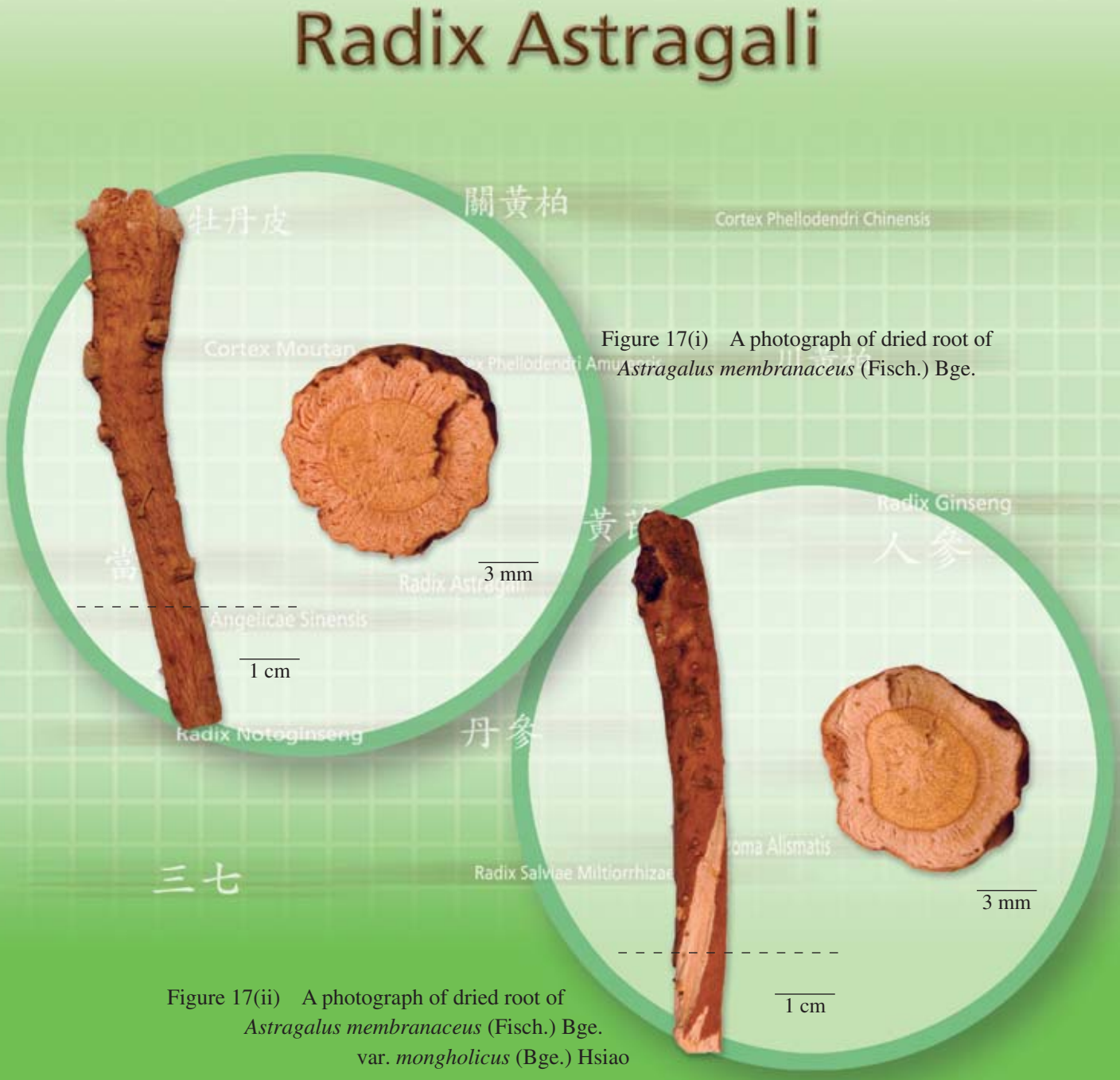

Figure 17(i) A photograph of dried root of *Astragalus membranaceus* (Fisch.) Bge.

Figure 17(ii) A photograph of dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao

1. NAMES

Official Name: Radix Astragali

Chinese Name: 黃芪

Chinese Phonetic Name: Huangqi

2. SOURCE

Radix Astragali is the dried root of *Astragalus membranaceus* (Fisch.) Bge. or *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (Fam. Leguminosae). The root is collected in spring and autumn, the rootlets and root stocks removed, then dried in the sun to obtain Radix Astragali.

3. DESCRIPTION

Cylindrical, some branched, usually upper part relatively thick, 5–35 mm in diameter. Externally pale brownish-yellow or dark brown, with irregular, longitudinal wrinkles or furrows. Texture hard and tenacious, uneasily broken, fracture highly fibrous and slightly starchy. Bark yellowish-white, wood pale-yellow, with radiate striations and fissures. The central part of older roots occasionally rotten-wood-shaped, blackish-brown or hollowed. Odour, slight; taste, slightly sweet. (Fig. 17)

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The transverse section shows cork consisting of several rows of cells. Phelloderm consisting of 3–5 rows of collenchymatous cells. Outer part of phloem rays often curved and fissured. Fibres in bundles, walls thickened and lignified or slightly lignified. Stone cells sometimes visible near phelloderm. Cambium in a ring. Xylem vessels scattered singly or 2–3 aggregated in groups (occasionally, 4–8 in groups), 8–231 μm in diameter. Xylem fibres found among vessels. (Fig. 18)

Powder

Yellowish-white to greyish-brown. The surface view of cork cells irregular or polygonal, sometimes with sinuous anticlinal walls. Fibers in bundles or scattered, 5–36 μm in diameter, thick-walled, with longitudinal fissures on the surface, both ends often broken, tassel-like, or slightly truncated; showing a polychrome when examined under a polarizing microscope. Bordered-pitted vessels colourless or yellowish, bordered pits arranged closely, 8–231 μm in diameter. Simple starch granules spheroidal or ellipsoid, 2–21 μm in diameter; compound granules of 2–4 units; showing a black, cross-shape when examined under a polarizing microscope. (Fig. 19)

4.2 Physicochemical Identification

Procedure

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 10 mL of dichloromethane. Sonicate the mixture for 30 min. Filter and transfer 0.5 mL of the filtrate to a test tube. Cautiously add about 0.5 mL of sulphuric acid along the inner wall of the tube. Allow to stand for about 20 min. A reddish-brown or yellowish-brown layer is observed in the interface of the two solvent layers.

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

Standard solutions

Astragaloside II standard solution

Weigh 1.0 mg of astragaloside II and dissolve in 1 mL of methanol.

Astragaloside IV standard solution

Weigh 1.0 mg of astragaloside IV and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of chloroform, ethyl acetate, methanol and water (20:40:22:10, v/v). Use the lower phase.

Spray reagent

Mix 1 mL of dilute sulphuric acid (50%, v/v) and 10 mL of *p*-hydroxybenzaldehyde in methanol (2%, w/v). Freshly prepare the reagent.

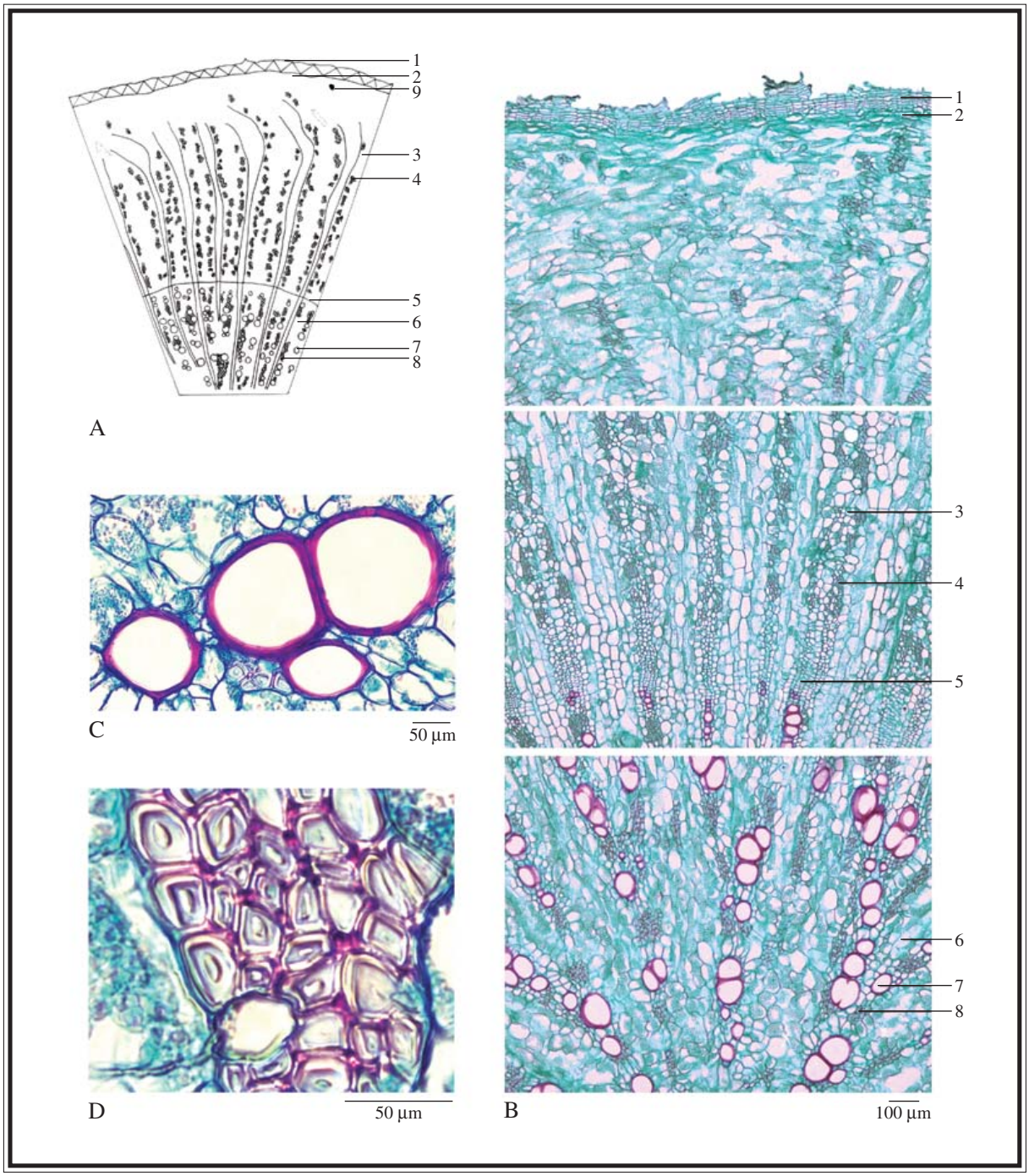

Figure 18(i) Microscopic features of transverse section of dried root of *Astragalus membranaceus* (Fisch.) Bge.

A. Sketch B. Section illustration C. Vessels D. Xylem fibres

1. Cork 2. Phelloderm 3. Phloem 4. Phloem fibres 5. Cambiums 6. Xylem 7. Vessels 8. Xylem fibres 9. Stone cells

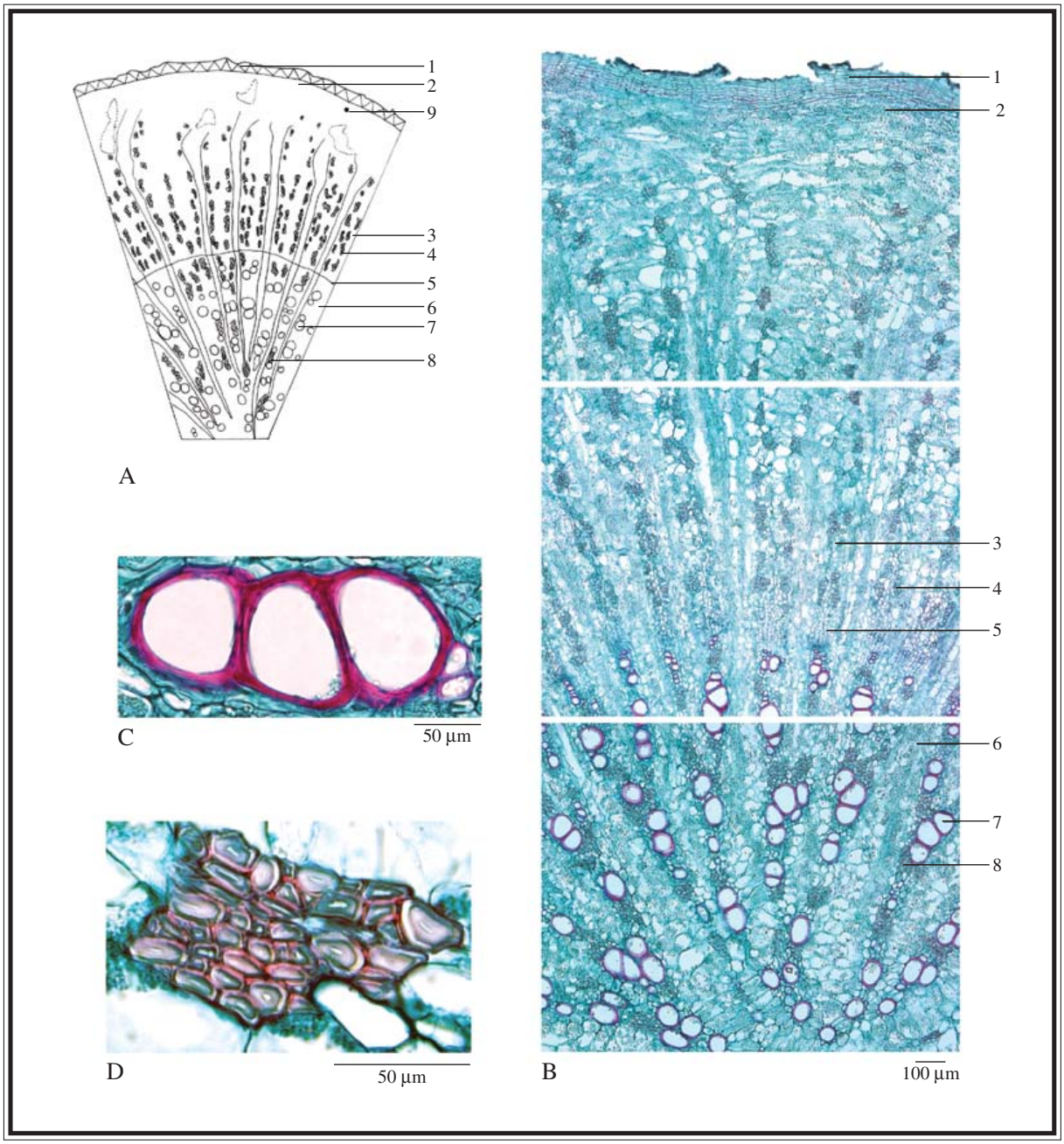

Figure 18(ii) Microscopic features of transverse section of dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao

A. Sketch B. Section illustration C. Vessels D. Xylem fibres

1. Cork 2. Phelloderm 3. Phloem 4. Phloem fibres 5. Cambiums 6. Xylem 7. Vessels 8. Xylem fibres 9. Stone cells

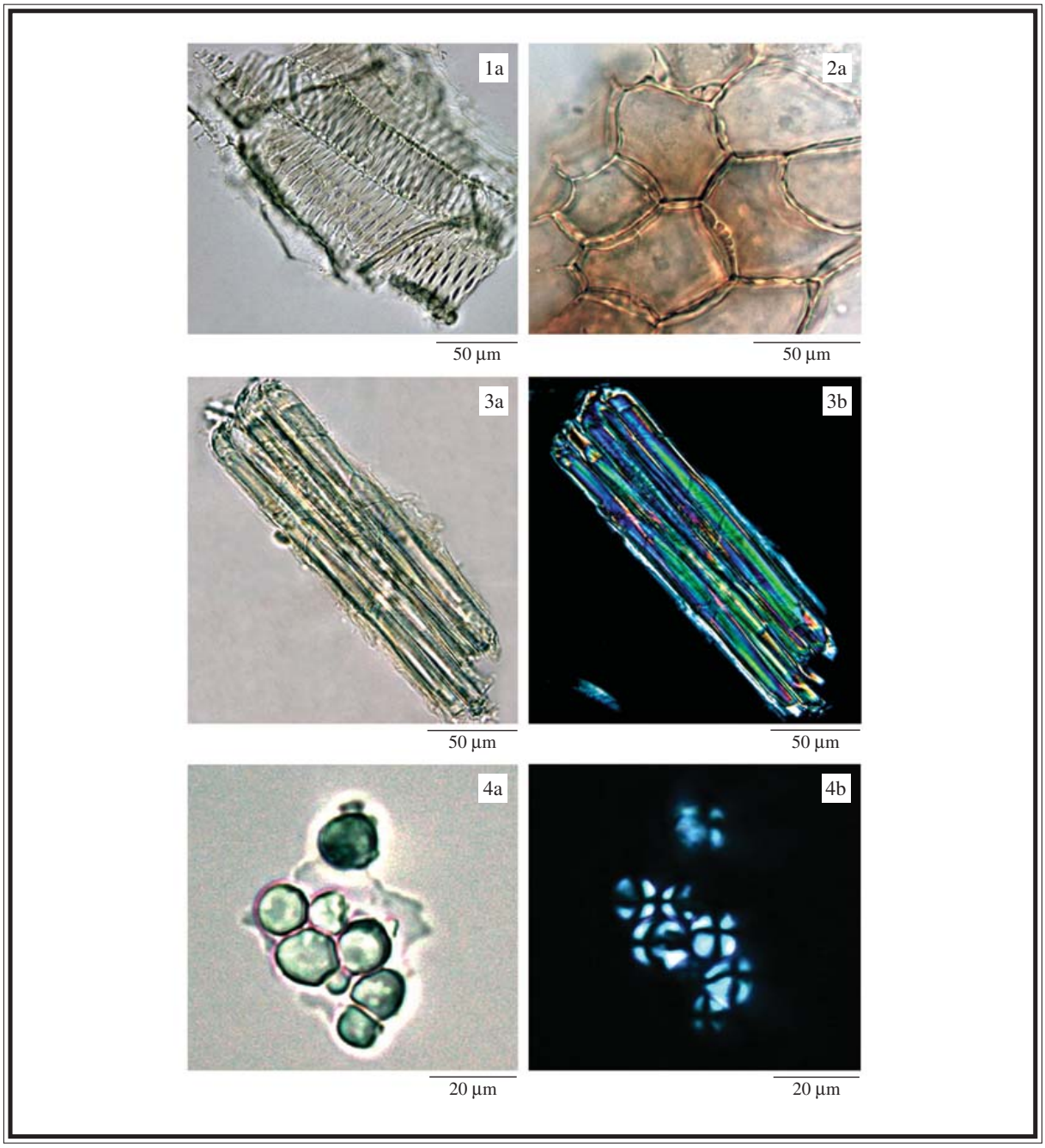

Figure19(i) Microscopic features of powder of dried root of *Astragalus membranaceus* (Fisch.) Bge.

1. Vessels 2. Cork cells 3. Fibres 4. Starch grains

a. Features under a light microscope b. Features under a polarizing microscope

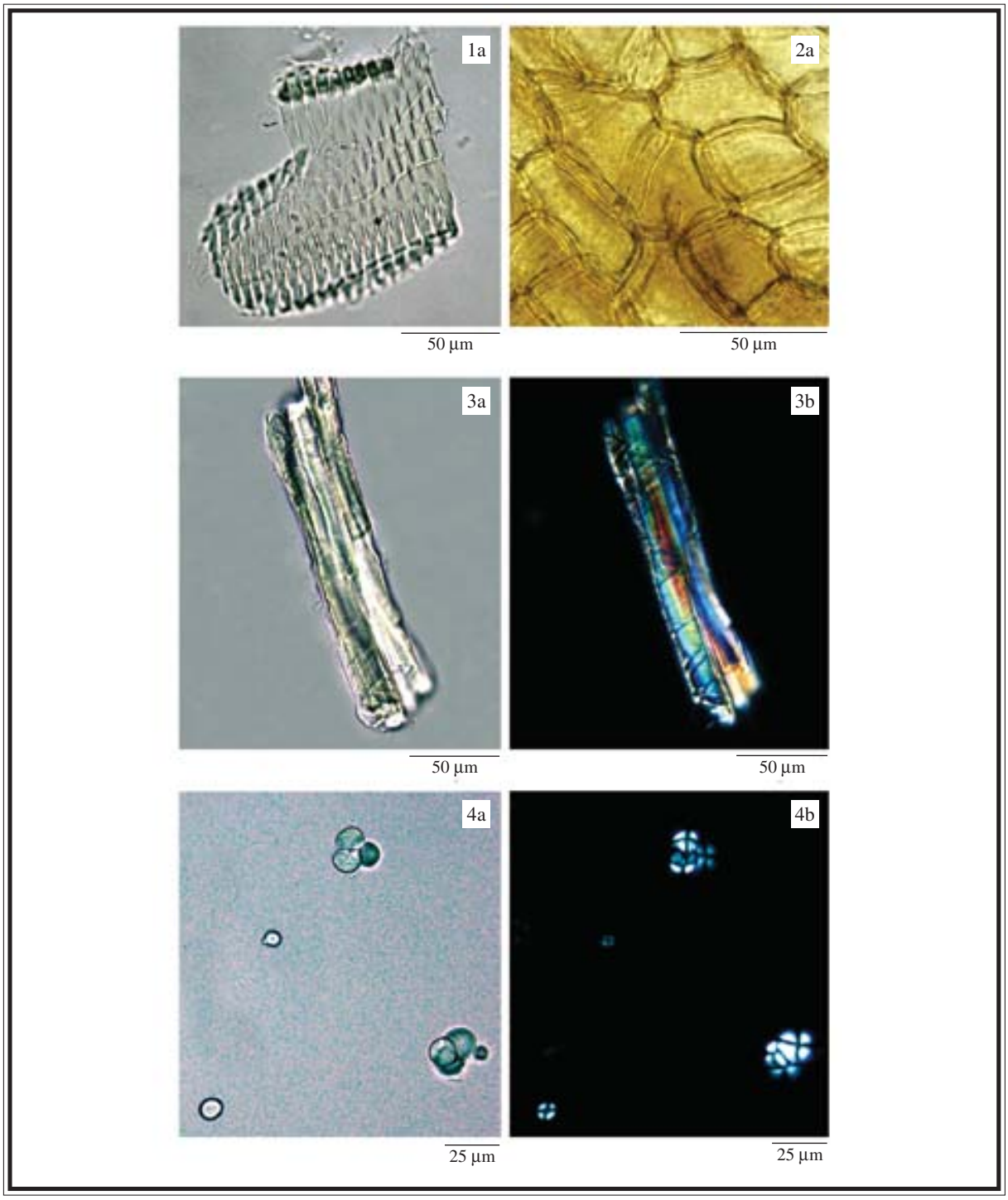

Figure 19(ii) Microscopic features of powder of dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao

1. Vessels 2. Cork cells 3. Fibres 4. Starch grains

a. Features under a light microscope b. Features under a polarizing microscope

Test solution

Weigh 3.0 g of the powdered sample and put into a 100-mL round-bottomed flask, then add 20 mL of methanol. Reflux the mixture for 30 min, filter and then pass the filtrate through a clean-up column (10 x 500 mm) packed with 5 g of neutral alumina (150 μm). Elute the column with 100 mL of methanol (40%). Collect the eluate and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 30 mL of water and extract twice each with 20 mL of 1-butanol. Combine the extracts and extract twice each with 20 mL of water and then discard the aqueous layer. Evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 0.5 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. Develop over a path of about 5 cm. Apply separately astragaloside II standard solution, astragaloside IV standard solution and the test solution (2 μL each) to the plate. After the development, remove the plate from the chamber, mark the solvent front and allow to dry in air. Spray the plate evenly with the spray reagent and heat at 80 ºC for about 10 min. Examine the plate in visible light. Calculate the R_f value 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 value, corresponding to those of astragaloside II and astragaloside IV.

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

Standard solution

Astragaloside IV standard stock solution, Std-Stock (1000 mg/L)

Weigh 4.0 mg of astragaloside IV and dissolve in 4 mL of methanol.

Astragaloside IV standard solution for fingerprinting, Std-FP (50 mg/L)

Pipette 0.25 mL of astragaloside IV Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 2.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 30 mL of methanol. Sonicate the mixture for 30 min. Centrifuge at about 3000 x *g* for 5 min. Filter the supernatant through a 0.45-μm PTFE filter. Wash the residue twice each with 15 mL of methanol, centrifuge and filter. Combine the filtrates and evaporate to dryness at reduced pressure in a rotary

evaporator. Dissolve the residue in 10 mL of aqueous ammonia solution (10%, v/v). Allow to stand for 10 min with occasional shaking. Transfer the solution to a separating funnel. Extract with 15 mL of water-saturated 1-butanol and repeat the extraction twice each with 10 mL of water-saturated 1-butanol. Combine the extracts and evaporate to dryness in a rotary evaporator. Dissolve the residue in 10 mL of methanol.

Chromatographic system

The liquid chromatograph is equipped with an ELSD and a column (4.6 x 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–10	100	0	isocratic
10–45	100 ➔ 40	0 ➔ 60	linear gradient
45–60	40	60	isocratic

System suitability requirements

Perform at least five replicate injections each with 20 µL of astragaloside IV Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of astragaloside IV should be not more than 5.0%; the RSD of the retention time of astragaloside IV peak should be not more than 2.0%; the column efficiency determined from astragaloside IV peak should be not less than 200,000 theoretical plates.

The *R* value between peaks 3 and 4 [Figs. 20(i) and (ii)] in the test solution should be not less than 1.5.

Procedure

Separately inject astragaloside IV Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of astragaloside IV peak in the chromatogram of astragaloside Std-FP and the retention times of the five characteristic peaks [Figs. 20(i) and 20(ii)] in the chromatogram of the test solution. Under the same HPLC conditions, identify astragaloside IV peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of astragaloside IV Std-FP. The retention times of astragaloside IV 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 five characteristic peaks of Radix Astragali extract are listed in Table 7.

Table 7 The RRTs and acceptable ranges of the five characteristic peaks of Radix Astragali extract

Peak No.	RRT	Acceptable Range
1	0.76	±0.03
2	0.87	±0.03
3	0.90	±0.03
4	0.91	±0.03
5 (marker, astragaloside IV)	1.00	-

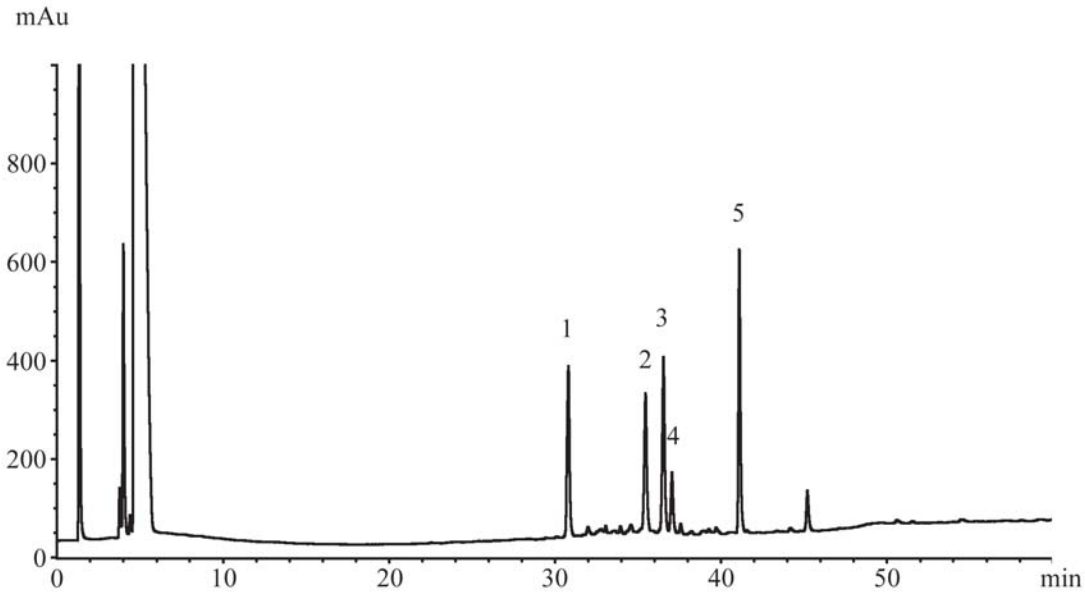

Figure 20(i) A reference fingerprint chromatogram of dried root of *Astragalus membranaceus* (Fisch.) Bge. extract

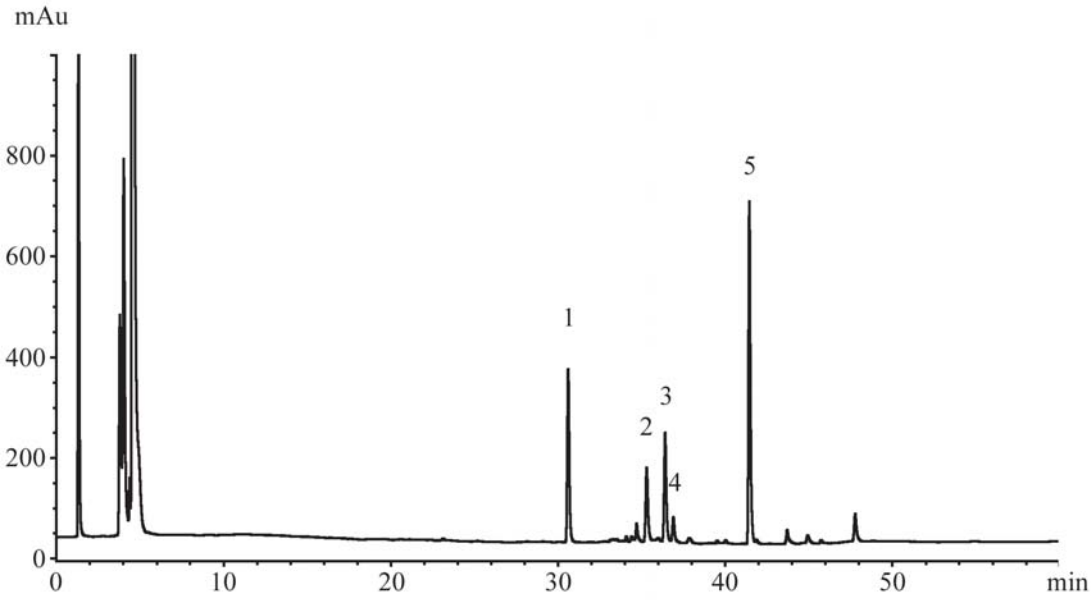

Figure 20(ii) A reference fingerprint chromatogram of dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference fingerprint chromatograms [Figs. 20(i) and 20(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 **Foreign Matter** *(Appendix VIII)*: not more than 2.0%.

5.5 **Ash** *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 1.0%.

5.6 **Water Content** *(Appendix X)*: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 17.0%.
Ethanol-soluble extractives (cold extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 5.0 mg of astragaloside IV and dissolve in 10 mL of methanol.

Astragaloside IV standard solution for assay, Std-AS

Measure accurately the volume of astragaloside IV Std-Stock, dilute with methanol to produce a series of solutions of 20, 60, 80, 100, 400 mg/L for astragaloside IV.

Test solution

Weigh accurately 2.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 30 mL of methanol. Sonicate the mixture for 30 min. Centrifuge at about 3000 x *g* for 5 min. Filter the supernatant through a 0.45-μm PTFE filter. Wash the residue twice each with 15 mL of methanol, centrifuge and filter. Combine the filtrates and evaporate to dryness in a rotary evaporator. Dissolve the residue in 10 mL of aqueous ammonia solution (10%, v/v). Allow to stand for 10 min with occasional shaking. Transfer the solution to a separating funnel. Extract with 15 mL of water-saturated 1-butanol and repeat the extraction twice each with 10 mL of water-saturated 1-butanol. Combine the extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of methanol.

Chromatographic system

The liquid chromatograph is equipped with an ELSD and a column (4.6 x 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. The mobile phase is a mixture of acetonitrile and water (4:6, v/v).

System suitability requirements

Perform at least five replicate injections each with 20 μL of astragaloside IV Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of astragaloside IV should be not more than 5.0%; the RSD of the retention time of astragaloside IV peak should be not more than 2.0%; the column efficiency determined from astragaloside IV peak should be not less than 10,000 theoretical plates.

The *R* value between astragaloside IV peak and the closest peak in the test solution should be not less than 1.5.

Calibration curve

Inject a series of astragaloside IV Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of astragaloside IV against the natural logarithm of the corresponding concentrations of astragaloside IV Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify astragaloside IV peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of astragaloside IV Std-AS. The retention times of astragaloside IV 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 astragaloside IV in the test solution by using the following equation –

$$\text{Concentration of astragaloside IV in the test solution} = e^{(\text{Ln}(A) - I) / m}$$

Where A = the peak area of astragaloside IV in the test solution,
I = the y-intercept of the 5-point calibration curve,
m = the slope of the 5-point calibration curve.

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

Limits

The sample contains not less than 0.040% of astragaloside IV ($C_{41}H_{68}O_{14}$), calculated with reference to the dried substance.