Gleditsiae Spina

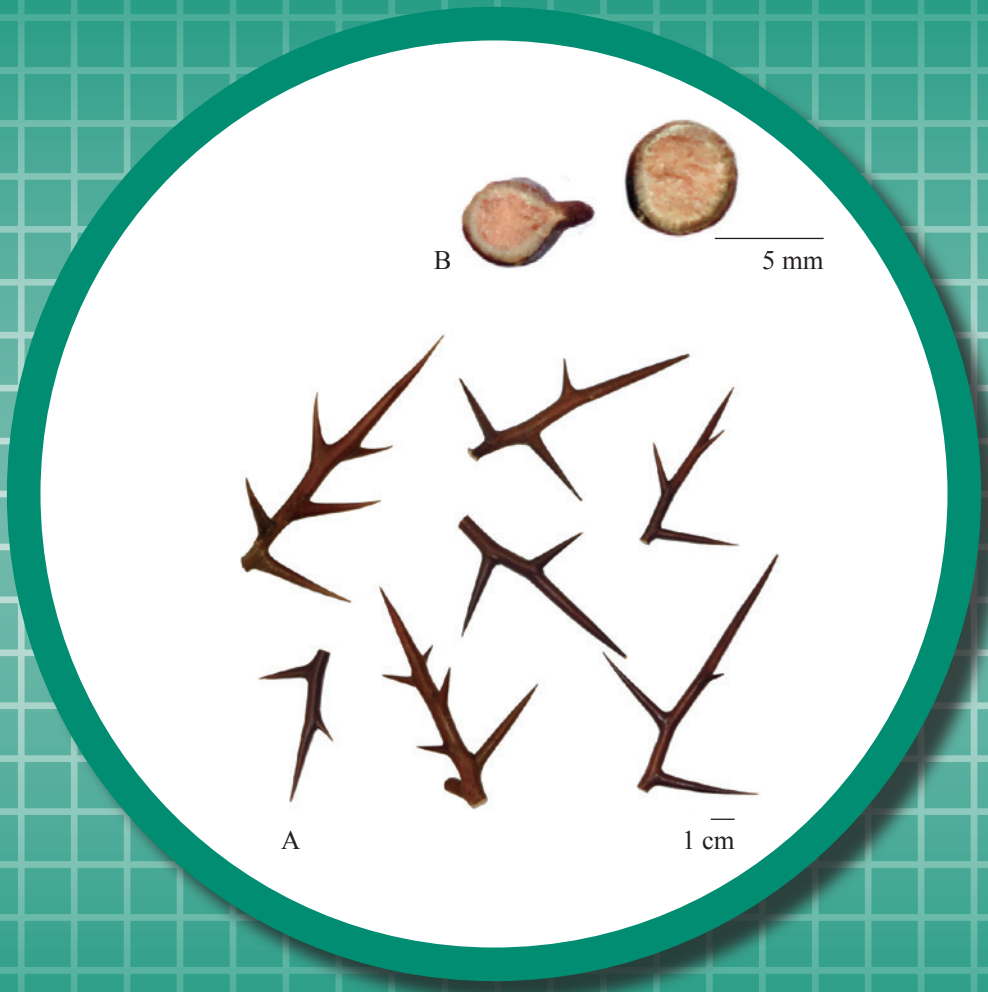

Figure 1 A photograph of Gleditsiae Spina

A. Gleditsiae Spina B. Magnified transverse sections of main spine

1. NAMES

Official Name: Gleditsiae Spina

Chinese Name: 皂角刺

Chinese Phonetic Name: Zaojiaoci

2. SOURCE

Gleditsiae Spina is the dried spine of *Gleditsia sinensis* Lam. (Fabaceae). The spine is collected all the year round, dried under the sun; or cut into slices when fresh, then dried under the sun to obtain Gleditsiae Spina.

3. DESCRIPTION

Spines (main and 1-2 branched spines) reddish-brown to dark-brown. Main spines long conical, 0.5-17.2 cm long, 1-9 mm in diameter, acute at apex. Branched spines 0.3-6.4 cm long. Smooth or with fine wrinkles on the surface. Fracture fibrous, wood yellowish-white to yellowish-brown; pith lax, pale reddish-brown. Texture hard and light in weight. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of cells, covered with cuticle, unicellular non-glandular hairs occasionally found. Cortex consists of 2-3 layers of parenchymatous cells, some containing reddish-brown contents. Pericycle fibre bundles arranged in an interrupted ring, prisms of calcium oxalate sometimes present in the surrounding parenchymatous cells, clusters of calcium oxalate rare. Stone cells mostly singly scattered or 2-3 in groups. Phloem narrow. Xylem consists of lignified cells; xylem rays consist of 1-2 rows of cells. Pith broad, some parenchymatous cells contain reddish-brown contents (Fig. 2).

Powder

Colour yellowish-brown to reddish-brown. Prisms of calcium oxalate subrectangular or irregular, 3-22 μm in diameter; bright white or polychromatic under the polarized microscope. Clusters of calcium oxalate occasionally found; polychromatic under the polarized microscope. Stone cells scattered or 2-3 in groups, oblong or subrounded, 17-120 μm long, 11-58 μm in diameter, walls thickened, pit canals distinct, striations visible; bright white or yellowish-white under the polarized microscope. Fibres single or in bundles, single fibres slender, 4-35 μm in diameter, fibre bundles sometimes surrounded by cells contain prisms of calcium oxalate, forming crystal fibres; yellowish-white under the polarized microscope. Epidermal cells subsquare or polygonal in surface view, containing yellowish-brown contents. Non-glandular hairs occasionally found, unicellular, with warty protuberance on the surface. Vessels rarely found, mainly spiral (Fig. 3).

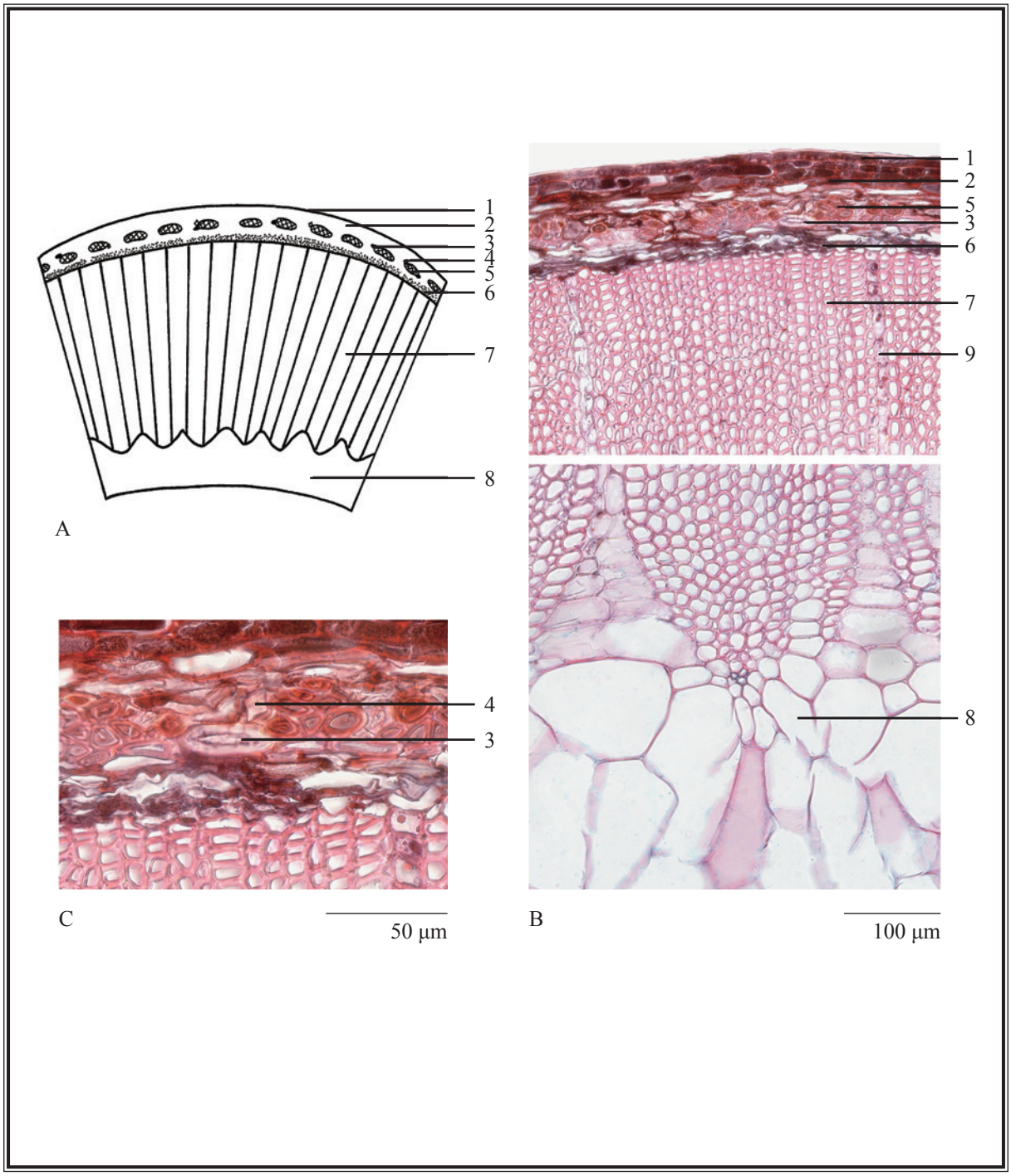

Figure 2 Microscopic features of transverse section of Gleditsiae Spina

A. Sketch B. Section illustration C. Prism of calcium oxalate and stone cell

1. Epidermis 2. Cortex 3. Stone cell 4. Prism of calcium oxalate
5. Pericycle fibre bundle 6. Phloem 7. Xylem 8. Pith 9. Xylem ray

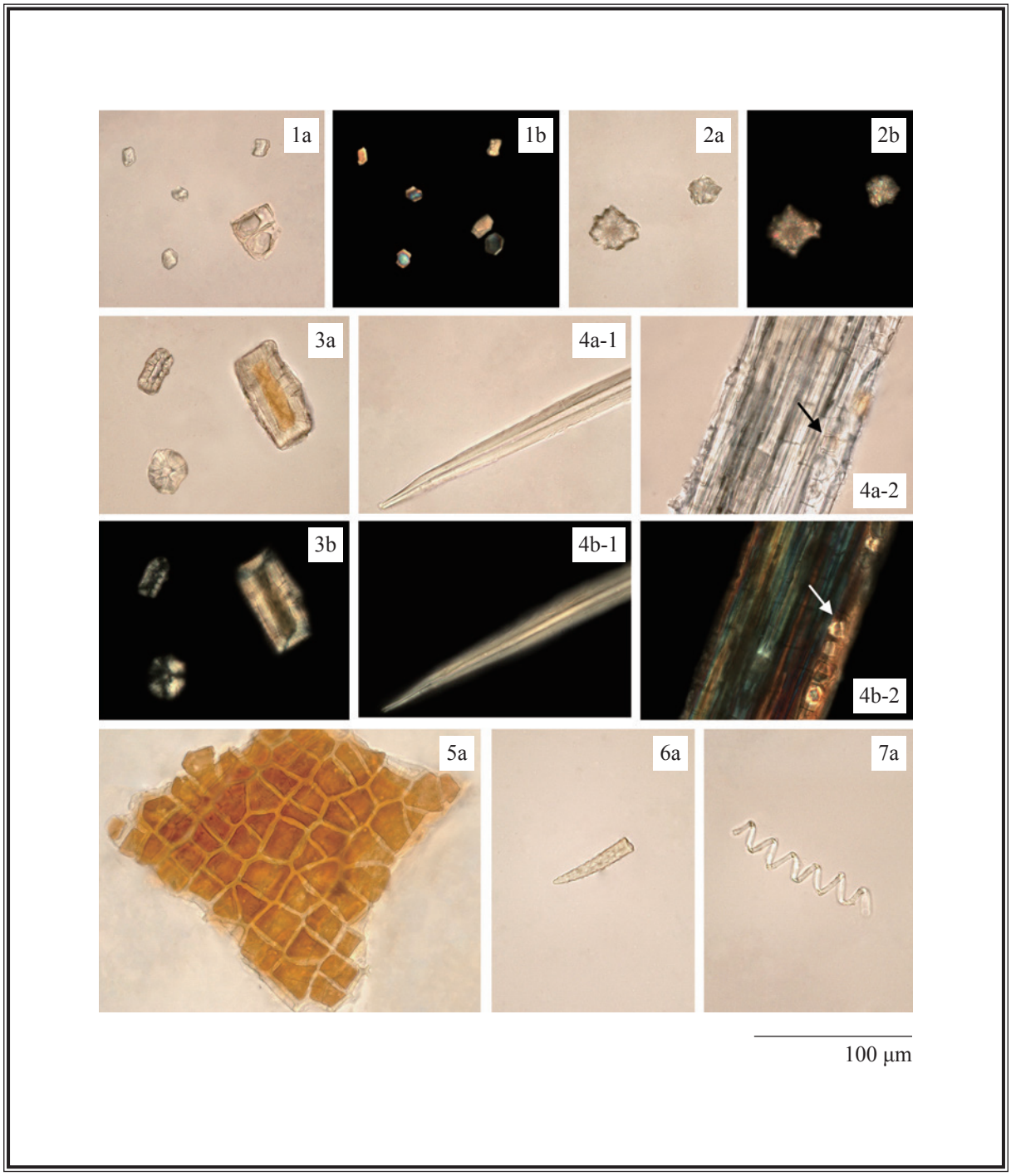

Figure 3 Microscopic features of powder of Gleditsiae Spina

1. Prisms of calcium oxalate 2. Clusters of calcium oxalate 3. Stone cells
4. Fibre (4-1 fibre, 4-2 crystal fibres, prism of calcium oxalate ⟶)
5. Epidermal cells 6. Non-glandular hair 7. Vessel

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

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

Standard solution

Taxifolin standard solution

Weigh 1.0 mg of taxifolin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane and glacial acetic acid (15:10:1, v/v).

Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of water. Transfer the solution to a 50-mL centrifuge tube. Add 10 mL of ethyl acetate. Centrifuge at about 2800 × *g* for 10 min. Transfer the upper layer to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately taxifolin standard solution and the test solution (4 μL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 6 cm. 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).

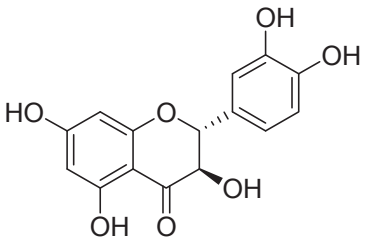

Figure 4 Chemical structure of taxifolin

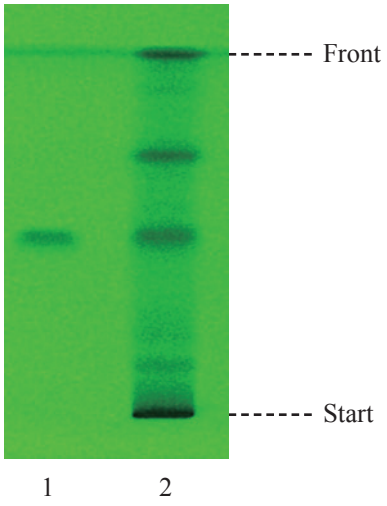

Figure 5 A reference HPTLC chromatogram of Gleditsiae Spina extract observed under UV light (254 nm)

1. Taxifolin standard solution 2. Test solution

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 taxifolin (Fig. 5).

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

Standard solution

Taxifolin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.5 mg of taxifolin CRS and dissolve in 50 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (288 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 50	88 → 75	12 → 25	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of taxifolin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of taxifolin should not be more than 5.0%; the RSD of the retention time of taxifolin peak should not be more than 2.0%; the column efficiency determined from taxifolin peak should not be less than 20000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject taxifolin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of taxifolin peak in the chromatogram of taxifolin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify taxifolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of taxifolin Std-FP. The retention times of taxifolin 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 Gleditsiae Spina extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Gleditsiae Spina extract

Peak No.	RRT	Acceptable Range
1 (marker, taxifolin)	1.00	-
2	1.05	± 0.03
3	1.10	± 0.03

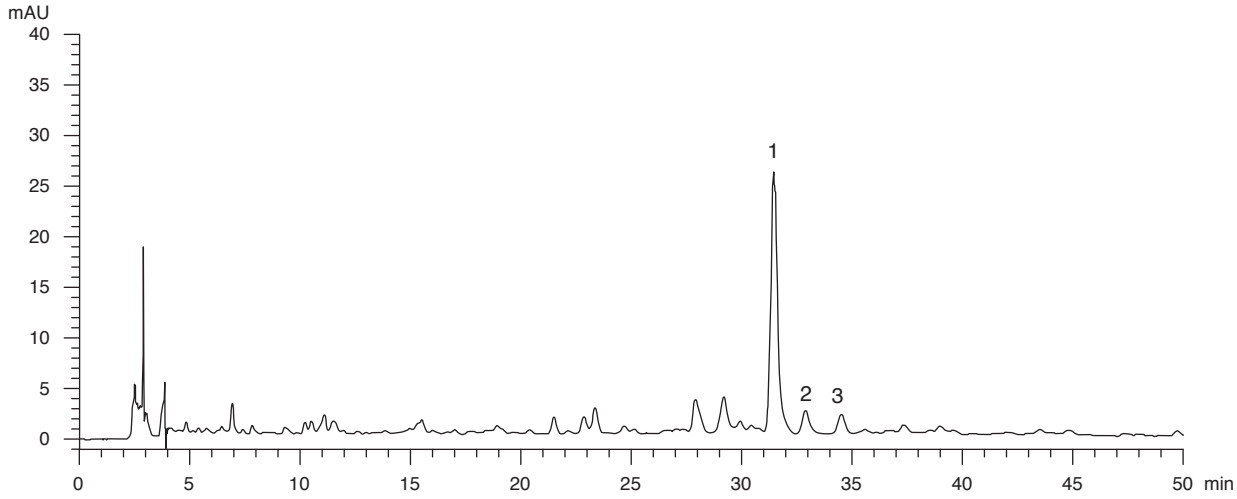

Figure 6 A reference fingerprint chromatogram of Gleditsiae Spina extract

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 reference fingerprint chromatogram (Fig. 6).

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 XVII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 3.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 5.0 mg of taxifolin CRS and dissolve in 10 mL of methanol (70%).

Taxifolin standard solution for assay, Std-AS

Measure accurately the volume of the taxifolin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 2.5, 5, 10, 50, 100 mg/L for taxifolin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (288 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 50	88 → 75	12 → 25	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of taxifolin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of taxifolin should not be more than 5.0%; the RSD of the retention time of taxifolin peak should not be more than 2.0%; the column efficiency determined from taxifolin peak should not be less than 20000 theoretical plates.

The *R* value between taxifolin 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 taxifolin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of taxifolin against the corresponding concentrations of taxifolin 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 taxifolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of taxifolin Std-AS. The retention times of taxifolin 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 taxifolin in the test solution, and calculate the percentage content of taxifolin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.063% of taxifolin ($C_{15}H_{12}O_7$), calculated with reference to the dried substance.