

Fibraureae Caulis

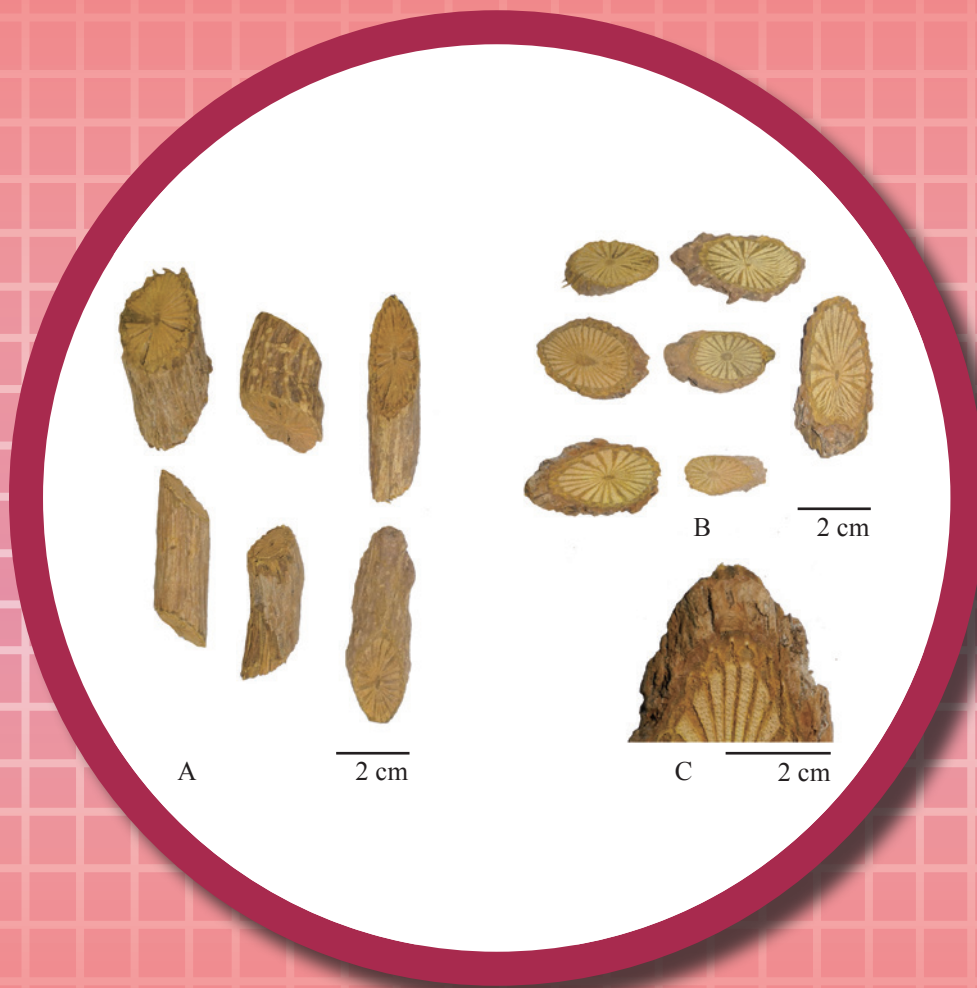


Figure 1 A photograph of Fibraureae Caulis

A. Fibraureae Caulis B. Oblique slices

C. Magnified image of transverse section of stem

1. NAMES

Official Name: *Fibraureae Caulis*

Chinese Name: 黃藤

Chinese Phonetic Name: Huangteng

2. SOURCE

Fibraureae Caulis is the dried lianoid stem of *Fibraurea recisa* Pierre (Menispermaceae). The lianoid stem is collected in autumn and winter, cut into section when fresh, then dried under the sun to obtain *Fibraureae Caulis*.

3. DESCRIPTION

Long cylindrical, slightly twisted, 6-30 mm in diameter. Externally greyish-brown to yellowish-brown, rough, with longitudinal furrows and transverse cracks, outer bark of the old stem easily fallen off. Texture hard, uneasily broken, dusting on breaking, fracture uneven, yellow, fibrous, showing radially arranged striations alternated with brownish-yellow and yellowish-brown colour, pores of vessels fine, xylem sometimes with cracks. Centre yellowish-brown or hollowed. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (*Appendix III*)

Transverse section

Cork consists of numerous layers of cells containing brown contents, sometimes the walls lignified and thickened. Cortex consists of several layers of flattened cells scattered with stone cell groups. Vascular bundles collateral. Fibres in bundle, scattered on the outside of phloem and inside of xylem; the ones outside form a sharply undulated ring circling the stem. Vessels large, up to about 30 µm in diameter. Xylem rays broad. Pith broad. Prisms of calcium oxalate scattered in the stone cells or in the neighboring cells of stone cells and fibre bundles. Parenchymatous cells filled with starch granules (Fig. 2).

Powder

Colour pale yellow. Stone cells scattered singly or in groups, subsquare or polygonal, 40-120 μm in diameter; polychromatic under the polarized microscope. Cork cells yellowish-brown, subpolygonal in surface view, some with lignified and thickened walls. Woody fibres mostly in bundles, with thick walls. Vessels mostly bordered-pitted, usually broken, intact vessels up to 150 μm in diameter. Medullary ray cells rectangular. Prisms of calcium oxalate 20-40 μm in diameter; bright white or polychromatic under the polarized microscope. Starch granules subrounded or oblong, hilum pointed, usually compound starch granules; black and cruciated-shaped under the polarized microscope (Fig. 3).

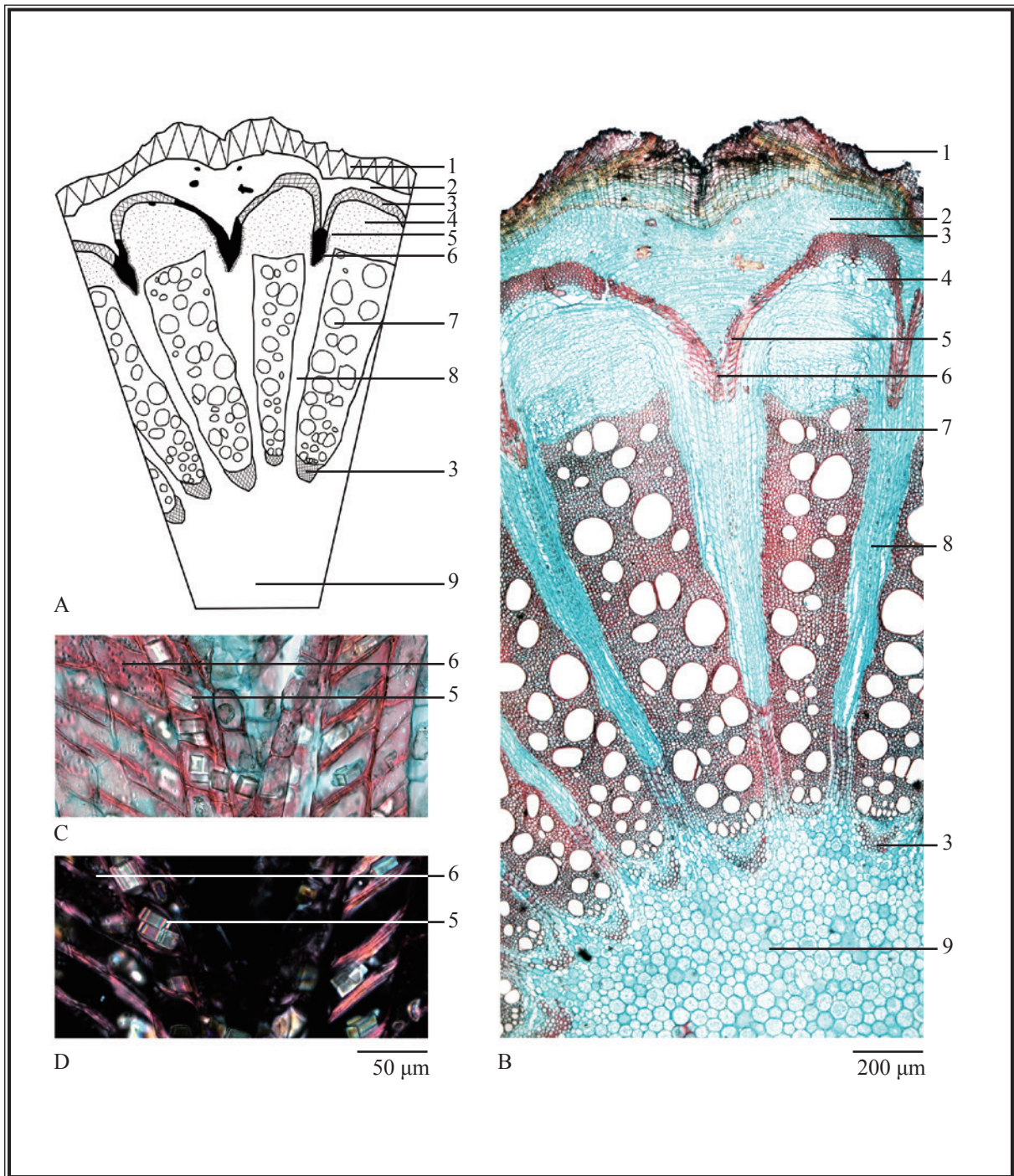


Figure 2 Microscopic features of transverse section of *Fibraureae Caulis*

A. Sketch B. Section illustration

C. Prisms of calcium oxalate (under the light microscope)

D. Prisms of calcium oxalate (under the polarized microscope)

1. Cork 2. Cortex 3. Fibre 4. Phloem 5. Prism of calcium oxalate

6. Stone cell 7. Xylem 8. Xylem ray 9. Pith

山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

天山雪蓮

白花丹

Polygoni Perfoliati Herba

杠板歸

北豆根

Menispermii Rhizoma

Loniceræ Flos

山銀花

Plantaginis Herba

Bruceae Fructus 鴉膽子

Plumbaginis Zeylanicae Radix

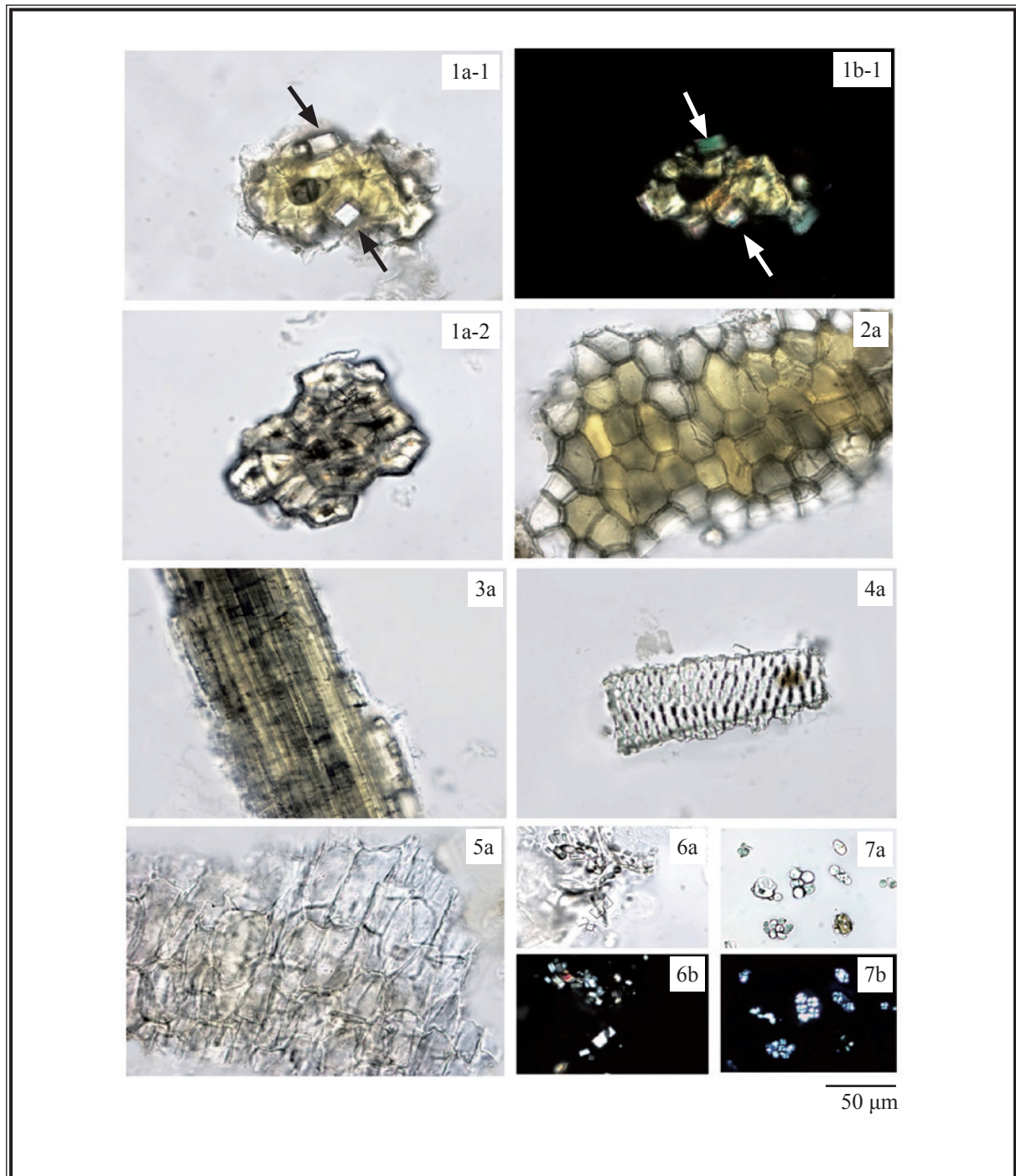
Fibraureae Caulis

Figure 3 Microscopic features of powder of *Fibraureae Caulis*

1. Stone cells (1-1 with prisms of calcium oxalate →, 1-2 in group)
2. Cork cells
3. Fibres
4. Bordered-pitted vessel
5. Medullary ray cells
6. Prisms of calcium oxalate
7. Starch granules

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

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

Standard solutions

Jatrorrhizine chloride standard solution

Weigh 0.5 mg of jatrorrhizine chloride CRS (Fig. 4) and dissolve in 5 mL of methanol.

Palmatine chloride standard solution

Weigh 0.5 mg of palmatine chloride CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of *n*-butanol, water and glacial acetic acid (7:2:1, v/v).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (100 W) the mixture for 20 min. Centrifuge at about $2000 \times g$ for 5 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately jatrorrhizine chloride standard solution, palmatine chloride standard solution and the test solution (2 μ L 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 6.5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

Polygoni Perfoliati Herba

Loniceræ Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

天山雪蓮

白花丹

杠板歸

北豆根
Menispermī Rhizoma

山銀花

Plumbaginis Zeylanicae Radix

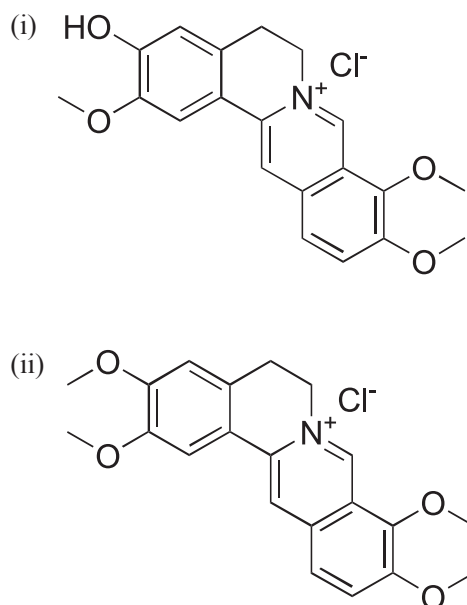
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Figure 4 Chemical structures of (i) jatrorrhizine chloride and (ii) palmatine chloride

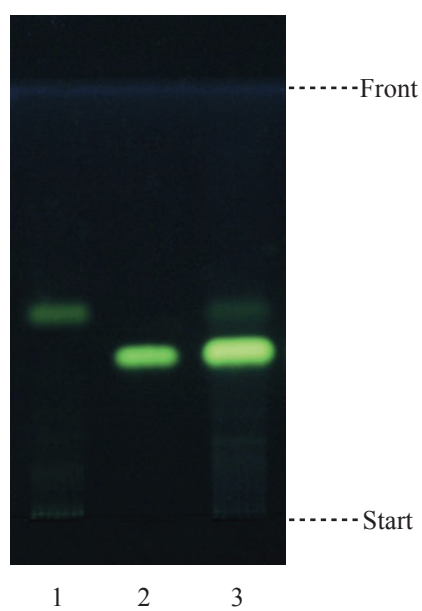


Figure 5 A reference HPTLC chromatogram of *Fibraureae Caulis* extract observed under UV light (366 nm)

1. Jatrorrhizine chloride standard solution 2. Palmatine chloride standard solution 3. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of jatrorrhizine and palmatine (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solutions

Jatrorrhizine chloride standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 0.6 mg of jatrorrhizine chloride CRS and dissolve in 2 mL of a mixture of methanol and hydrochloric acid (99:1, v/v).

Palmatine chloride standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 0.6 mg of palmatine chloride CRS and dissolve in 2 mL of a mixture of methanol and hydrochloric acid (99:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of a mixture of methanol and hydrochloric acid (99:1, v/v). Sonicate (100 W) the mixture for 1 h. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (225 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m particle size). The column temperature is maintained at 30°C 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)	0.4% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 60	85 \rightarrow 70	15 \rightarrow 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μ L of jatrorrhizine chloride Std-FP and palmatine chloride Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of jatrorrhizine and palmatine should not be more than 5.0%; the RSD of the retention times of jatrorrhizine and palmatine peaks should not be more than 2.0%; the column efficiencies determined from jatrorrhizine and palmatine peaks should not be less than 8000 and 3000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject jatrorrhizine chloride Std-FP, palmatine chloride Std-FP and the test solution (5 μ L each) into the HPLC system and record the chromatograms. Measure the retention times of jatrorrhizine and palmatine peaks in the chromatograms of jatrorrhizine chloride Std-FP, palmatine chloride Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify jatrorrhizine and palmatine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of jatrorrhizine chloride Std-FP and palmatine chloride Std-FP. The retention times of jatrorrhizine and palmatine peaks in the chromatograms of the test solution and the corresponding Std-FP 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 *Fibraureae Caulis* extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of *Fibraureae Caulis* extract

Peak No.	RRT	Acceptable Range
1 (jatrorrhizine)	0.76	± 0.03
2 (marker, palmatine)	1.00	-
3	1.20	± 0.03
4	1.49	± 0.03

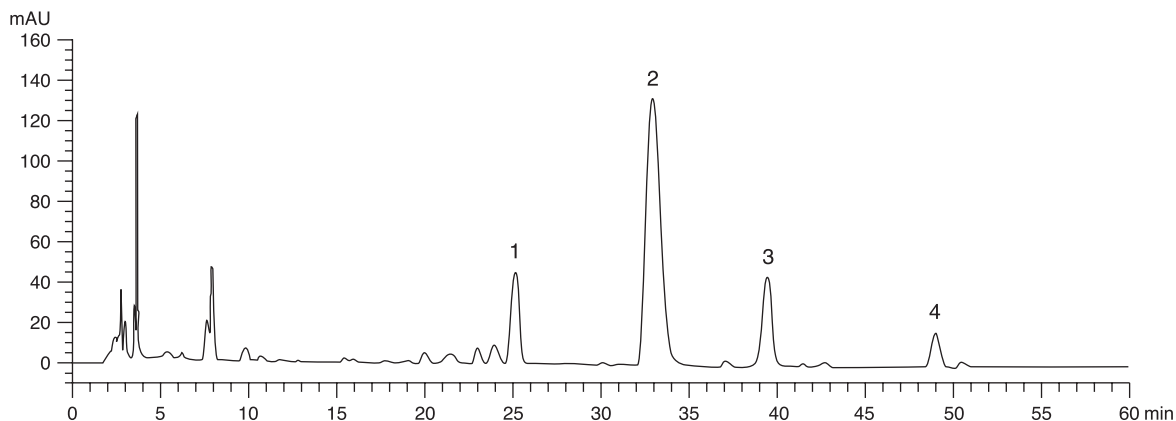


Figure 6 A reference fingerprint chromatogram of *Fibraureae Caulis* extract

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. 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 XVI*): meet the requirements.

5.5 Foreign Matter (*Appendix VIII*): not more than 3.0%.

5.6 Ash (*Appendix IX*)

Total ash: not more than 7.0%.

Acid-insoluble ash: not more than 2.0%.

5.7 Water Content (*Appendix X*)

Oven dried method: not more than 11.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 13.0%.

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

Mixed jatrorrhizine chloride and palmatine chloride standard stock solution, Std-Stock (200 mg/L for jatrorrhizine chloride and 800 mg/L for palmatine chloride)

Weigh accurately 1.0 mg of jatrorrhizine chloride CRS and 4.0 mg of palmatine chloride CRS, and dissolve in 5 mL of a mixture of methanol and hydrochloric acid (99:1, v/v).

Mixed jatrorrhizine chloride and palmatine chloride standard solution for assay, Std-AS

Measure accurately the volume of the mixed jatrorrhizine chloride and palmatine chloride Std-Stock, dilute with a mixture of methanol and hydrochloric acid (99:1, v/v) to produce a series of solutions of 10, 30, 40, 60, 100 mg/L for jatrorrhizine chloride and 120, 160, 200, 240, 400 mg/L for palmatine chloride.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 45 mL of a mixture of methanol and hydrochloric acid (99:1, v/v). Sonicate (90 W) the mixture for 1 h. Centrifuge at about $3000 \times g$ for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with a mixture of methanol and hydrochloric acid (99:1, v/v). Combine the solutions and make up to the mark with a mixture of methanol and hydrochloric acid (99:1, v/v). Filter through a 0.45- μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (345 nm) and a column (4.6 \times 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Trifluoroacetic acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 10	75 → 70	25 → 30	linear gradient
10 – 30	70 → 30	30 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μ L of the mixed jatrorrhizine chloride and palmatine chloride Std-AS (40 mg/L for jatrorrhizine chloride and 200 mg/L for palmatine chloride). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of jatrorrhizine and palmatine should not be more than 5.0%; the RSD of the retention times of jatrorrhizine and palmatine peaks should not be more than 2.0%; the column efficiencies determined from jatrorrhizine and palmatine peaks should not be less than 37000 and 66000 theoretical plates respectively.

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

Calibration curves

Inject a series of the mixed jatrorrhizine chloride and palmatine chloride Std-AS (5 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of jatrorrhizine and palmatine against the corresponding concentrations of the mixed jatrorrhizine chloride and palmatine chloride Std-AS. Obtain the slopes, *y*-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 μ L of the test solution into the HPLC system and record the chromatogram. Identify jatrorrhizine and palmatine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed jatrorrhizine chloride and palmatine chloride Std-AS. The retention times of jatrorrhizine and palmatine peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of jatrorrhizine chloride and palmatine chloride in the test solution, and calculate the percentage contents of jatrorrhizine (the percentage content of jatrorrhizine chloride \times 0.91, where 0.91 is the molar mass ratio of jatrorrhizine and jatrorrhizine chloride) and palmatine (the percentage content of palmatine chloride \times 0.91, where 0.91 is the molar mass ratio of palmatine and palmatine chloride) in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 2.0% of the total content of jatrorrhizine (C₂₀H₂₀NO₄) and palmatine (C₂₁H₂₂NO₄), calculated with reference to the dried substance.