Radix et Rhizoma Gentianae

Figure 1(i) A photograph of dried root and rhizome of *Gentiana scabra* Bge.

1. Rhizome 2. Root

Figure 1(ii) A photograph of dried root and rhizome of *Gentiana rigescens* Franch.

1. Rhizome 2. Root

1. NAMES

Official Name: Radix et Rhizoma Gentianae

Chinese Name: 龍膽

Chinese Phonetic Name: Longdan

2. SOURCE

Radix et Rhizoma Gentianae is the dried root and rhizome of *Gentiana scabra* Bge. or *Gentiana rigescens* Franch. (Gentianaceae). The roots with the rhizome is collected in the spring and autumn. After removal of remnants of the above-ground stems. The root and rhizome were dried under the sun to obtain Radix et Rhizoma Gentianae.

3. DESCRIPTION

***Gentiana scabra* Bge. :** Rhizomes irregularly bundle-shaped, 1-3 cm in length, 3-10 mm in diameter. Outer surface dull dark greyish-brown or dark brown, the top end marked by stem scars or remnants of stems, all sides and the lower end with numerous slender roots. Root cylindrical, slightly twisted, 4-21 cm in length, 1-6 mm in diameter. Outer surface pale yellow or yellowish-brown, upper part usually with conspicuous transverse wrinkles, lower part thinner and marked with longitudinal wrinkles and scars of branch roots. Texture brittle, easily broken. Broken surface slightly even. Bark yellowish-white or pale yellowish-brown. Xylem paler in colour. Odour, faint; taste, quite bitter [Fig. 1(i)].

***Gentiana rigescens* Franch. :** Rhizomes in the form of irregular pieces, smaller than that of *Gentiana scabra* Bge. Root 3.5-23 cm in length, 1-4 mm in diameter, externally yellowish-brown or yellowish-red without transverse wrinkles. The outer layer membranaceous, easily falling off. Wood yellowish-white, easily separated from the bark [Fig. 1(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

***Gentiana scabra* Bge. :** Exodermis consists of a layer of cells, the outer walls relatively thicker. Cortex narrow, often with clefts. Endodermis distinct, cells elongated tangentially, each cell divided by longitudinal walls into several subsquare small cells. Phloem broad with cleft, occupying 1/3 of the radius. Cambium indistinct. Xylem bundles 5-10 grouped with vessels. Pith distinct. Parenchyma cells containing minute needles of calcium oxalate crystals are occasionally found [Fig. 2(i)].

***Gentiana rigescens* Franch. :** The tissues outside the endodermis mostly falling off. Endodermis distinct. Phloem occupying 2/3 of the radius. Vessels in the xylem densely distributed with radial orientation. Pith absent [Fig. 2(ii)].

Powder

***Gentiana scabra* Bge. :** Colour pale yellowish-brown. Exodermis cells easily observed externally spindle-shaped or subrectangular, 42-536 μm in length, 16-134 μm in diameter, each cell divided by longitudinal wall into several small rectangular cells. Endodermis cells can be observed easily, rectangular in surface view and somewhat larger in size, 36-558 μm in length, 34-255 μm in diameter. Periclinal wall shows minute transverse striations, each cell divided by longitudinal septum into 5-36 small palisade-like cells, with the longitudinal septum beaded. Vessels reticulate and scalariform, 7-52 μm in diameter. Some parenchyma cells contain minute needles of calcium oxalate crystals which appear as firework polychrome under the polarized microscope [Fig. 3(i)].

***Gentiana rigescens* Franch. :** Colour reddish-brown to yellowish-brown. Exodermis cells absent. Endodermis cells 67-381 μm in length, 36-305 μm in diameter, transverse striations of the periclinal wall thicker and dense, each cell divided by longitudinal wall into 3-34 subsquare, palisade-like cells. Vessels reticulate, scalariform and spiral, 9-28 μm in diameter. Fibre-tracheid easily observed, short, spindle-shaped or rectangular. Some parenchymatous cells containing minute needles of calcium oxalate which showing firework polychrome under a polarizing microscope [Fig. 3(ii)].

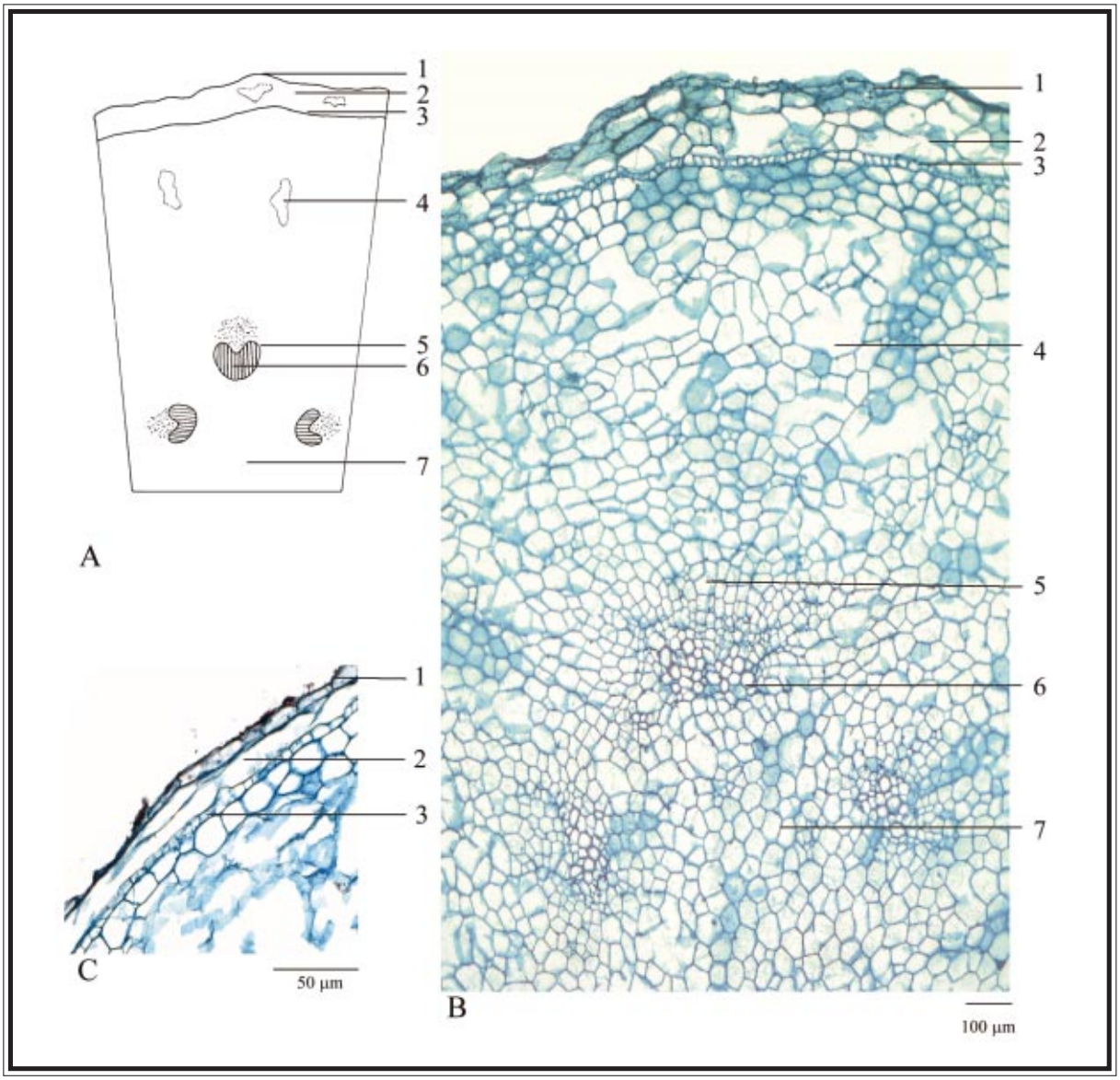


Figure 2(i) Microscopic features of transverse section of dried root and rhizome of *Gentiana scabra* Bge.

A. Sketch B. Section illustration C. Cortex

1. Exodermis 2. Cortex 3. Endodermis 4. Cleft 5. Phloem 6. Xylem 7. Pith

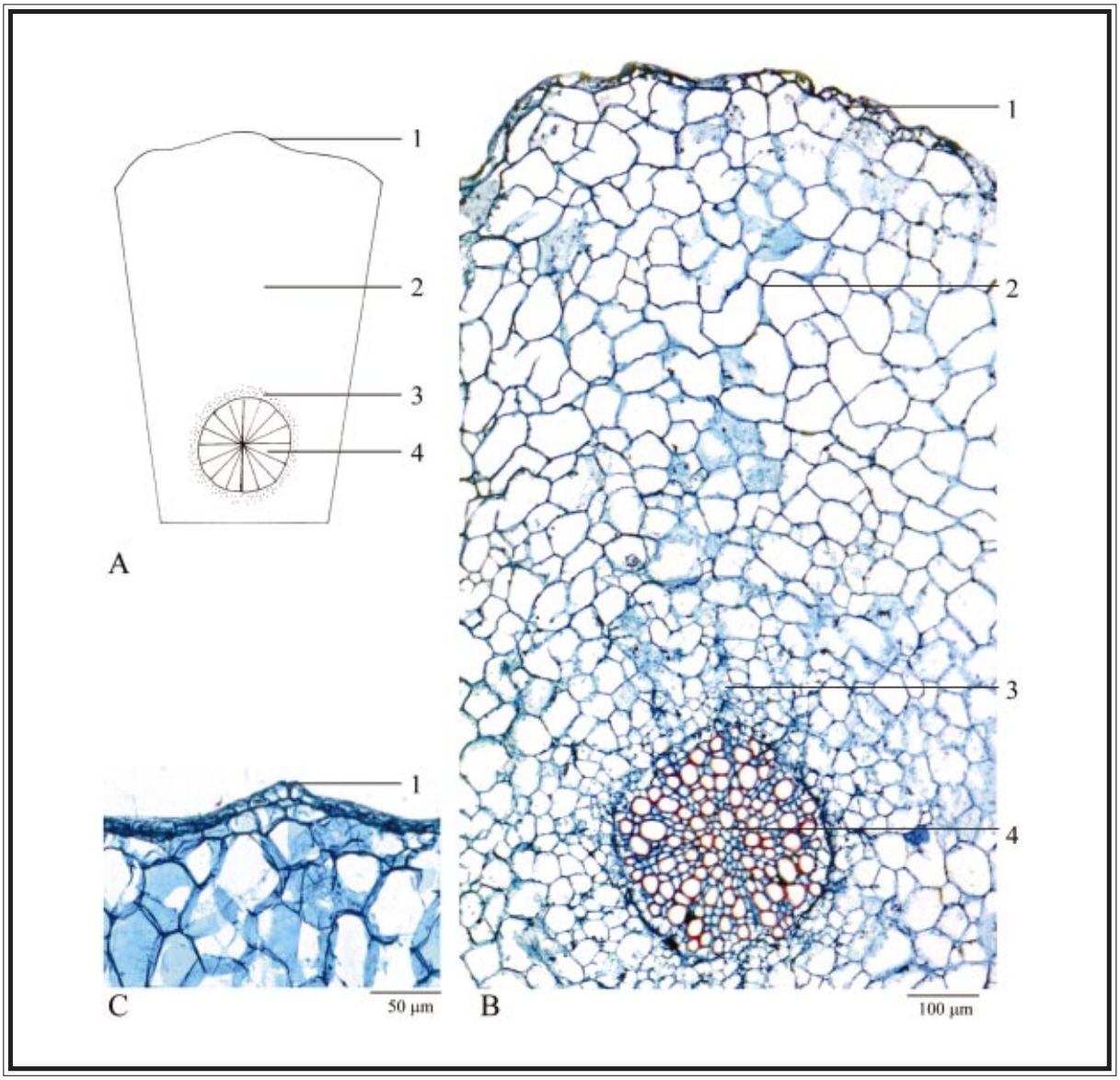


Figure 2(ii) Microscopic features of transverse section of dried root and rhizome of *Gentiana rigescens* Franch.

A. Sketch B. Section illustration C. Endodermis

1. Endodermis 2. Phloem 3. Sieve tube group 4. Xylem

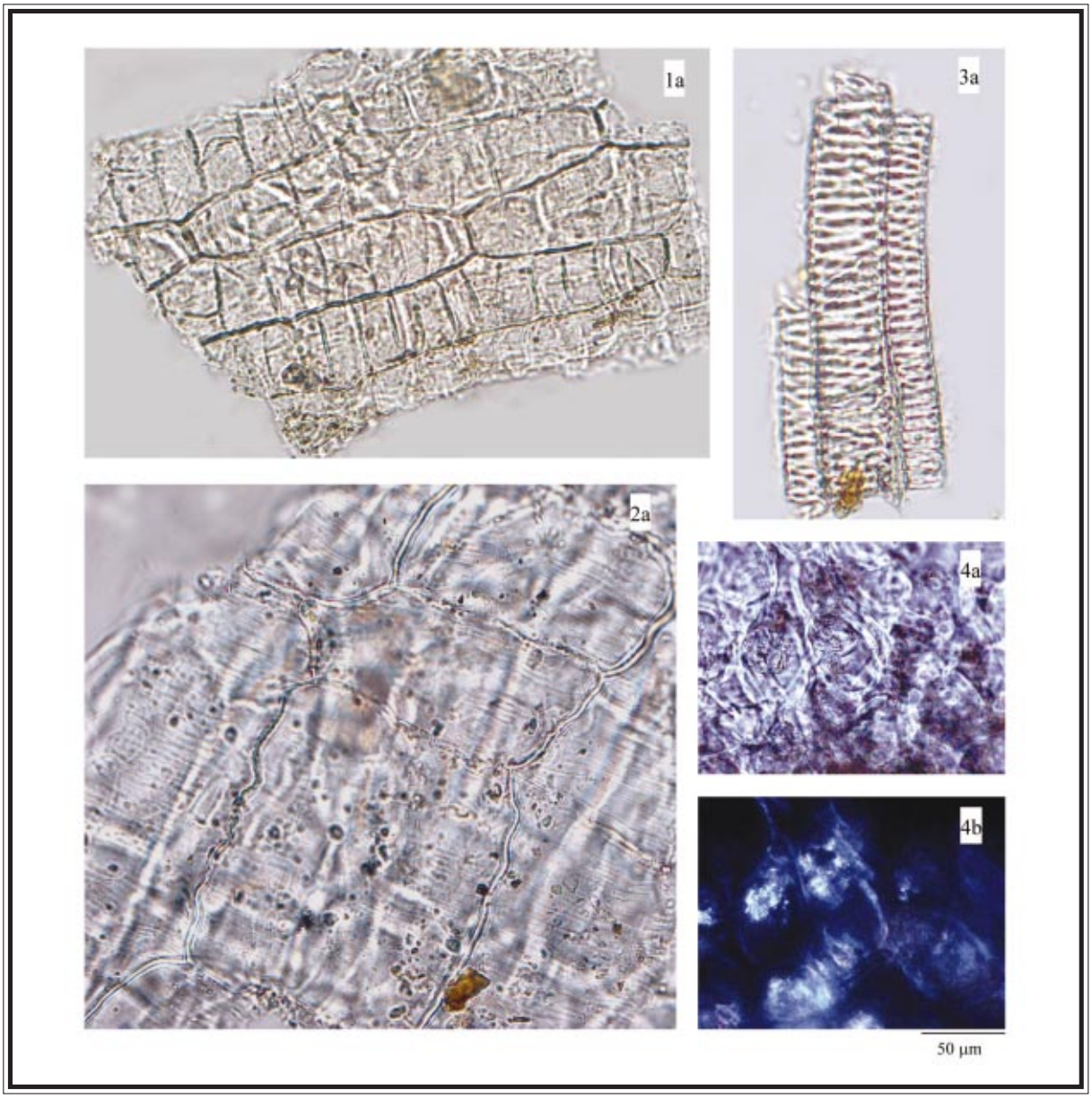


Figure 3(i) Microscopic features of powder of dried root and rhizome of *Gentiana scabra* Bge.

1. Exodermis 2. Endodermis 3. Reticulate vessel 4. Needles of calcium oxalate

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

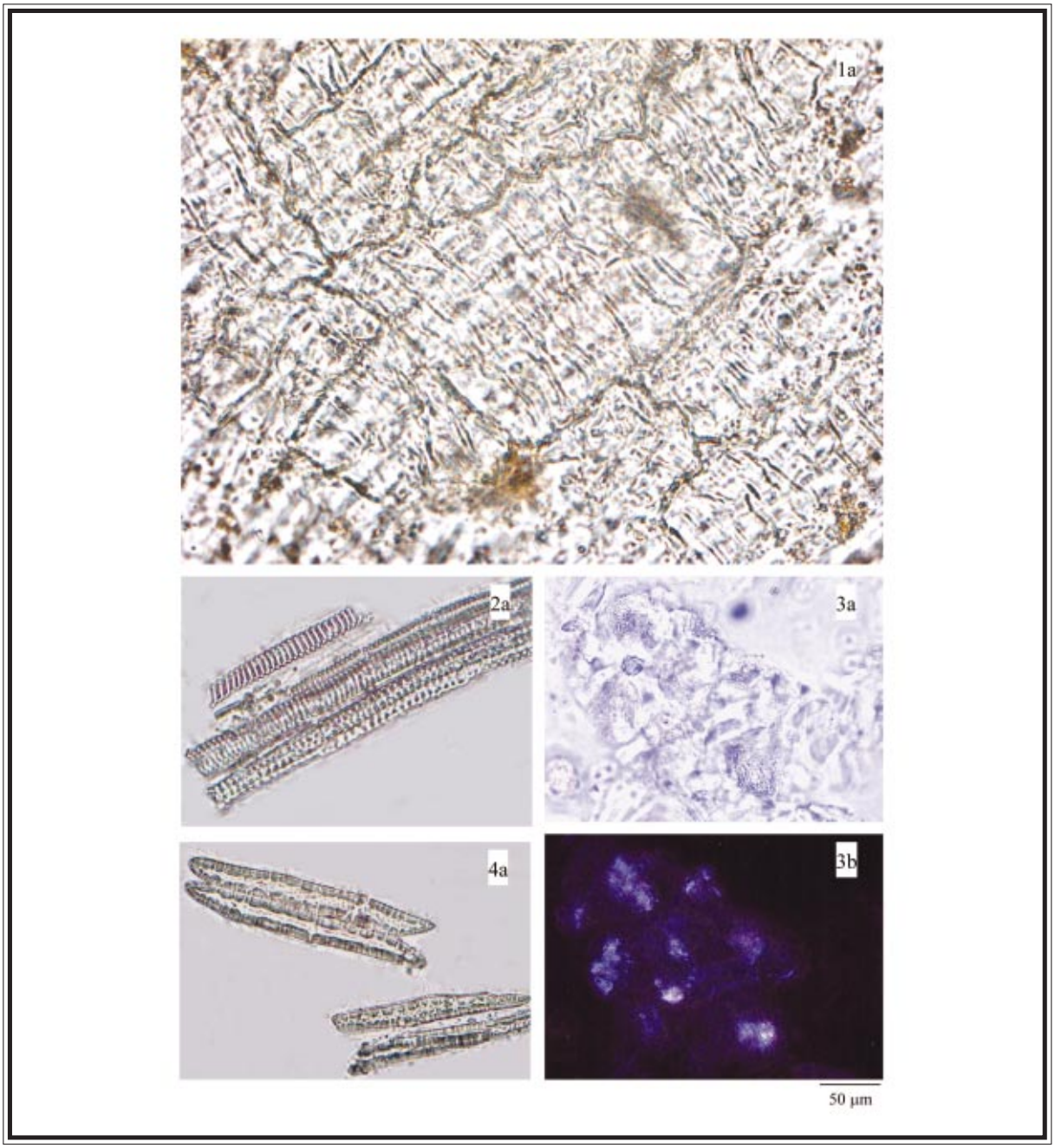


Figure 3(ii) Microscopic features of powder of dried root and rhizome of *Gentiana rigescens* Franch.

1. Endodermis 2. Reticulate, scalariform and spiral vessels 3. Needles of calcium oxalate 4. Fibre-tracheid

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

4.2 Physicochemical Identification

Reagent

Potassium iodobismuthate solution R_1

Dissolve 3.8 g of potassium iodide and 0.85 g of bismuth subnitrate in 35 mL of acetic acid (14%, v/v).

Procedure

Weigh 3.0 g of the powdered sample and put into a 50-mL conical flask, then add 10 mL of methanol. Sonicate (240 W) the mixture for 30 min. Filter and transfer the filtrate to a test tube. Evaporate the solvent to dryness on a water bath. Dissolve the residue in 1 mL of methanol. Transfer 250 μL of the solution to another test tube and add 1 drop of acetic acid (14%, v/v). Add 2 drops of potassium iodobismuthate solution R_1 into the mixture. Allow to stand for about 1 min. An orange precipitate is observed.

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

Standard solution

Gentiopicrin standard solution

Weigh 4.0 mg of gentiopicrin CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, methanol and water (20:2:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and put into a 25-mL conical flask, then add 10 mL of methanol. Sonicate (240 W) the mixture for 45 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Concentrate the mixture to about 1.5 mL at reduced pressure in a rotary evaporator. Transfer the mixture into a 2-mL volumetric flask and make up to the mark with 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. Apply separately gentiopicrin standard solution (3 μL) and the test solution (2 μL) to the plate. Develop over a path of about 7 cm. After the development, remove the plate from the chamber. After drying in air, place the plate in the chamber and re-develop over a path of about 7 cm in the same mobile phase. After the development, remove the

plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). 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 gentiopicrin.

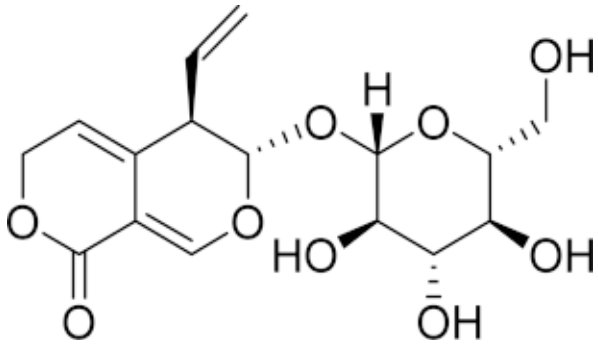


Figure 4 Chemical structure of gentiopicrin

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

Standard solution

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

Weigh 5.0 mg of gentiopicrin CRS and dissolve in 5 mL of methanol.

Gentiopicrin standard solution for fingerprinting, Std-FP (200 mg/L)

Pipette 2 mL of gentiopicrin Std-Stock into a 10-mL volumetric flask and make up to the mark with mixed solvent of 0.4% phosphoric acid and methanol (1:1, v/v).

Test solution

Weigh 0.25 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of mixed solvent of 0.4% phosphoric acid and methanol (1:1, v/v). Sonicate (240 W) the mixture for 45 min. Centrifuge at about 3000 × *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (230 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)	0.4% of Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 5	95 → 94	5 → 6	linear gradient
5 – 40	94 → 40	6 → 60	linear gradient
40 – 55	40 → 0	60 → 100	linear gradient
55 – 60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 20 µL of gentiopicrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of gentiopicrin should not be more than 2.0%; the RSD of the retention time of gentiopicrin peak should not be more than 3.0%; the column efficiency determined from gentiopicrin peak should not be less than 3000 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) or (ii)].

Procedure

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

Table 1 The RRTs and acceptable ranges of the three characteristic peaks of Radix et Rhizoma Gentianae extract

Peak No.	RRT	Acceptable Range
1	0.90	±0.03
2	0.93	±0.03
3 (marker, gentiopicrin)	1.00	-

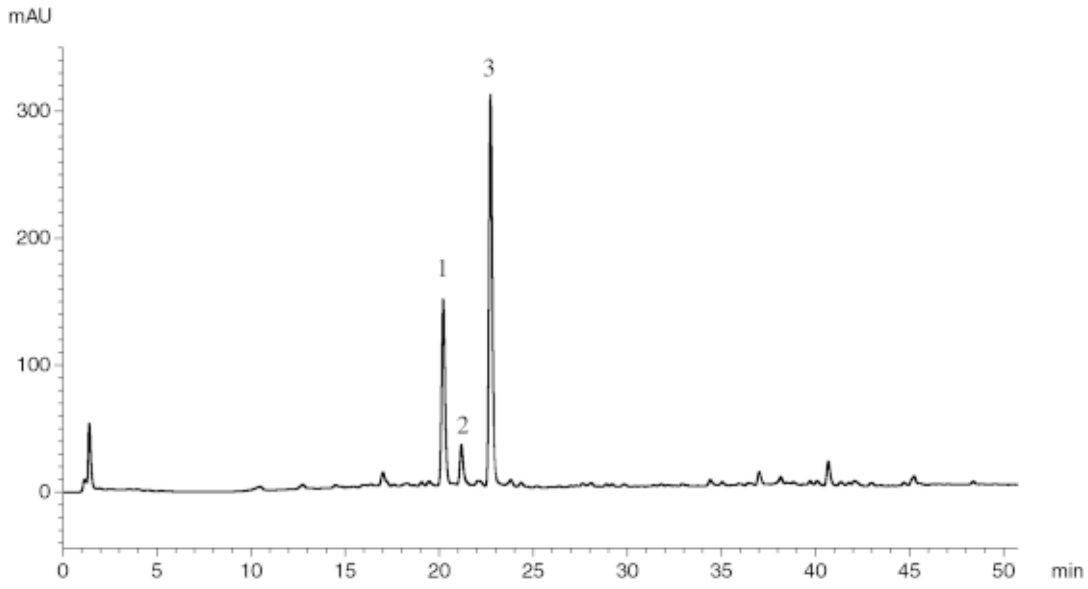


Figure 5(i) A reference fingerprint chromatogram of dried root and rhizome of *Gentiana scabra* Bge. extract

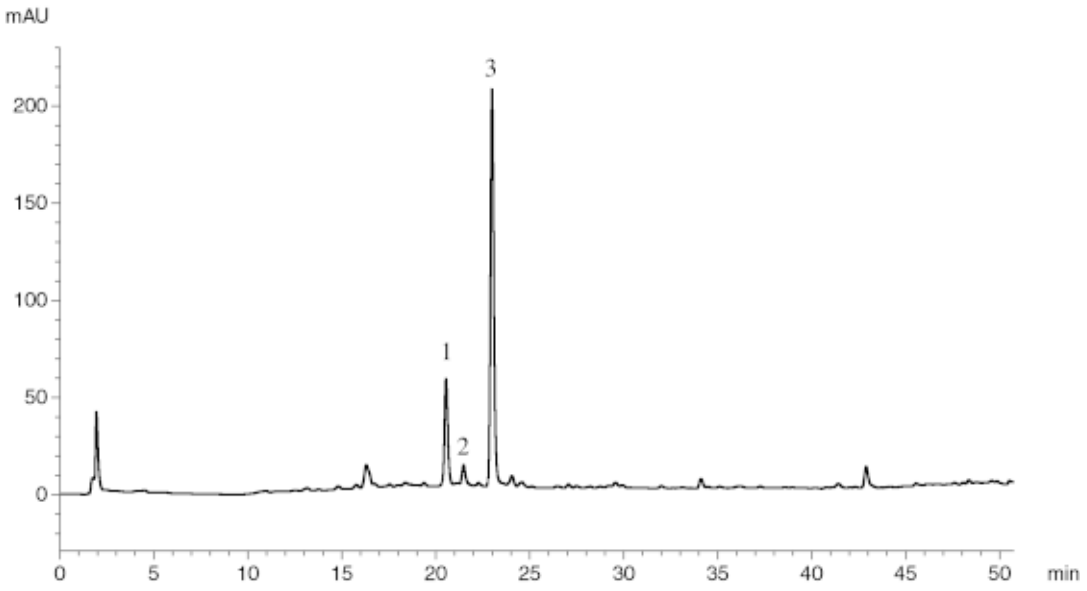


Figure 5(ii) A reference fingerprint chromatogram of dried root and rhizome of *Gentiana rigescens* Franch. extract

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 – Aflatoxins** *(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 7.5%.
Acid-insoluble ash: not more than 3.5%.

5.7 **Water Content** *(Appendix X)*: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 37.0%.
Ethanol-soluble extractives (cold extraction method): not less than 36.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 10.0 mg of gentiopicrin CRS and dissolve in 10 mL of methanol.

Gentiopicrin standard solution for assay, Std-AS

Measure accurately the volume of the gentiopicrin Std-Stock, dilute with methanol to produce a series of solutions of 20, 60, 200, 400, 600 mg/L for gentiopicrin.

Test solution

Weigh accurately 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add 15 mL of mixed solvent of 0.4% phosphoric acid in methanol (1:1, v/v). Sonicate (240 W) the mixture for 45 min. Centrifuge at about 3000 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction twice. Combine the extracts and make up to the mark with same solvent. Mix and filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (270 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)	0.4% of Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 40	90 → 40	10 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 μL of gentiopicrin Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of gentiopicrin should not be more than 5.0%; the RSD of the retention time of gentiopicrin peak should not be more than 2.0%; the column efficiency determined from gentiopicrin peak should not be less than 3000 theoretical plates.

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

Calibration curve

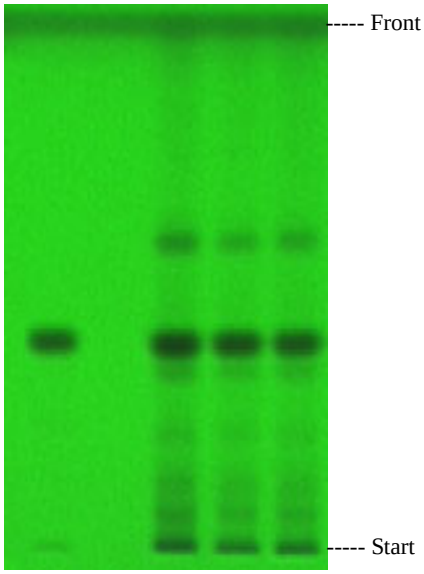
Inject a series of gentiopicrin Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of gentiopicrin against the corresponding concentrations of gentiopicrin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

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

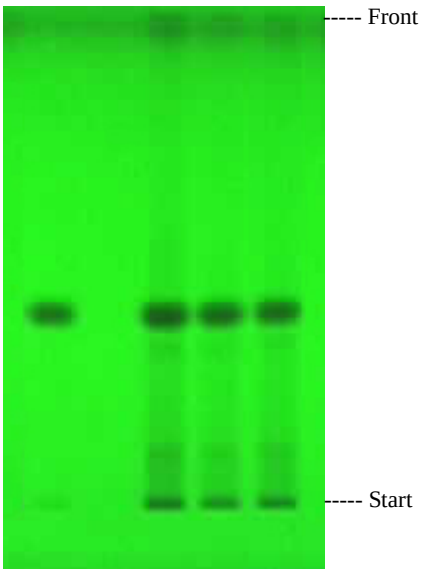
Limits

The sample contains not less than 1.0% of gentiopicrin ($C_{16}H_{20}O_9$), calculated with reference to the dried substance.



Lane	Sample	Results
1	Standard (Gentiopicrin)	Gentiopicrin positive
2	Blank (Methanol)	Negative
3	Spiked sample (Sample plus gentiopicrin)	Gentiopicrin positive
4	Sample (*Gentiana scabra* Bge.)	Gentiopicrin positive
5	Sample duplicate (*Gentiana scabra* Bge.)	Gentiopicrin positive

Figure 1 TLC results of dried root and rhizome of *Gentiana scabra* Bge. extract observed under UV light (254 nm)



Lane	Sample	Results
1	Standard (Gentiopicrin)	Gentiopicrin positive
2	Blank (Methanol)	Negative
3	Spiked sample (Sample plus gentiopicrin)	Gentiopicrin positive
4	Sample (*Gentiana rigescens* Franch.)	Gentiopicrin positive
5	Sample duplicate (*Gentiana rigescens* Franch.)	Gentiopicrin positive

Figure 2 TLC results of dried root and rhizome of *Gentiana rigescens* Franch. extract observed under UV light (254 nm)