Spatholobi Caulis

Figure 1 A photograph of Spatholobi Caulis

A. Spatholobi Caulis B. Oblique slices C. Magnified transverse section

1. NAMES

Official Name: Spatholobi Caulis

Chinese Name: 雞血藤

Chinese Phonetic Name: Jixueteng

2. SOURCE

Spatholobi Caulis is the dried lianoid stem of *Spatholobus suberectus* Dunn (Fabaceae). The stem is collected in autumn and winter. After branches and leaves removed, the stem is cut into slices and dried under the sun to obtain Spatholobi Caulis.

3. DESCRIPTION

Cylindrical, slightly twisted, 30-90 mm in diameter. Externally greyish-white to greyish-brown, appearing reddish-brown when the cork exfoliated. Texture compact and hard. Oblique slices elliptical, oblong or irregular, 2-5 mm thick, xylem brownish-yellow to reddish-brown, showing numerous pores; phloem with resinous secretion, reddish-brown to blackish-brown, arranged alternately with xylem, forming 2-10 eccentric semi-circular or circular rings; pith inclined to one side. Odour slight; taste astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of rectangular cells. Cortex relatively narrow; groups of stone cells present in cortex, with brownish-red contents in the lumen; some parenchymatous cells contain prisms of calcium oxalate. Phloem arranged alternately with xylem in several whorls forming abnormal vascular bundles. Sclerenchymatous cell layer present at the outer side of

phloem, consisting of 1 to several layers of stone cells. Phloem rays often compressed; secretory cells numerous, often several in groups, containing brownish-red contents; phloem fibre bundles abundant, surrounded by cells containing prisms of calcium oxalate, forming crystal fibres. In the xylem, the vessels usually scattered singly or 2-3 in groups; bundles of xylem fibres also form crystal fibres, and cells of xylem rays often filled with brownish-red contents. Pith relatively small, consisting of parenchymatous cells, some of which are filled with brownish-red contents (Fig. 2).

Powder

Colour brownish-red. Stone cells subsquare, subrectangular, suborbicular or polygonal, 14-81 μm in diameter, with distinct pits, pit canals and striations, sometimes filled with brownish-red contents; bright yellowish-white under the polarized microscope. Prisms of calcium oxalate scattered or present in sclerenchymatous cells, 4-26 μm in diameter; polychromatic under the polarized microscope. Fibres mainly in bundles, surrounded by cells containing prisms of calcium oxalate, forming crystal fibres; single fibres slender, 4-31 μm in diameter; yellowish-white or polychromatic under the polarized microscope. Secretory cells contain brownish-red contents. Cork cells polygonal in surface view, with slit-shaped pits. Clumps of resinous secretion yellowish-brown to reddish-brown, stick-shaped or irregular in shape. Vessels mainly bordered-pitted, 18-394 μm in diameter, often broken into fragments (Fig. 3).

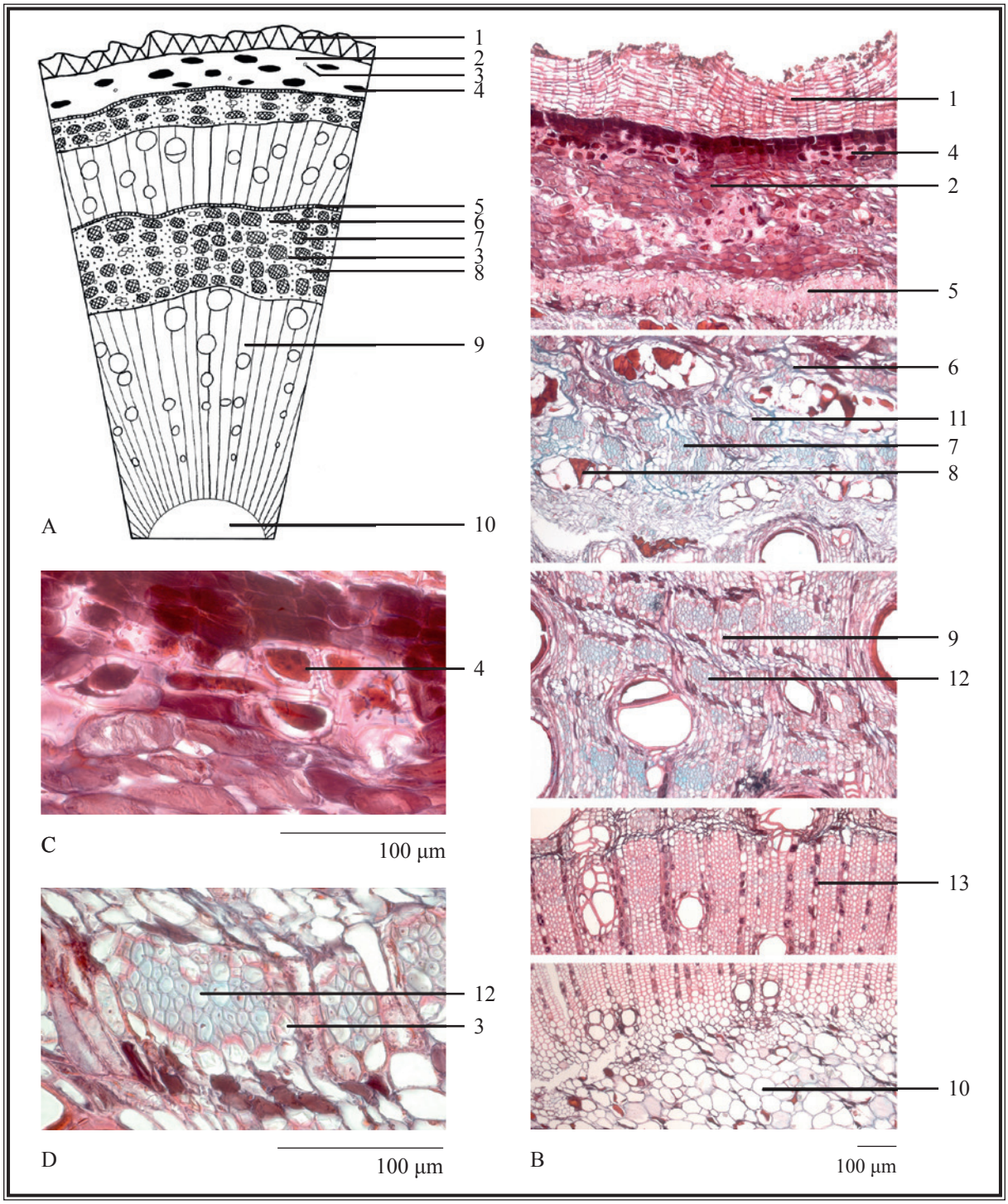


Figure 2 Microscopic features of transverse section of Spatholobi Caulis

A. Sketch B. Section illustration C. Stone cells in cortex D. Crystal fibres in xylem

1. Cork 2. Cortex 3. Prism of calcium oxalate 4. Stone cell 5. Sclerenchymatous cell layer
6. Phloem 7. Phloem fibre 8. Secretory cell 9. Xylem 10. Pith 11. Phloem ray
12. Xylem fibre 13. Xylem ray

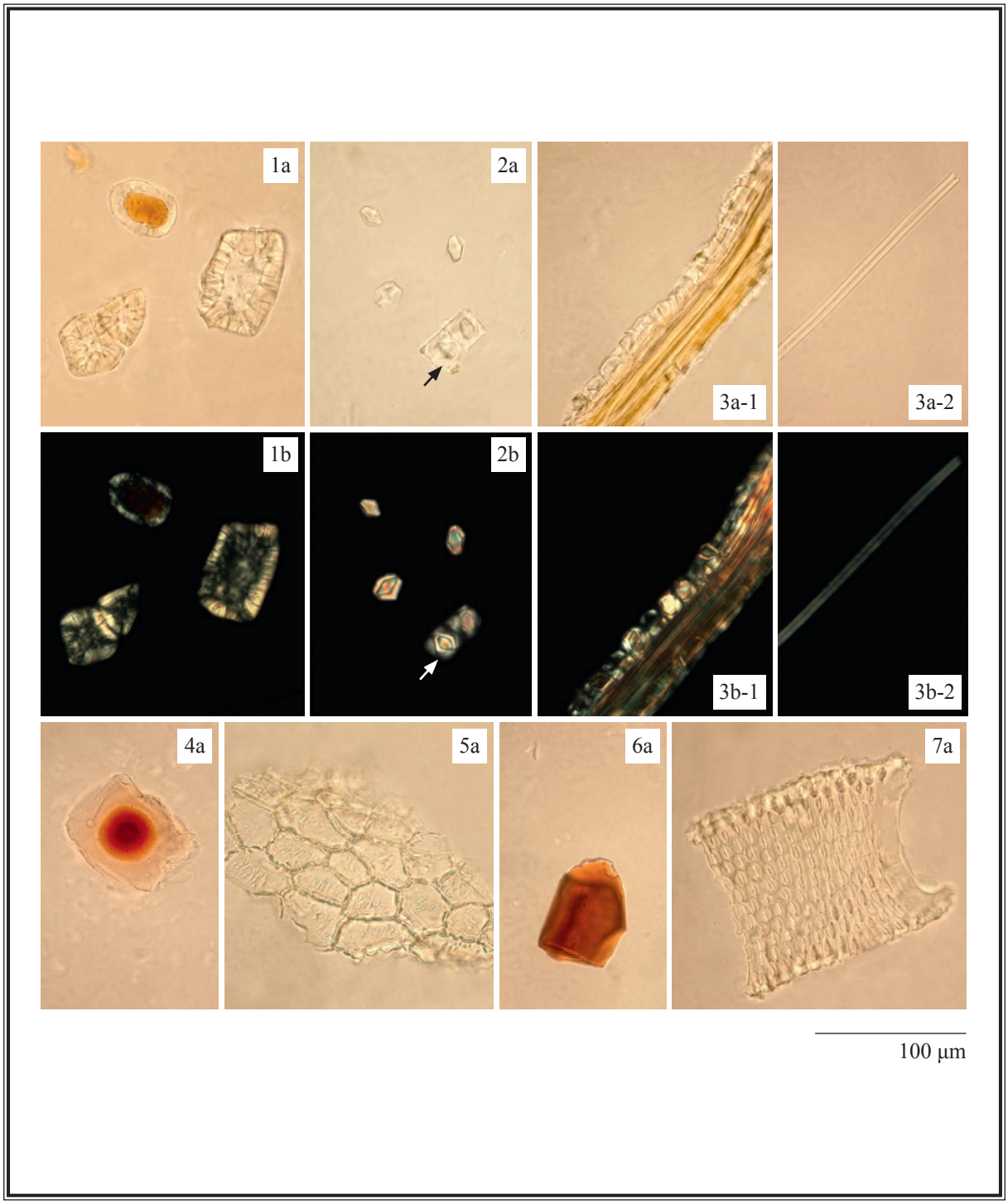


Figure 3 Microscopic features of powder of Spatholobi Caulis

1. Stone cells 2. Prisms of calcium oxalate in sclerenchymatous cells
3. Fibres (3-1 crystal fibres, 3-2 single fibre) 4. Secretory cell 5. Cork cells
6. Clump of resinous secretion 7. Bordered-pitted vessel

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

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

Standard solution

Formononetin standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane and methanol (30:1, v/v).

Test solution

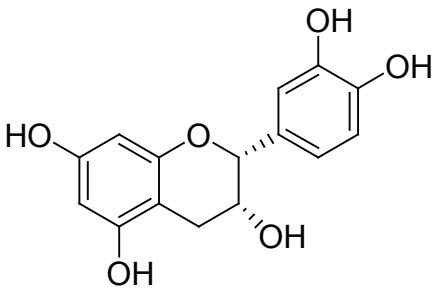
Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in 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 supernatant 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 formononetin standard solution (1 μL) and the test solution (10 μL) 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 (366 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 formononetin.

(i)



(ii)

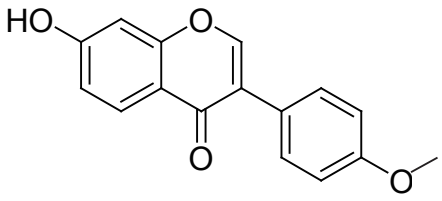


Figure 4 Chemical structures of (i) (-)-epicatechin and (ii) formononetin

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

Standard solution

Formononetin standard solution for fingerprinting, Std-FP (2 mg/L)

Weigh 0.2 mg of formononetin CRS and dissolve in 100 mL of methanol.

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 (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3800 × *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 (60%). Centrifuge at about 3800 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (60%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 40ºC 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)	Methanol (%, v/v)	Elution
0 – 10	60	40	isocratic
10 – 60	60 → 40	40 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of formononetin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of formononetin should not be more than 5.0%; the RSD of the retention time of formononetin peak should not be more than 2.0%; the column efficiency determined from formononetin peak should not be less than 20000 theoretical plates.

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

Procedure

Separately inject formononetin Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of formononetin peak in the chromatogram of formononetin Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify formononetin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of formononetin Std-FP. The retention times of formononetin 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 six characteristic peaks of Spatholobi Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Spatholobi Caulis extract

Peak No.	RRT	Acceptable Range
1	0.40	± 0.03
2	0.53	± 0.03
3	0.65	± 0.03
4	0.71	± 0.03
5	0.74	± 0.03
6 (marker, formononetin)	1.00	-

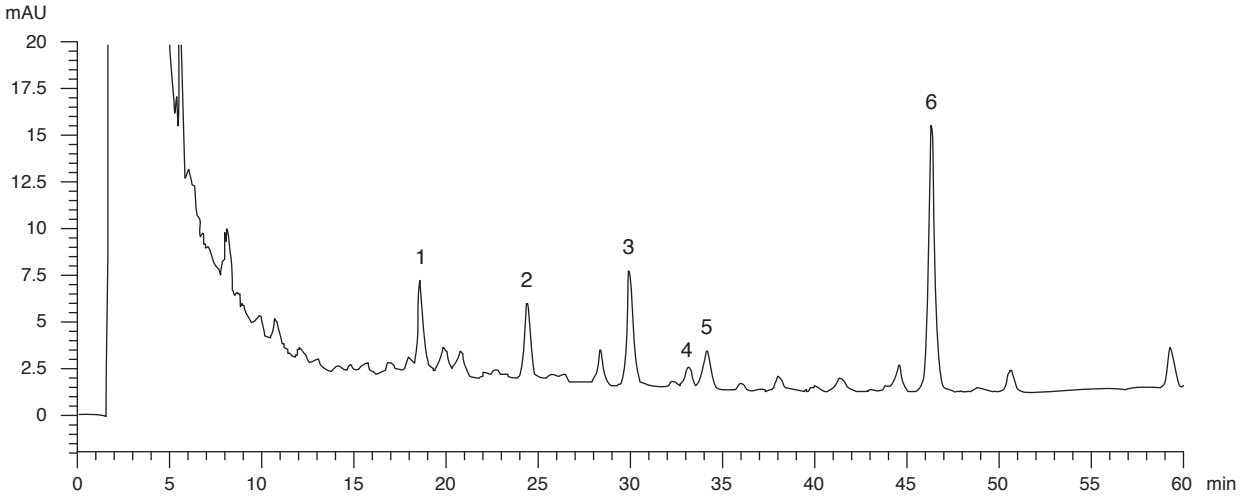


Figure 5 A reference fingerprint chromatogram of Spatholobi Caulis extract

For positive identification, the sample must give the above six 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 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 4.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 6.0%.
Ethanol-soluble extractives (hot extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 1.0 mg of (-)-epicatechin CRS (Fig. 4) and dissolve in 10 mL of methanol.

(-)-Epicatechin standard solution for assay, Std-AS

Measure accurately the volume of the (-)-epicatechin Std-Stock, dilute with methanol to produce a series of solutions of 1, 2, 5, 10, 50 mg/L for (-)-epicatechin.

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 (60%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3800 × *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 (60%). Centrifuge at about 3800 × *g* for 10 min. Combine the supernatants and make up to the mark with methanol (60%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (202 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.2 mL/min. The mobile phase is a mixture of 0.1% phosphoric acid and methanol (80:20, v/v). The elution time is about 60 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of (-)-epicatechin Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of (-)-epicatechin should not be more than 5.0%; the RSD of the retention time of (-)-epicatechin peak should not be more than 2.0%; the column efficiency determined from (-)-epicatechin peak should not be less than 5000 theoretical plates.

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

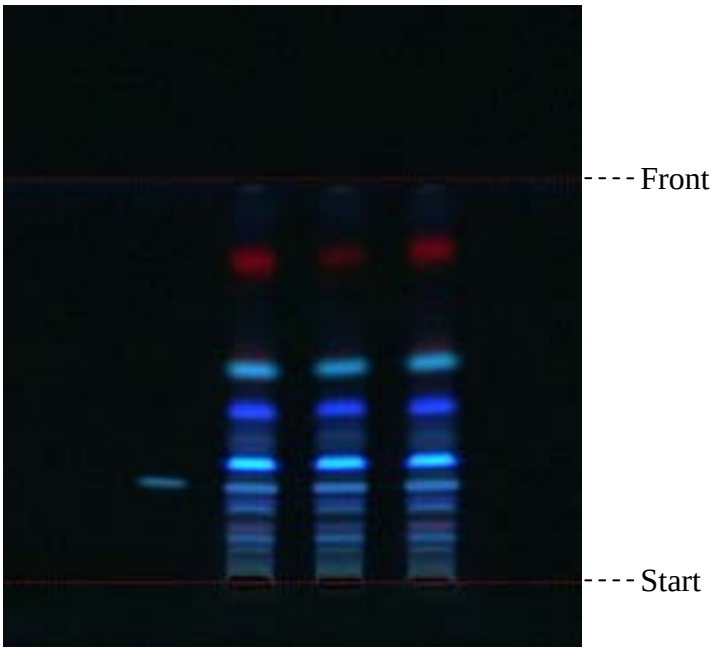
Procedure

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

Limits

The sample contains not less than 0.021% of (-)-epicatechin ($C_{15}H_{14}O_6$), calculated with reference to the dried substance.

Spatholobi Caulis (雞血藤)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Formononetin)	Formononetin positive
3	Sample (Spatholobi Caulis)	Formononetin positive
4	Sample duplicate (Spatholobi Caulis)	Formononetin positive
5	Spiked sample (Sample plus formononetin)	Formononetin positive

Figure 1 TLC results of Spatholobi Caulis extract observed under UV light (366 nm)