* (Schrenk) Wight contains not less than 4.2% of galactitol ($C_6H_{14}O_6$), calculated with reference to the dried substance.