

Radix Codonopsis



Figure 1(i) A photograph of dried root of *Codonopsis pilosula* (Franch.) Nannf.



Figure 1(ii) A photograph of dried root of *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T. Shen



Figure 1(iii) A photograph of dried root of *Codonopsis tangshen* Oliv.

1. NAMES

Official Name: Radix Codonopsis

Chinese Name: 黨參

Chinese Phonetic Name: Dangshen

2. SOURCE

Radix Codonopsis is the dried root of *Codonopsis pilosula* (Franch.) Nannf., *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T. Shen or *Codonopsis tangshen* Oliv. (Campanulaceae). The root is collected in autumn, washed clean, then dried under the sun to obtain Radix Codonopsis.

3. DESCRIPTION

***Codonopsis pilosula* (Franch.) Nannf. :** The root is long and cylindrical, slightly curved, occasionally branched, 11-34 cm in length, 3-18 mm in diameter, outer surface yellowish-brown to greyish-yellow. The upper portion with numerous verruciform (wart-shaped), protuberant stem scars, each stem scar dented and dotted, commonly known as "*shizi-pantou*", dense transverse annulations occur below the root stock, gradually becoming sparse towards the lower part, some up to half the length of the root, few or no transverse annulations on cultivated roots. Whole root marked with longitudinal wrinkles and scattered transverse lenticels, scars of branch roots often with gelatinous, dull blackish-brown substances. Texture slightly hard or tenacious, fracture somewhat even, cleft or with striated radial markings, bark pale yellowish-white to pale brown, slightly thick, xylem pale yellow, in the shape of a chrysanthemum. Odour, with a distinctive fragrance; taste, sweet [Fig. 1(i)].

***Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T. Shen :** The root is 6-32 cm in length, 6-25 mm in diameter, outer surface yellowish-brown to greyish-yellow, with dense transverse annulations occurring below the root stock, frequently up to over half the length of the root. Fracture shows more cleft [Fig. 1(ii)].

***Codonopsis tangshen* Oliv. :** The root is 10-45 cm in length, 5-18 mm in diameter, outer surface yellowish-brown to greyish-brown with distinctly longitudinal wrinkles. Texture slightly soft and pliable, fracture shows less cleft [Fig. 1(iii)].

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

***Codonopsis pilosula* (Franch.) Nannf. :** Cork consists of 3-10 layers of cork cells, stone cells present in the outer layer of the cork tissue, single or grouped. Cortex narrow. Phloem broad, often with cleft, with groups of laticiferous tubes frequently alternated with sieve tubes. Cambium in a ring. Xylem vessels scattered singly or aggregated in groups, arranged radially. Inulin and starch grains contained in parenchyma cells [Fig. 2(i)].

***Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T. Shen :** Stone cells present in a complete ring in the outer layer of cork tissue, composed of 2-5 layers of cells. Group of laticiferous tubes arranged radially in the inner part of the phloem, curved in the outer part, sometimes arranged tangentially in an interrupted ring [Fig. 2(ii)].

***Codonopsis tangshen* Oliv. :** Stone cells present in the outer layer of the cork tissue, singly scattered or several groups arranged in an interrupted ring. Groups of laticiferous tubes arranged irregularly [Fig. 2(iii)].

Powder

Colour, yellowish-white, pale yellow or greyish-yellow. Stone cells frequent, in polygonal, rhombic, rectangular or spindle shapes, occurring scattered singly or aggregated in groups, some attached to the cork cells, 8-68 µm in diameter, 15-152 µm long, cells wall with occasional pits and pit traps. Cork cells frequent, subrectangular and polygonal in surface view. Vessels mostly of reticulate and scalariform bordered-pitted types, 8-142 µm in diameter. Inulin abundant, fan-shaped or irregular, with radial striation; showing bright mass with radial striation when examined under the polarized microscope. Groups of laticiferous tubes frequent, consisting yellowish-brown matter in granules or oily drops. Starch grains infrequent [Fig. 3(i), (ii) and (iii)].

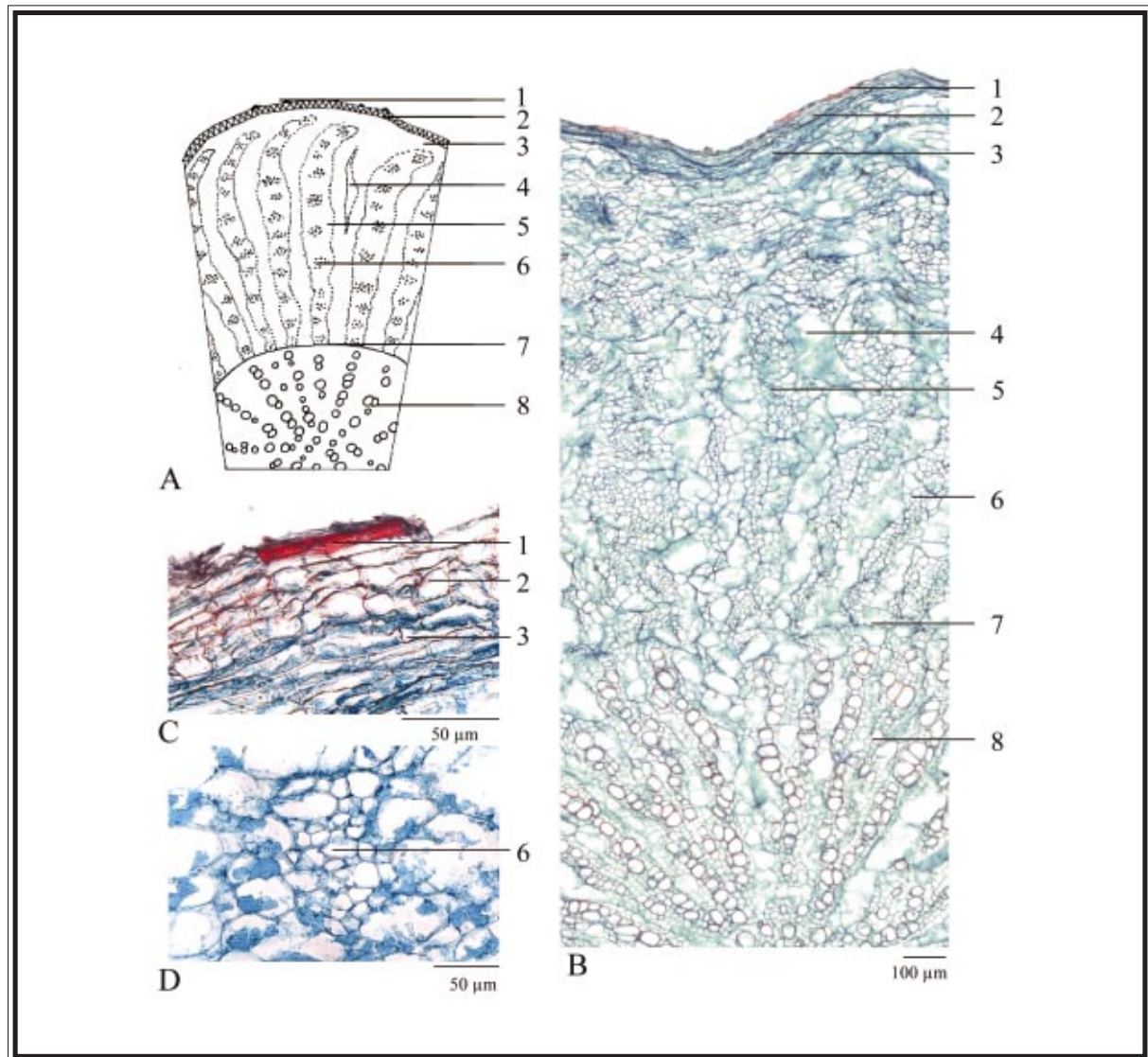


Figure 2(i) Microscopic features of transverse section of dried root of *Codonopsis pilosula* (Franch.) Nannf.

A. Sketch B. Section illustration C. Stone cell D. Laticiferous tube group

1. Stone cell 2. Cork 3. Cortex 4. Cleft 5. Phloem 6. Laticiferous tube group 7. Cambium 8. Xylem

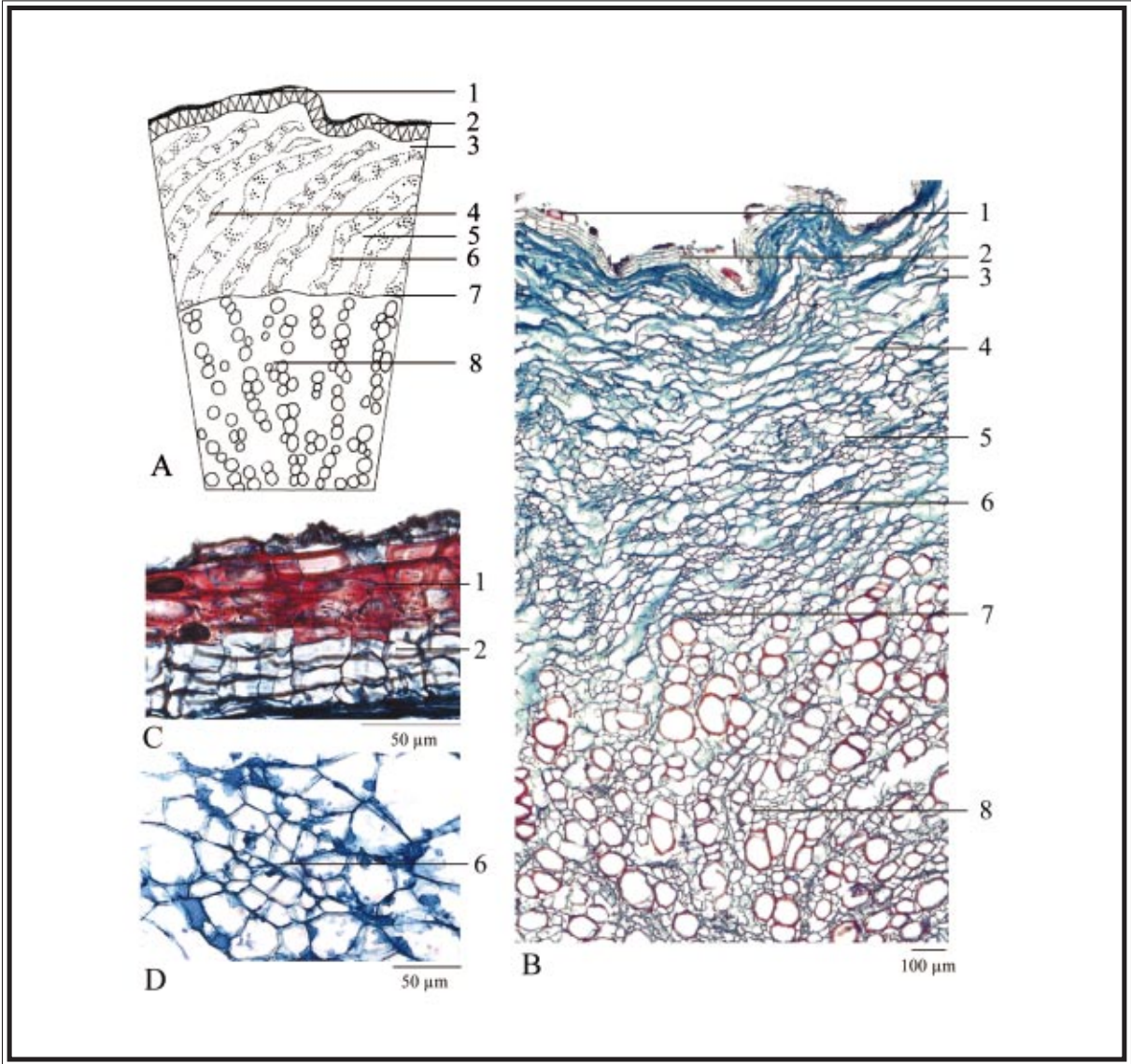


Figure 2(ii) Microscopic features of transverse section of dried root of *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T. Shen

A. Sketch B. Section illustration C. Stone cell D. Laticiferous tube group

1. Stone cell 2. Cork 3. Cortex 4. Cleft 5. Phloem 6. Laticiferous tube group 7. Cambium 8. Xylem

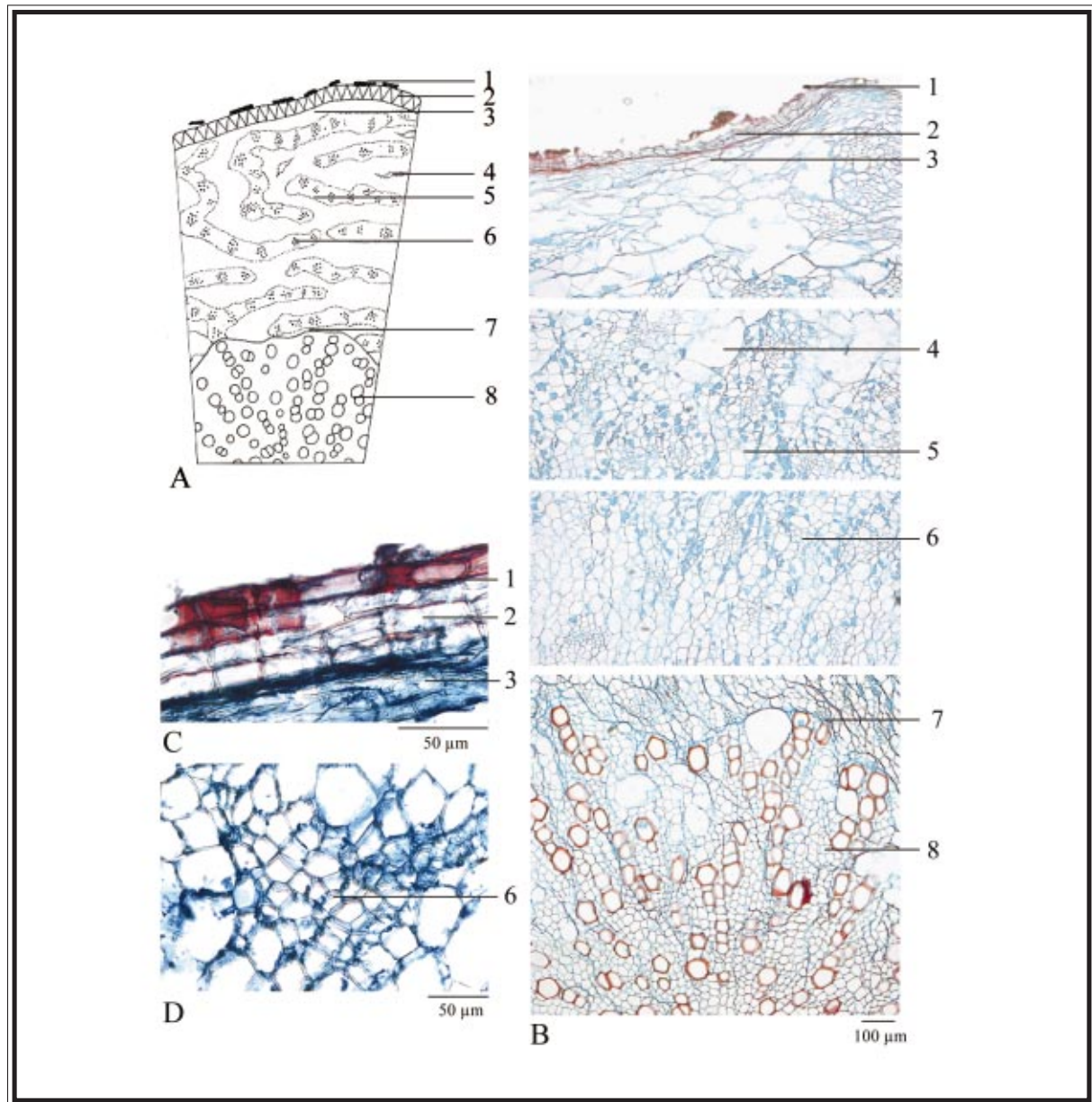


Figure 2(iii) Microscopic features of transverse section of dried root of *Codonopsis tangshen* Oliv.

A. Sketch B. Section illustration C. Stone cell D. Laticiferous tube group

1. Stone cell 2. Cork 3. Cortex 4. Cleft 5. Phloem 6. Laticiferous tube group 7. Cambium 8. Xylem

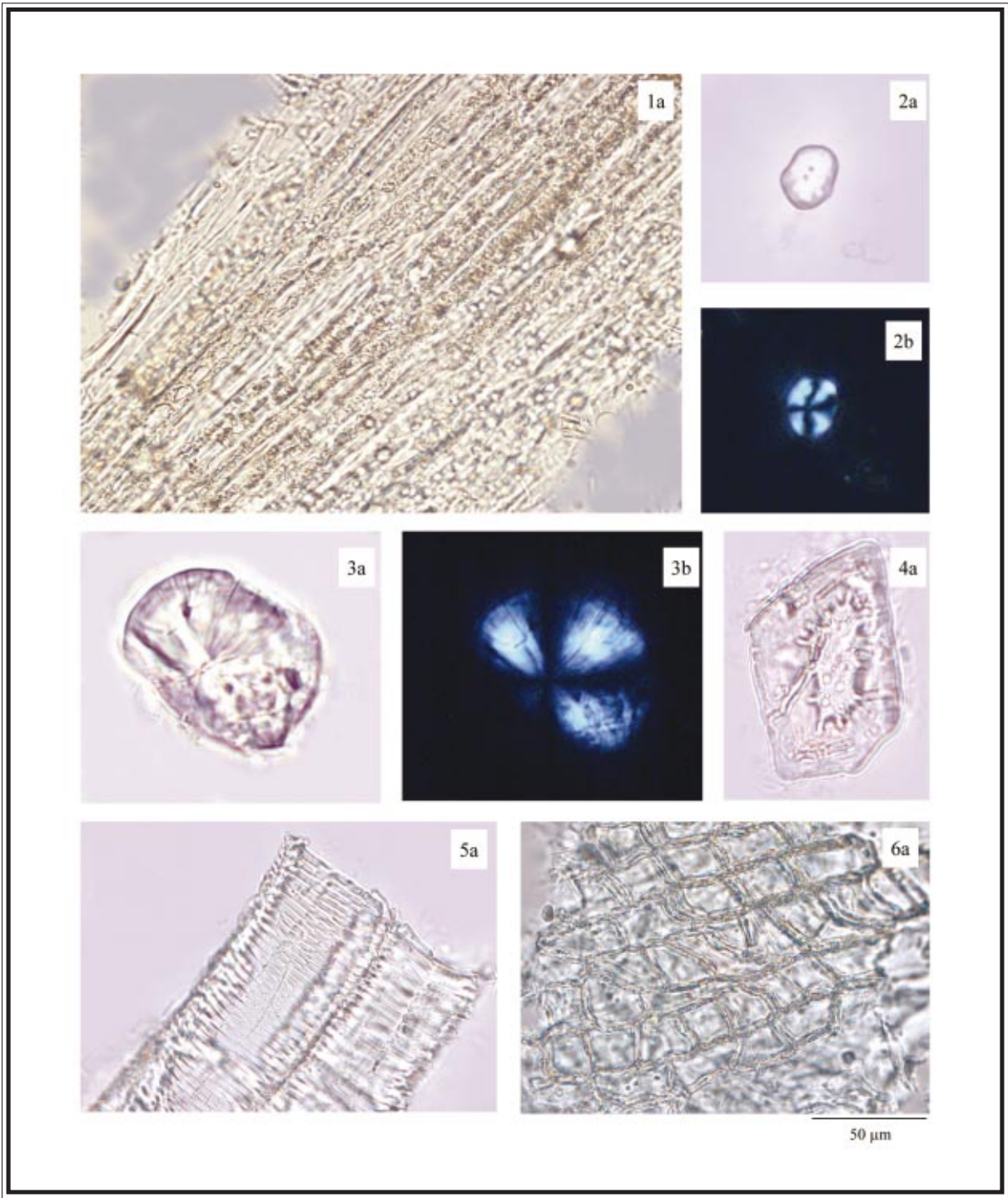


Figure 3(i) Microscopic features of powder of dried root of *Codonopsis pilosula* (Franch.) Nannf.

1. Laticiferous tube 2. Starch grain 3. Inulin 4. Stone cell 5. Reticulate bordered pits vessels 6. Cork cells
a. Features under the light microscope b. Features under the polarized microscope

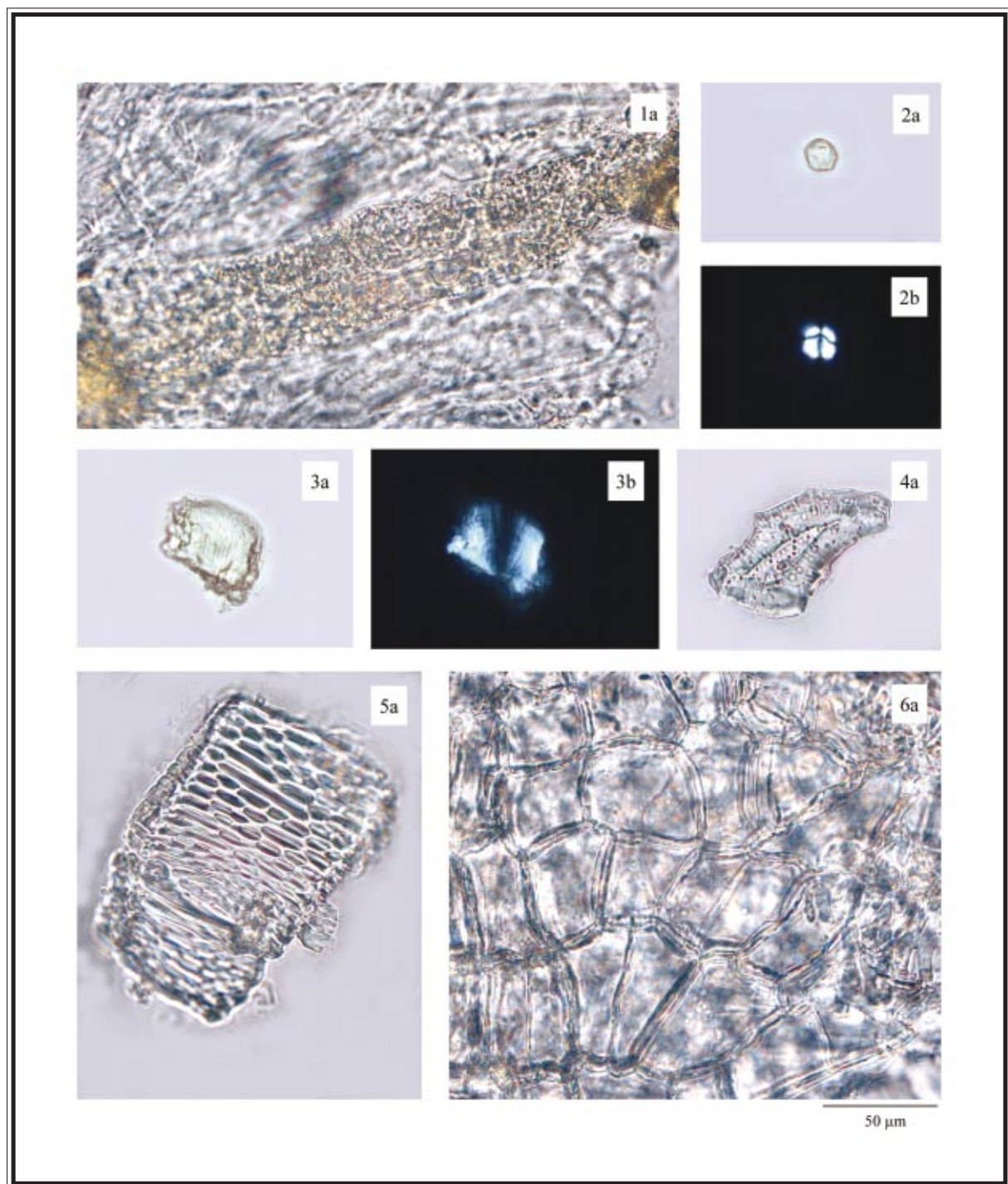


Figure 3(ii) Microscopic features of powder of dried root of *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.)
L.T. Shen

1. Laticiferous tube 2. Starch grain 3. Inulin 4. Stone cell 5. Reticulate bordered pits vessel 6. Cork cells
a. Features under the light microscope b. Features under the polarized microscope

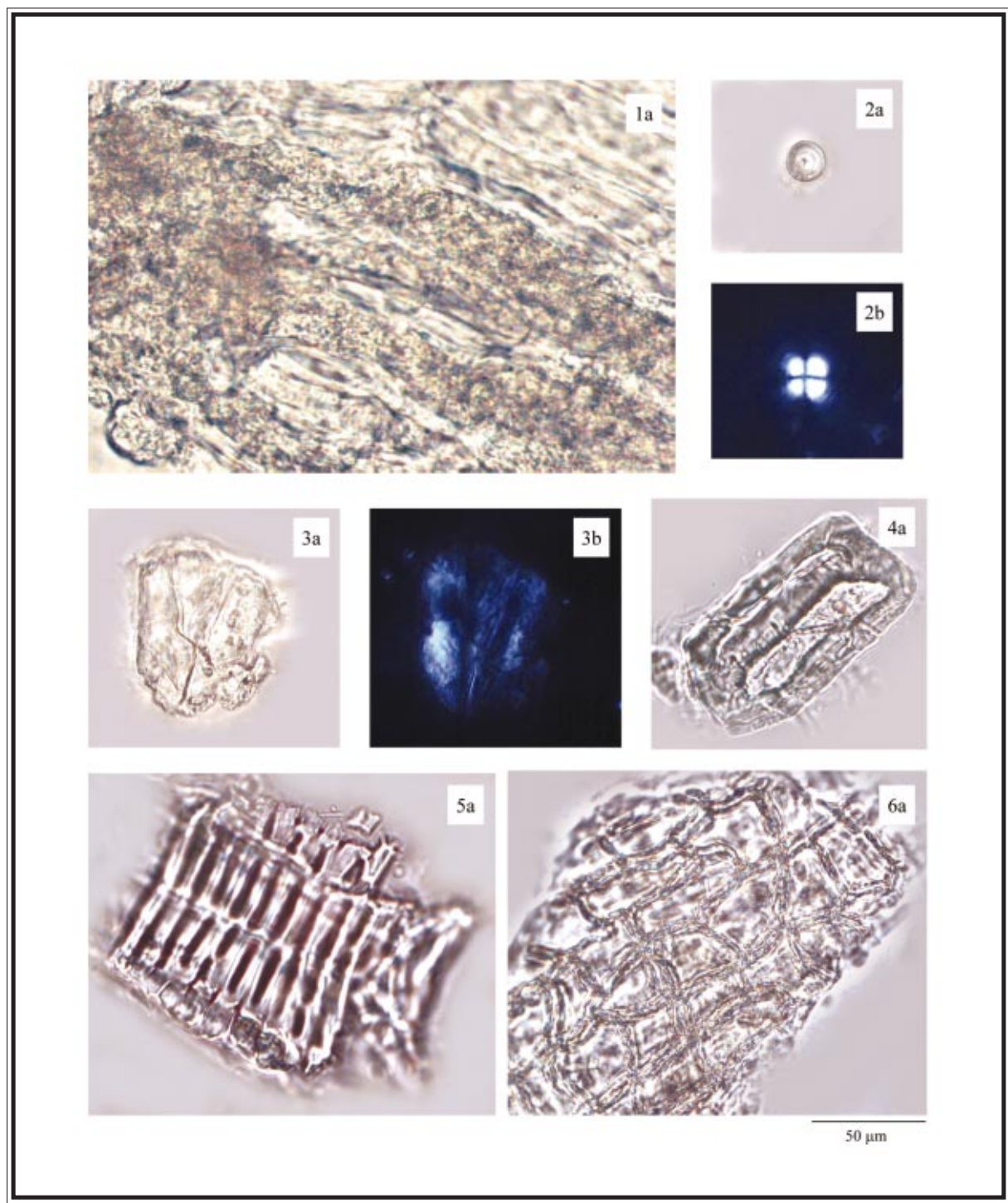


Figure 3(iii) Microscopic features of powder of dried root of *Codonopsis tangshen* Oliv.

1. Laticiferous tube 2. Starch grain 3. Inulin 4. Stone cells 5. Scalariform bordered pits vessel
6. Cork cells

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

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 (240 W) the mixture for 30 min. Centrifuge at about $2000 \times g$ for 15 min. Filter and transfer 1 mL of the filtrate to a test tube. Cautiously add about 1 mL of sulphuric acid along the inner wall of the test tube. Allow to stand for about 20 min. An orange-brown ring is observed at the interface of the two solvent layers.

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

Standard solution

Lobetyolin standard solution

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

Developing solvent system

Prepare a mixture of 1-butanol, acetic acid and water (7:1:0.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 2.5 g of the powdered sample and put into a 100-mL conical flask, then add 50 mL of methanol. Sonicate (240 W) the mixture for 60 min. Centrifuge at about $2000 \times g$ for 10 min. Transfer 25 mL of the supernatant to a 100-mL round-bottomed flask and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 3 mL of water. Load the water extract onto a Hydrophilic-Lipophilic Balance (HLB) extraction cartridge (3 mL, 60 mg). Wash the cartridge with 6 mL of water, then elute with 6 mL of methanol. Transfer the methanol solution to a 100-mL round-bottomed flask, and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate and a freshly prepared developing solvent system as described above. Apply separately lobetyolin standard solution and the test solution (5 μ L each) 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, then heat at about 105°C until the spots or bands become visible (about 5–

10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV(A).

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 lobetyolin.

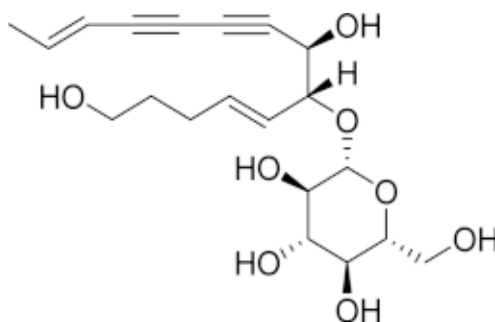


Figure 4 Chemical structure of lobetyolin

4.4 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Lobetyolin standard stock solution, Std-Stock (3600 mg/L)

Weigh 3.6 mg of lobetyolin CRS and dissolve in 1 mL of methanol.

Lobetyolin standard solution for fingerprinting, Std-FP (360 mg/L)

Pipette 1 mL of the lobetyolin Std-Stock into a 10-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 1.5 g of the powdered sample and put into a 250-mL conical flask, then add 30 mL of 0.1 M HCl-methanol (1:1, v/v). Sonicate (240 W) the mixture for 60 min. Centrifuge at about $3000 \times g$ for 10 min. Filter the supernatant through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (267 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time (min)	0.2% of Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	90 → 80	10 → 20	linear gradient
20 – 60	80 → 0	20 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of lobetyolin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of lobetyolin should not be more than 5.0%; the RSD of the retention time of lobetyolin peak should not be more than 2.0%; the column efficiency determined from lobetyolin peak should not be less than 50000 theoretical plates.

The *R* value between peak 2 and peak 3 in the chromatogram of the test solution should not be less than 1.5 [Fig. 5 (i), (ii) or (iii)].

Procedure

Separately inject lobetyolin Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of lobetyolin peak in the chromatogram of lobetyolin Std-FP and the retention times of the four characteristic peaks [Fig. 5 (i), (ii) or (iii)] in the chromatogram of the test solution. Under the same HPLC conditions, identify lobetyolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of lobetyolin Std-FP. The retention times of lobetyolin 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 four characteristic peaks of Radix Codonopsis extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Radix Codonopsis extract

Peak No.	RRT	Acceptable Range
1	0.64	±0.05
2	0.90	±0.03
3 (marker, lobetyolin)	1.00	-
4	1.11	±0.03

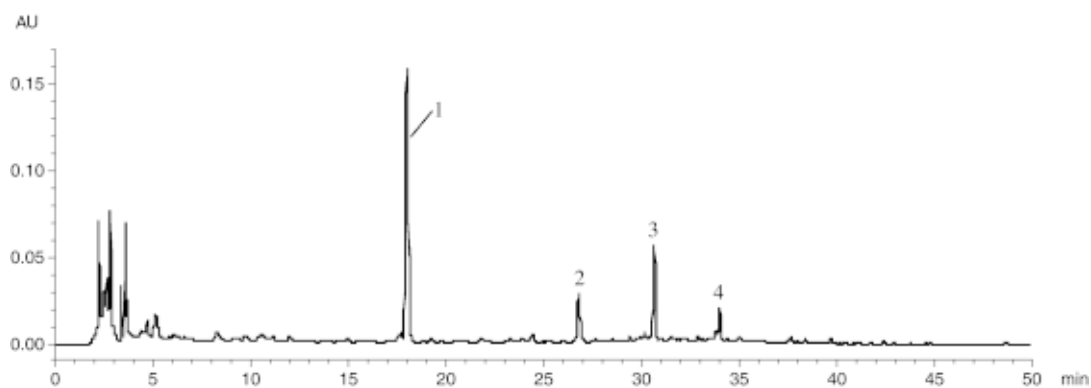


Figure 5(i) A reference fingerprint chromatogram of dried root of *Codonopsis pilosula* (Franch.) Nannf. extract

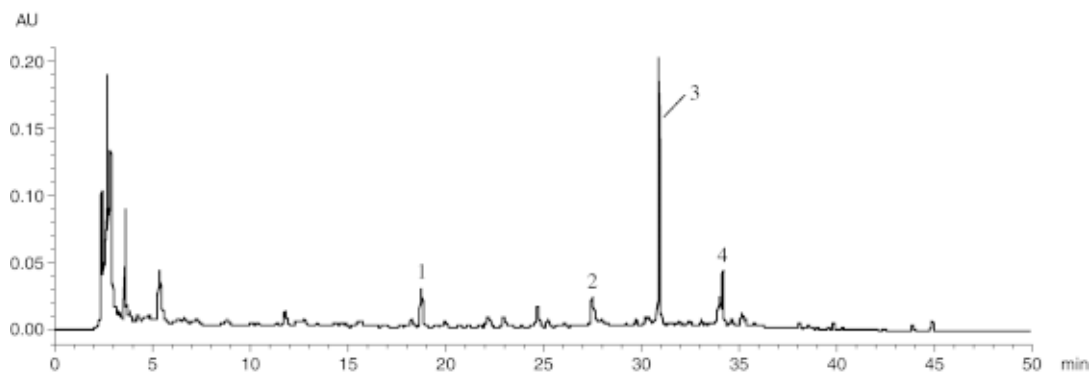


Figure 5(ii) A reference fingerprint chromatogram of dried root of *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T. Shen extract

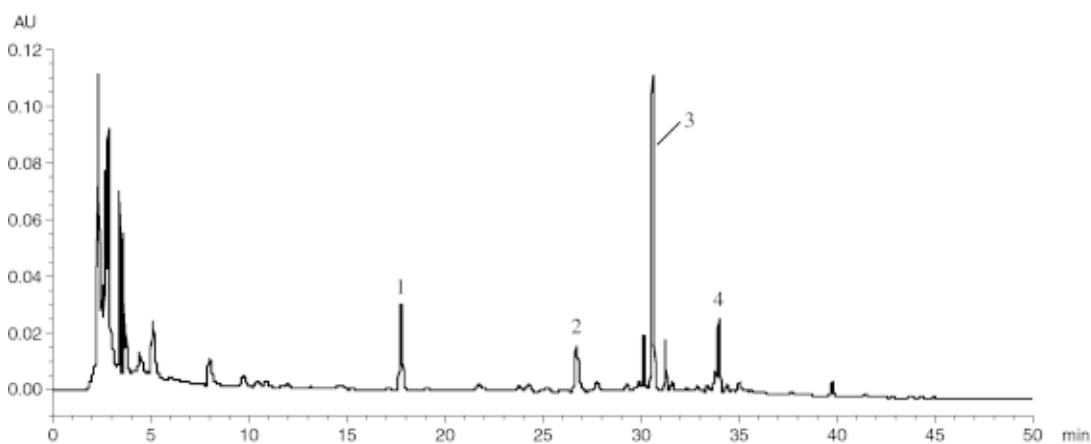


Figure 5(iii) A reference fingerprint chromatogram of dried root of *Codonopsis tangshen* Oliv. extract

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

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 XVII*): not more than 400 mg/kg.

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

5.6 Ash (*Appendix IX*)

Total ash: not more than 6.0%.

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 (cold extraction method): not less than 41.0%.

Ethanol-soluble extractives (cold extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

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

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

Lobetyolin standard solution for assay, Std-AS

Measure accurately the volume of the lobetyolin Std-Stock, dilute with methanol to produce a series of solutions of 10, 30, 50, 100, 200 mg/L for lobetyolin.

Test solution

Weigh accurately 2.5 g of the powdered sample and put into a 50-mL conical flask, then add 20 mL of methanol. Sonicate (240 W) the mixture for 60 min. Collect the extract. Repeat the extraction twice each with 12 mL of methanol. Combine the extracts. Filter and transfer the filtrate to a 50-mL volumetric flask and make up to the mark with methanol. Pipette 25 mL of extract into a round-bottomed flask and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (214 nm) and a column (3.9 × 150 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 – 40	100 ➔ 60	0 ➔ 40	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 μL of lobetyolin Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of lobetyolin should not be more than 5.0%; the RSD of the retention time of lobetyolin peak should not be more than 2.0%; the column efficiency determined from lobetyolin peak should not be less than 10000 theoretical plates.

The *R* value between lobetyolin 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 lobetyolin Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of lobetyolin against the corresponding concentrations of lobetyolin Std-AS. Obtain the slope, y-intercept and the *r*² value from the 5-point calibration curve.

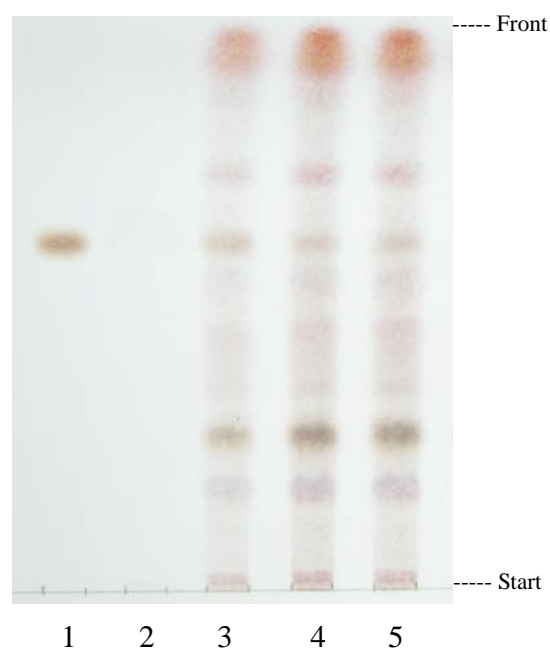
Procedure

Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify lobetyolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of lobetyolin Std-AS. The retention times of lobetyolin 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 lobetyolin in the test solution, and calculate the percentage content of lobetyolin in the sample by using the equations indicated in Appendix IV (B).

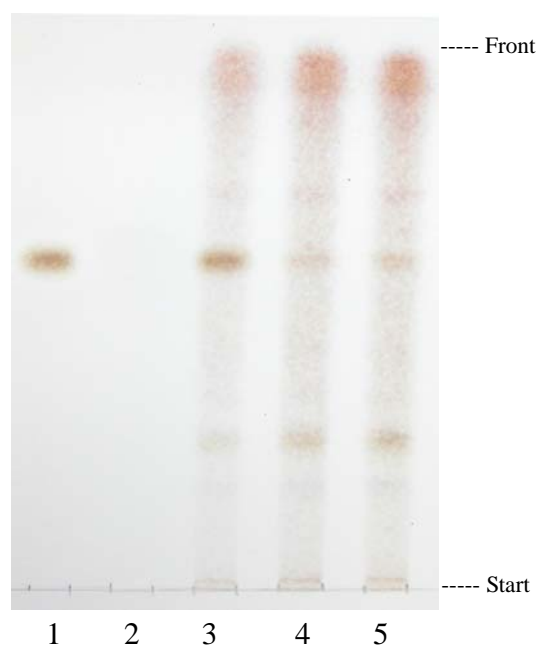
Limits

The sample contains not less than 0.029% of lobetyolin (C₂₀H₂₈O₈), calculated with reference to the dried substance.



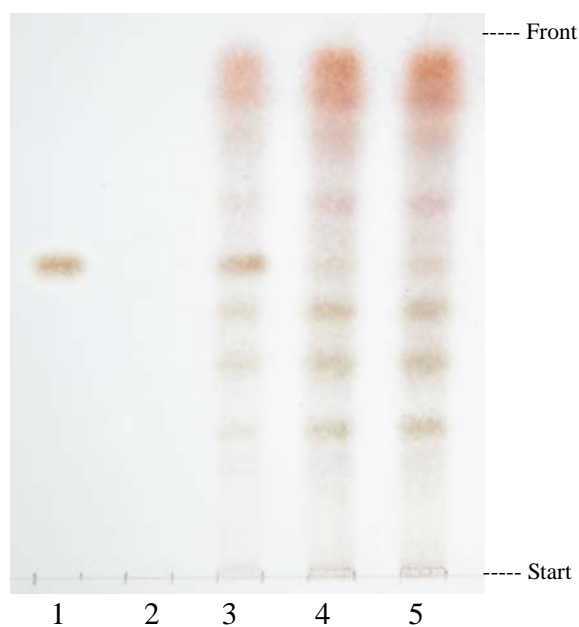
Lane	Sample	Results
1	Standard (Lobetyolin)	Lobetyolin positive
2	Blank (Methanol)	Negative
3	Spiked sample (Sample plus lobetyolin)	Lobetyolin positive
4	Sample [<i>Codonopsis pilosula</i> (Franch.) Nannf.]	Lobetyolin positive
5	Sample duplicate [<i>Codonopsis pilosula</i> (Franch.) Nannf.]	Lobetyolin positive

Figure 1 TLC results of dried root of *Codonopsis pilosula* (Franch.) Nannf. extract observed under visible light after staining



Lane	Sample	Results
1	Standard (Lobetyolin)	Lobetyolin positive
2	Blank (Methanol)	Negative
3	Spiked sample (Sample plus lobetyolin)	Lobetyolin positive
4	Sample [<i>Codonopsis pilosula</i> Nannf. var. <i>modesta</i> (Nannf.) L. T. Shen]	Lobetyolin positive
5	Sample duplicate [<i>Codonopsis pilosula</i> Nannf. var. <i>modesta</i> (Nannf.) L. T. Shen]	Lobetyolin positive

Figure 2 TLC results of dried root of *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L.T. Shen extract observed under visible light after staining



Lane	Sample	Results
1	Standard (Lobetyolin)	Lobetyolin positive
2	Blank (Methanol)	Negative
3	Spiked sample (Sample plus lobetyolin)	Lobetyolin positive
4	Sample (<i>Codonopsis tangshen</i> Oliv.)	Lobetyolin positive
5	Sample duplicate (<i>Codonopsis tangshen</i> Oliv.)	Lobetyolin positive

Figure 3 TLC results of dried root of *Codonopsis tangshen* Oliv. extract observed under visible light after staining