Sargentodoxae Caulis

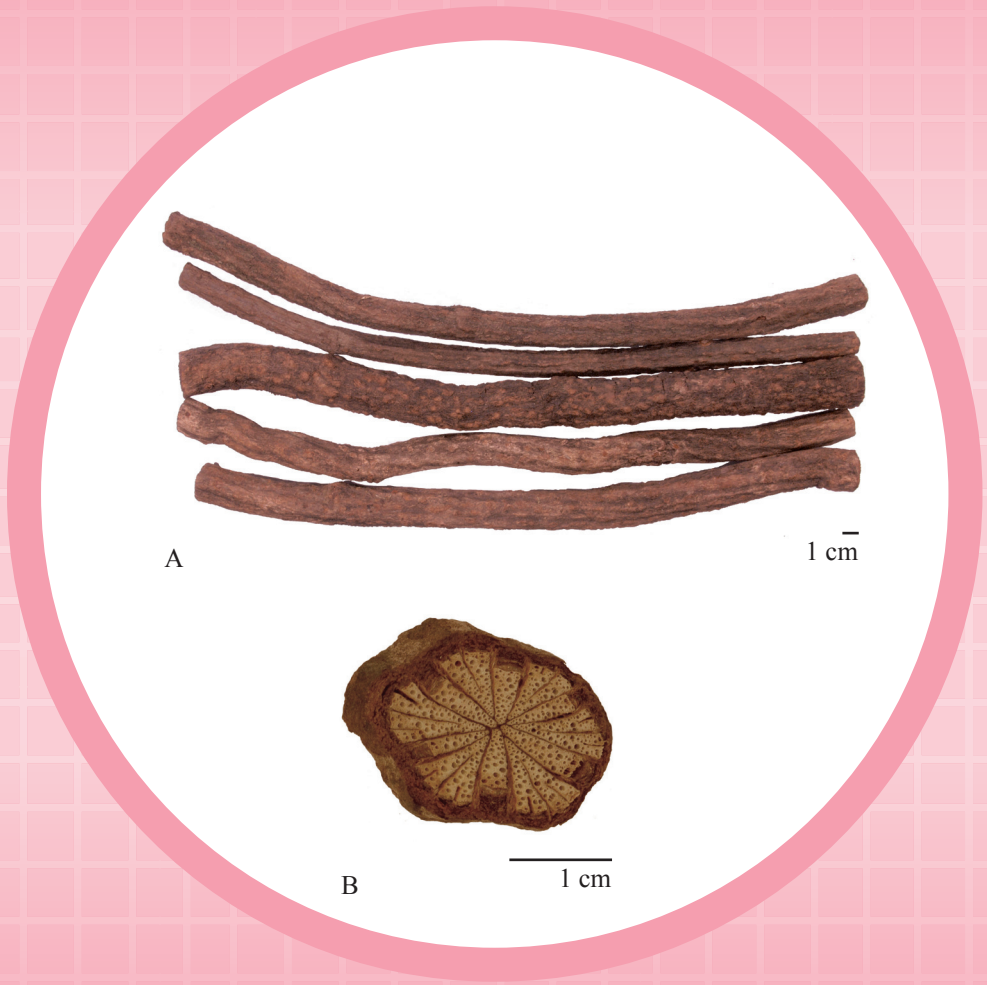


Figure 1 A photograph of Sargentodoxae Caulis

A. Sargentodoxae Caulis B. Magnified image of transverse section of stem

1. NAMES

Official name: Sargentodoxae Caulis

Chinese name: 大血藤

Chinese phonetic name: Daxueteng

2. SOURCE

Sargentodoxae Caulis is the dried lianoid stem of *Sargentodoxa cuneata* (Oliv.) Rehd. et Wils. (Lardizabalaceae). The stem is collected in autumn and winter. Removed from lateral branch, cut into sections, then dried under the sun to obtain Sargentodoxae Caulis.

3. DESCRIPTION

Cylindrical, slightly curved, up to 60 cm long, 10-30 mm in diameter. Externally greyish-brown, rough, the outer layer often scaling off, the exposed surface dark reddish-brown, sometimes showing inflated nodes and slightly dented branch scars or leaf scars. Texture hard, in fracture, bark reddish-brown, some inlaid into the wood, wood yellowish-white, with numerous fine pores of vessels and rays arranged radially. Odour slight, taste slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells containing brown to brownish-red contents. Stone cells in cortex usually in groups, some of them containing prisms of calcium oxalate. Group of sieve tubes scattered in phloem. Secretory cells contain brown to brownish-red content, arranged tangentially and alternated with sieve tubes. Vascular bundles collateral. Fascicular cambium obvious. Xylem ray broad. Vessels mostly single and scattered, subrounded, up to about 400 μm in diameter, surrounded by xylem fibres. Stone cells in pith sometimes visible. Paranchymatous cells containing brown to brownish-red contents and prisms of calcium oxalate (Fig. 2).

Powder

Colour reddish-brown. Cork cells brown, polygonal in surface view. Stone cells elliptical, subtriangular, sub-rectangular, fusiform or irregular in shape, some containing prisms of calcium oxalate; polychromatic under the polarized microscope. Prisms of calcium oxalate scattered or present in parenchymatous cells, up to 39 μm in diameter; polychromatic under the polarized microscope. Secretory cells elliptical or irregular in shape, containing brown to brownish-red content. Diameter of bordered-pitted vessels varying, up to 400 μm. Fibres pit distinct, 20-42 μm in diameter (Fig. 3).

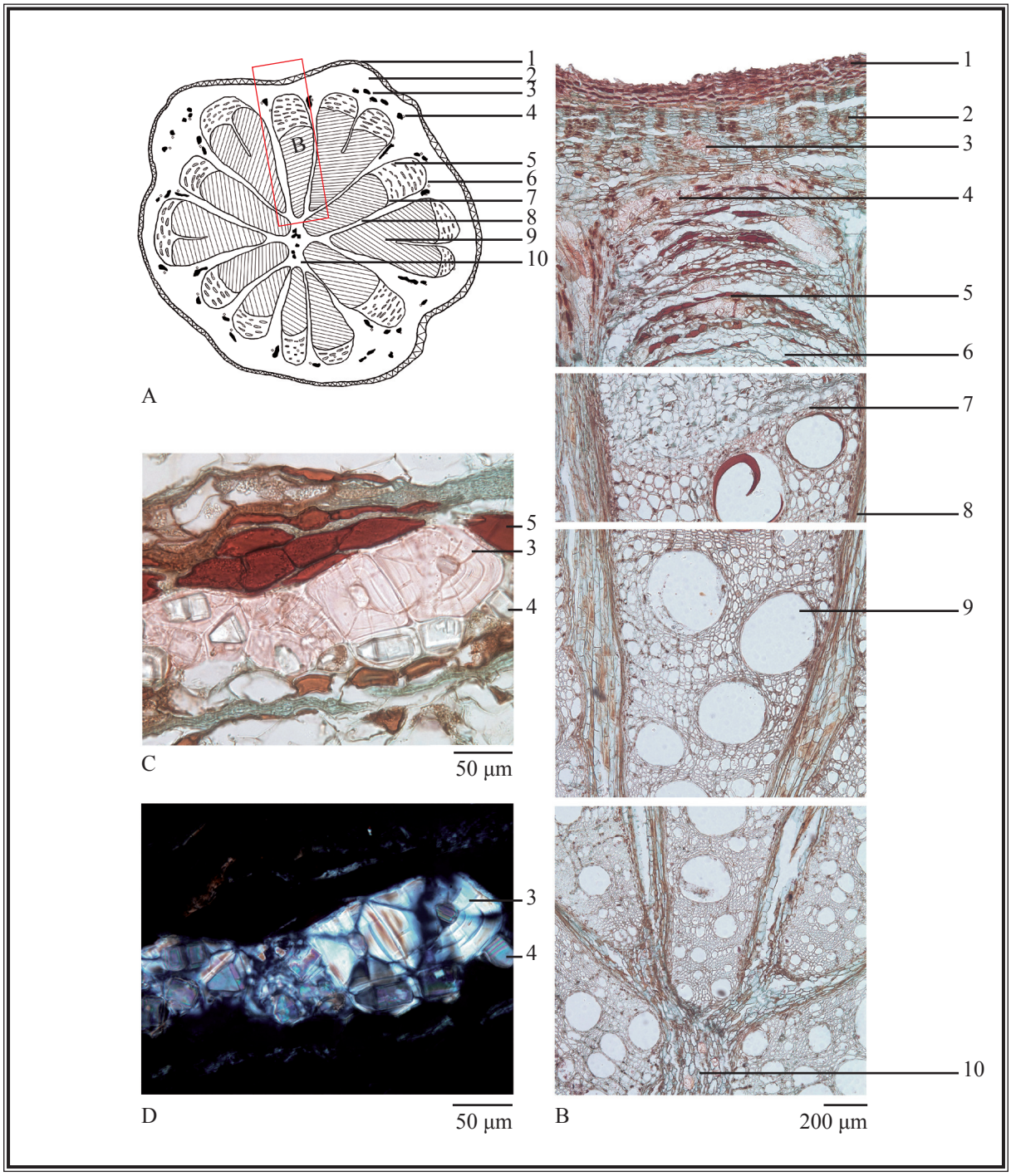


Figure 2 Microscopic features of transverse section of Sargentodoxae Caulis

A. Sketch B. Section illustration C. Section magnified (under the light microscope)
D. Section magnified (under the polarized microscope)

1. Cork 2. Cortex 3. Stone cell 4. Prism of calcium oxalate
5. Secretory cell 6. Phloem 7. Cambium 8. Ray 9. Xylem 10. Pith

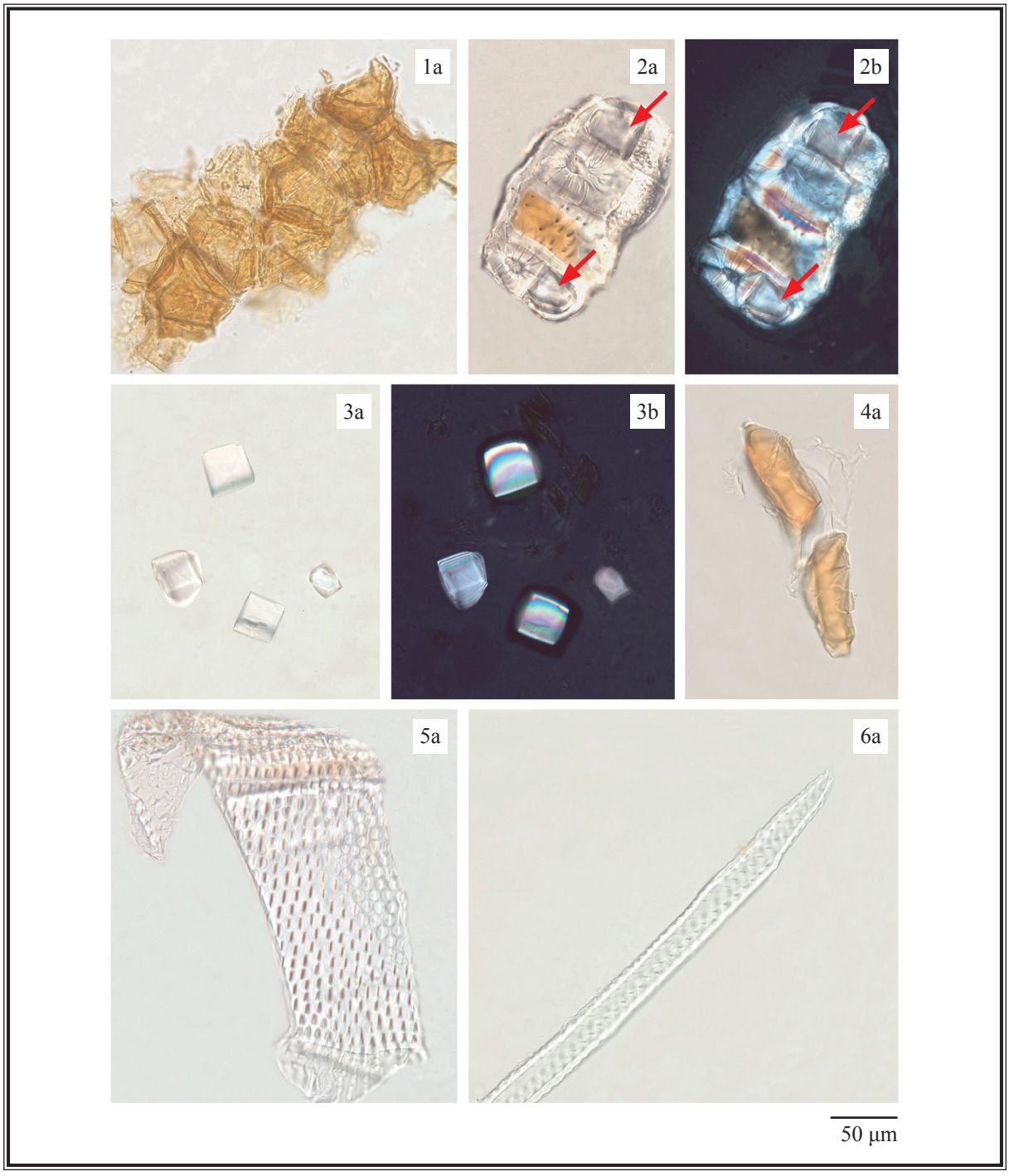


Figure 3 Microscopic features of powder of Sargentodoxae Caulis

1. Cork cells 2. Stone cells and stone cells containing prisms of calcium oxalate (→)
3. Prisms of calcium oxalate 4. Secretory cells 5. Bordered-pitted vessel
6. Fibre

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

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

Standard solution

Liriodendrin standard solution

Weigh 1.0 mg of liriodendrin CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (7 : 1.5 : 1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol and dissolve 1 g of vanillin.

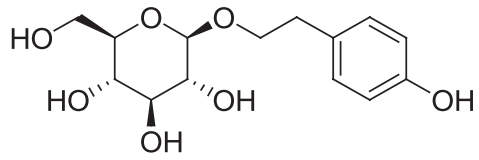
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of water.

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 liriodendrin standard solution (2 μL) and the test solution (3 μL) to the plate. 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. Spray the plate evenly with the spray reagent and heat at about 110ºC (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

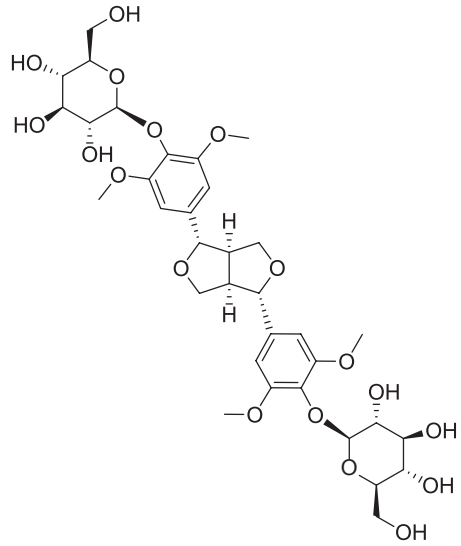


Figure 4 Chemical structures of (i) *p*-hydroxyphenethyl glucopyranoside (salidroside) and (ii) liriodendrin

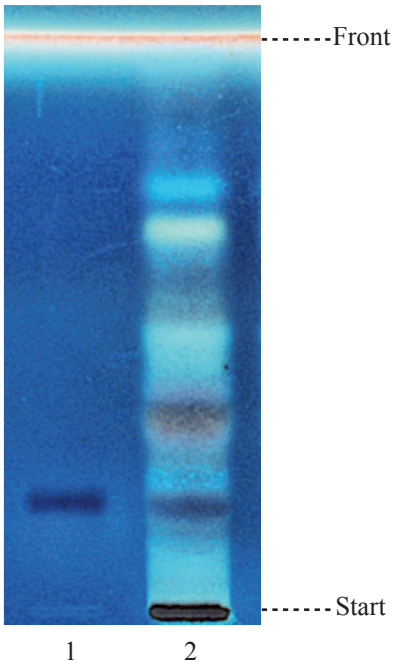


Figure 5 A reference HPTLC chromatogram of Sargentodoxae Caulis extract observed under UV light (366 nm) after staining

1. Liriodendrin 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 liriodendrin (Fig. 5).

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

Standard solutions

p-Hydroxyphenethyl glucopyranoside (salidroside) standard solution for fingerprinting, Std-FP (15 mg/L)

Weigh 1.5 mg of *p*-hydroxyphenethyl glucopyranoside CRS (Fig. 4) and dissolve in 100 mL of water.

Liriodendrin standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.5 mg of liriodendrin CRS and dissolve in 100 mL of water.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.2-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (2.1 × 50 mm) packed with ODS bonded silica gel (1.7 µm particle size, 130 Å pore size and 185 m^2/g surface area). The flow rate is about 0.2 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 – 5	95	5	Isocratic
5 – 20	95 → 85	5 → 15	linear gradient
20 – 30	85 → 70	15 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of *p*-hydroxyphenethyl glucopyranoside Std-FP and liriodendrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of *p*-hydroxyphenethyl glucopyranoside and liriodendrin should not be more than 5.0%; the RSD of the retention times of *p*-hydroxyphenethyl glucopyranoside and liriodendrin peaks should not be more than 2.0%; the column efficiencies determined from *p*-hydroxyphenethyl glucopyranoside and liriodendrin peaks should not be less than 8000 and 100000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject *p*-hydroxyphenethyl glucopyranoside Std-FP, liriodendrin Std-FP and the test solution (5 µL each) into the UHPLC system and record the chromatograms. Measure the retention times of *p*-hydroxyphenethyl glucopyranoside and liriodendrin peaks in the chromatograms of *p*-hydroxyphenethyl glucopyranoside Std-FP, liriodendrin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify *p*-hydroxyphenethyl glucopyranoside and liriodendrin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of *p*-hydroxyphenethyl glucopyranoside Std-FP and liriodendrin Std-FP. The retention times of *p*-hydroxyphenethyl glucopyranoside and liriodendrin 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 five characteristic peaks of Sargentodoxae Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Sargentodoxae Caulis extract

Peak No.	RRT	Acceptable Range
1	0.46	± 0.03
2	0.57	± 0.03
3 (marker, *p*-hydroxyphenethyl glucopyranoside)	1.00	-
4	1.76	± 0.04
5 (liriodendrin)	3.88	± 0.09

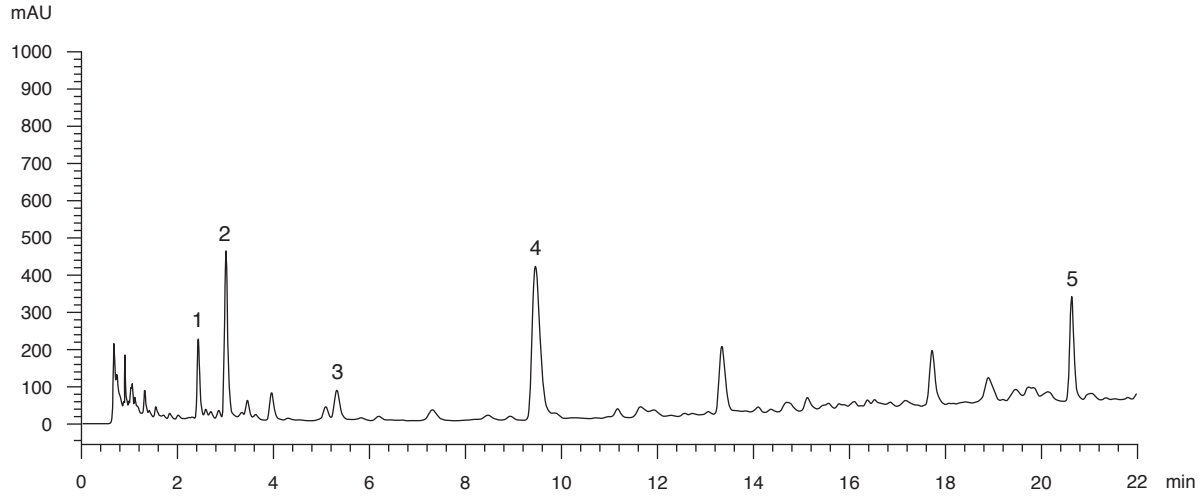


Figure 6 A reference fingerprint chromatogram of Sargentodoxae Caulis extract

For positive identification, the sample must give the above five 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 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)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 5.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution

Liriodendrin standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 mg of liriodendrin CRS and dissolve in 10 mL of water.

Liriodendrin standard solution for assay, Std-AS

Measure accurately the volume of the liriodendrin Std-Stock, dilute with water to produce a series of solutions of 10, 20, 40, 60, 80 mg/L for liriodendrin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.2-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (2.1 × 50 mm) packed with ODS bonded silica gel (1.7 μm particle size, 130 Å pore size and 185 m^2/g surface area). The flow rate is about 0.2 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 – 5	95	5	isocratic
5 – 20	95 → 85	5 → 15	linear gradient
20 – 30	85 → 70	15 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of liriodendrin Std-AS (40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of liriodendrin should not be more than 5.0%; the RSD of the retention time of liriodendrin peak should not be more than 2.0%; the column efficiency determined from liriodendrin peak should not be less than 100000 theoretical plates.

The *R* value between liriodendrin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

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

Procedure

Inject 5 µL of the test solution into the UHPLC system and record the chromatogram. Identify liriodendrin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of liriodendrin Std-AS. The retention times of liriodendrin 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 liriodendrin in the test solution, and calculate the percentage content of liriodendrin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.068% of liriodendrin ($C_{34}H_{46}O_{18}$), calculated with reference to the dried substance.

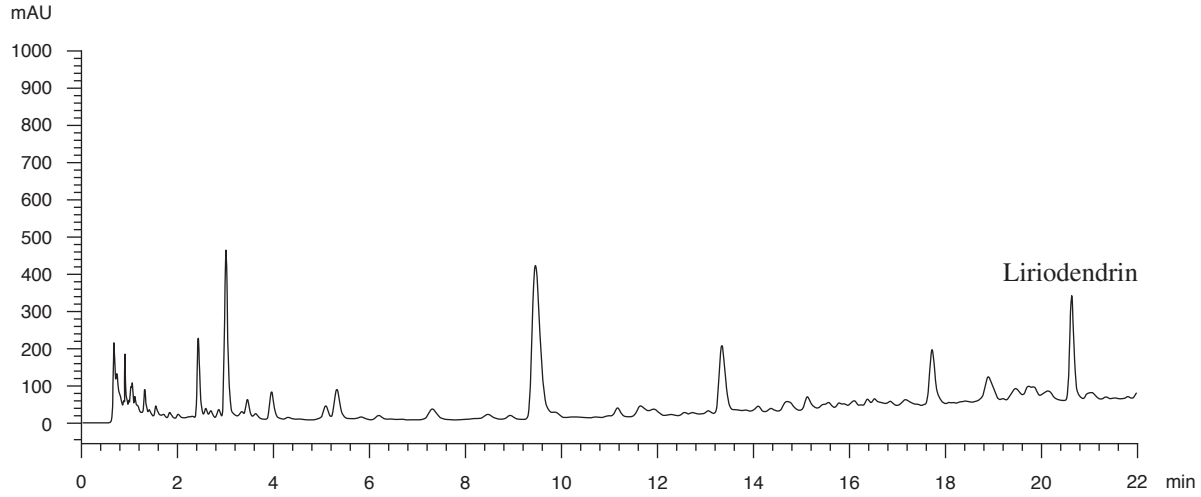


Figure 7 A reference assay chromatogram of Sargentodoxae Caulis extract