Radix Glehniae

Figure 1 A photograph of Radix Glehniae

1. NAMES

Official Name: Radix Glehniae

Chinese Name: 北沙參

Chinese Phonetic Name: Beishashen

2. SOURCE

Radix Glehniae is the dried peeled root of *Glehnia littoralis* Fr. Schmidt ex Miq. (Apiaceae). The root is collected in summer and autumn, the rootlet removed, washed clean, treated with boiling water for 2-3 min, afterwards the cork is peeled, then dried under the sun to obtain Radix Glehniae.

3. DESCRIPTION

Slender-cylindrical, 9-30 cm long, 2-15 mm in diameter. Outer surface pale yellowish-white to yellowish-brown, somewhat rough, the cork often peeled; fine longitudinal wrinkles and grooves, brownish-yellow punctiform protuberances scars of rootlets cover the surface throughout. The top slender, often marked with remnants of yellowish-brown rhizome base, the greater portion of the middle section of the root is thick, then becoming slender again towards the distal end. Texture hard and fragile, easily broken; fracture yellowish-white in the bark and brown in the wood. Odour distinctively fragrant; taste slightly sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cortex consists of several rows of parenchyma cells. Phloem broad, cleft; phloem rays and groups of sieve tubes in the inner part are encrusted with densely arranged secretory canals, each surrounded by 5-8 secretory cells containing yellowish-brown secretions. Cambium prominent, in a ring. Xylem rays broad, vessels occur singly and scattered or arranged in a V-shape configuration. Parenchyma cells contain gelatinous starch masses (Fig. 2).

Powder

Colour yellowish-white. Fragments of secretory canals containing yellowish-brown secretion are found frequently. Yellowish-brown secretion and gelatinous starch masses, their shape irregular, are also found. Vessel elements appear singly or in groups, 16-88 μm in diameter, with reticulate wall thickening, long and wide pits. Parenchyma cells sub-rectangular, abundant (Fig. 3).

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

Standard solution

Falcarinol standard solution

Carefully open the sealed brown ampoule containing 2 mg/mL of falcarinol CRS (Fig. 4). Pipette 500 µL of falcarinol CRS solution into a 2-mL brown volumetric flask and make up to mark with methanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C) and ethyl acetate (5:1, v/v).

Spray reagent

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

Test solution

Weigh 3.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of ethanol. Sonicate (240 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 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. Apply separately falcarinol standard solution (4 µL) and the test solution (6 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. 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 falcarinol.

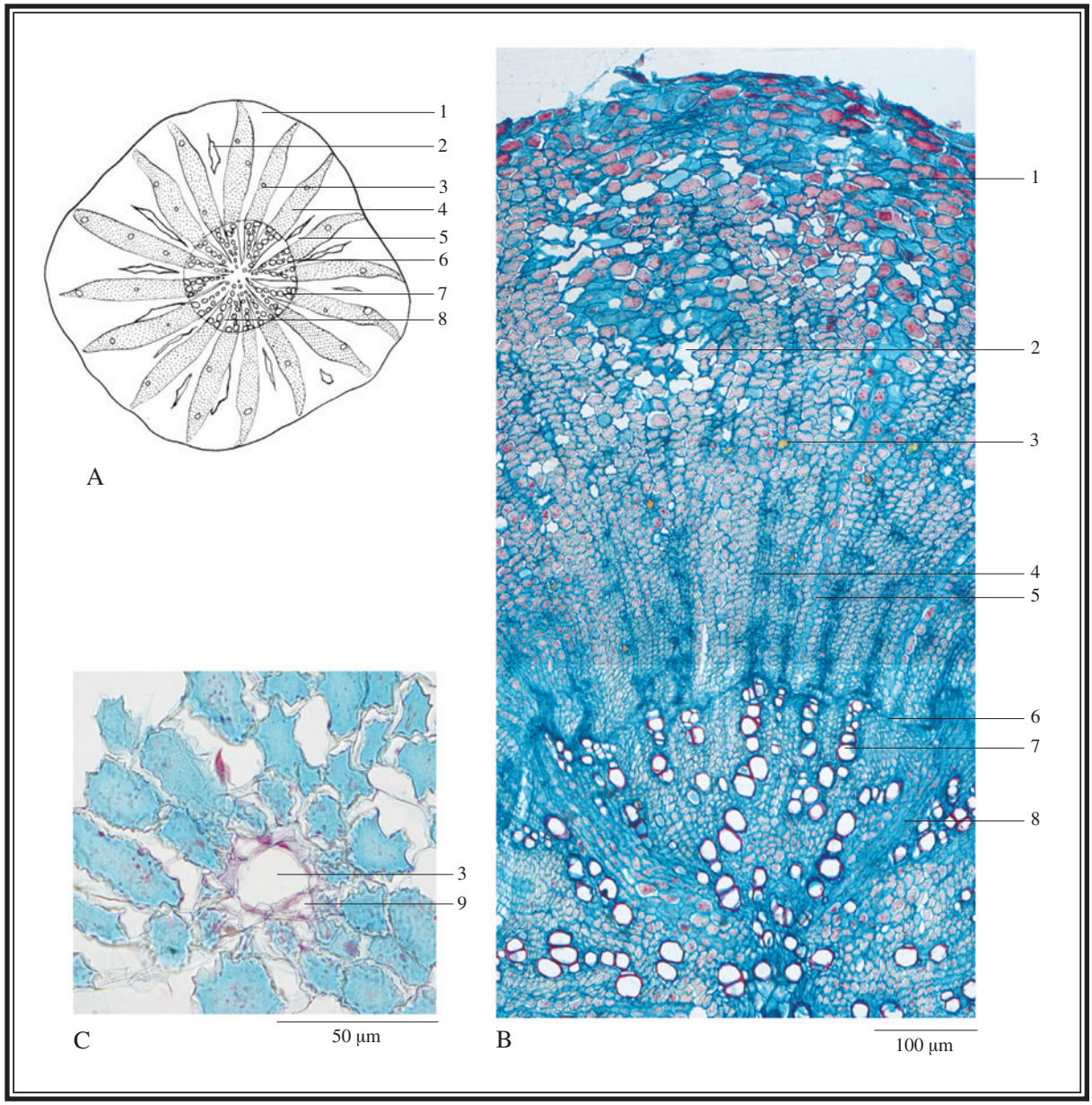


Figure 2 Microscopic features of transverse section of Radix Glehniae

A. Sketch B. Section illustration C. Secretory canals

1. Cortex 2. Cleft 3. Secretory canal 4. Phloem 5. Phloem ray
6. Cambium 7. Xylem 8. Xylem ray 9. Secretory cell

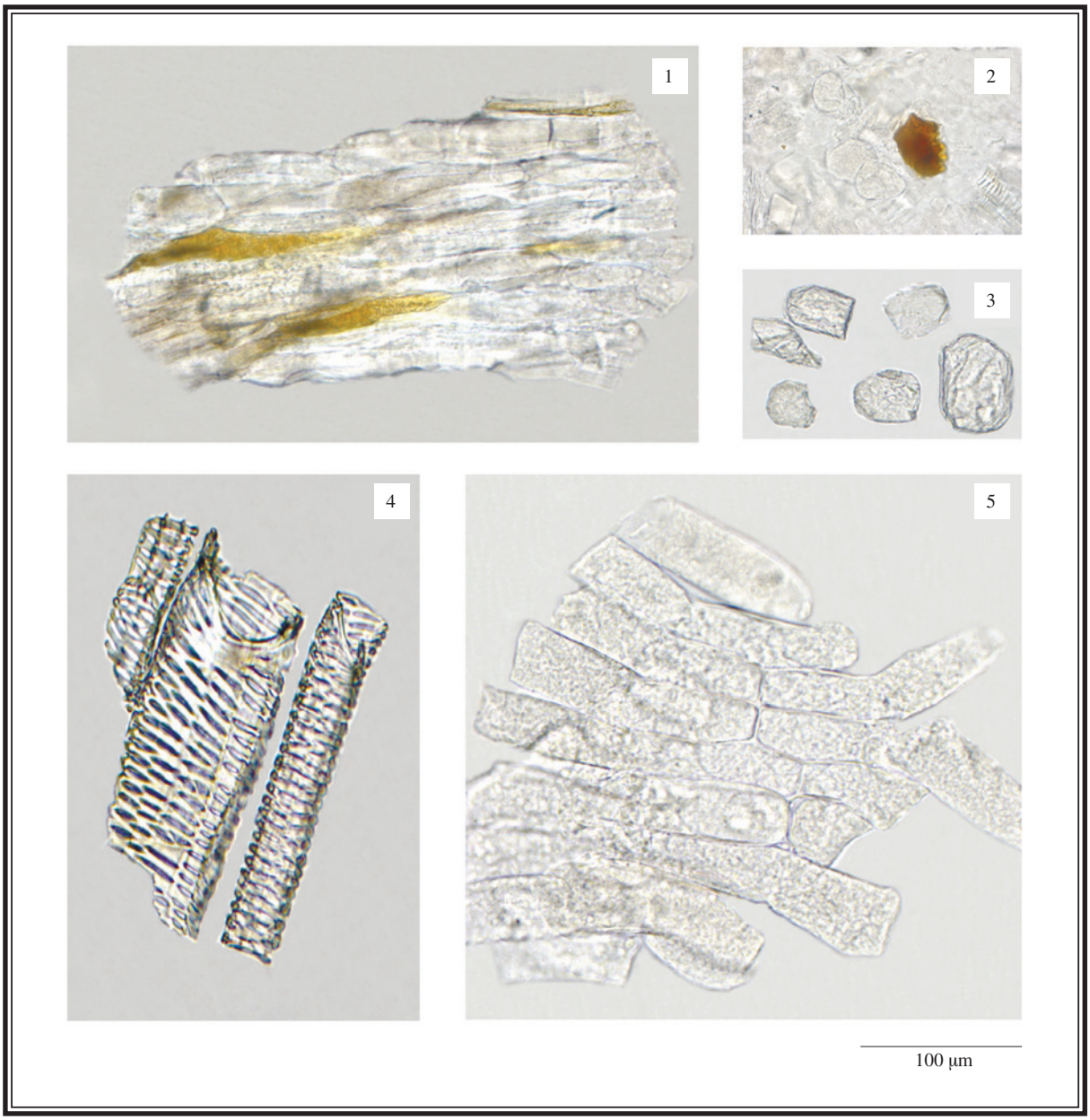


Figure 3 Microscopic features of powder of Radix Glehniae (under the light microscope)

1. Secretory canals 2. Yellowish-brown secretion 3. Starch gelatinous masses
4. Reticulate vessels 5. Parenchyma cells

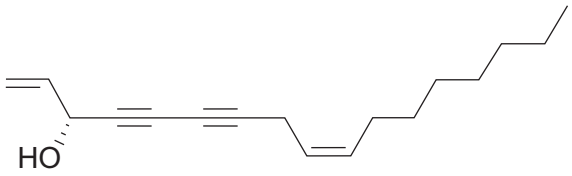


Figure 4 Chemical structure of falcarinol

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

Standard solution

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

Carefully open the sealed brown ampoule containing 2 mg/mL of falcarinol CRS. Pipette 1 mL of falcarinol CRS solution into a 10-mL brown volumetric flask and make up to mark with methanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of dichloromethane. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 150-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatant. Evaporate the solvent to dryness at about 50˚C at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (260 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–25	53→40	47→60	linear gradient
25–60	40→15	60→85	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of falcarinol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of falcarinol should not be more than 5.0%; the RSD of the retention time of falcarinol peak should not be more than 2.0%; the column efficiency determined from falcarinol peak should not be less than 30000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject falcarinol Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of falcarinol peak in the chromatogram of falcarinol Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify falcarinol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of falcarinol Std-FP. The retention times of falcarinol 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 Glehniae extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Radix Glehniae extract

Peak No.	RRT	Acceptable Range
1	0.61	±0.03
2	0.67	±0.03
3	0.79	±0.03
4 (marker, falcarinol)	1.00	-

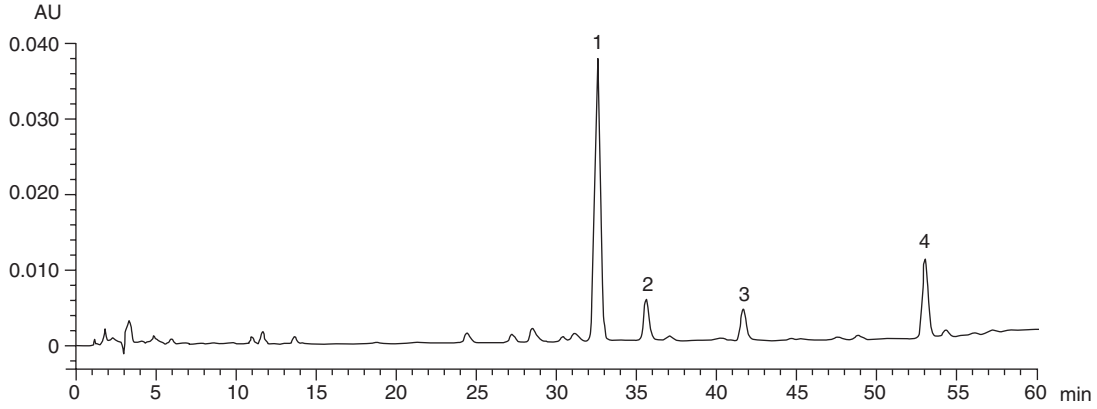


Figure 5 A reference fingerprint chromatogram of Radix Glehniae extract

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 reference fingerprint chromatogram (Fig. 5).

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 XV)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 0.5%.

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

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 22.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

Falcarinol standard stock solution, Std-Stock (400 mg/L)

Carefully open the sealed brown ampoule containing 2 mg/mL of falcarinol CRS. Pipette 2 mL of falcarinol CRS solution into a 10-mL brown volumetric flask and make up to mark with methanol.

Falcarinol standard solution for assay, Std-AS

Measure accurately the volume of the falcarinol Std-Stock, dilute with methanol to produce a series of solutions of 40, 100, 150, 200, 300 mg/L for falcarinol.

Test solution

Weigh accurately 3.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of methanol. Reflux the mixture for 1.5 h. Cool to room temperature. Filter and transfer the filtrate to a 150-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-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 DAD (205 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. The mobile phase is a mixture of acetonitrile and water (72:28, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of falcarinol Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of falcarinol should not be more than 5.0%; the RSD of the retention time of falcarinol peak should not be more than 2.0%; the column efficiency determined from falcarinol peak should not be less than 2500 theoretical plates.

The *R* value between falcarinol 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 falcarinol Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of falcarinol against the corresponding concentrations of falcarinol Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

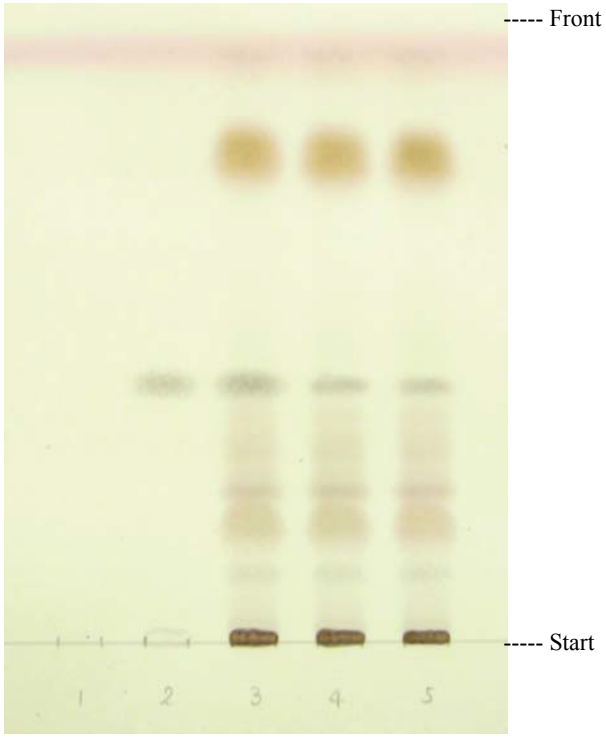
Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify falcarinol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of falcarinol Std-AS. The retention times of falcarinol 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 falcarinol in the test solution, and calculate the percentage content of falcarinol in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.023% of falcarinol ($C_{17}H_{24}O$), calculated with reference to the dried substance.

Radix Glehniae (北沙參)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Falcarinol)	Falcarinol positive
3	Spiked sample (Sample plus falcarinol)	Falcarinol positive
4	Sample (Radix Glehniae)	Falcarinol positive
5	Sample duplicate (Radix Glehniae)	Falcarinol positive

Figure 1 TLC results of Radix Glehniae extract observed under visible light after staining