Polygoni Multiflori Caulis

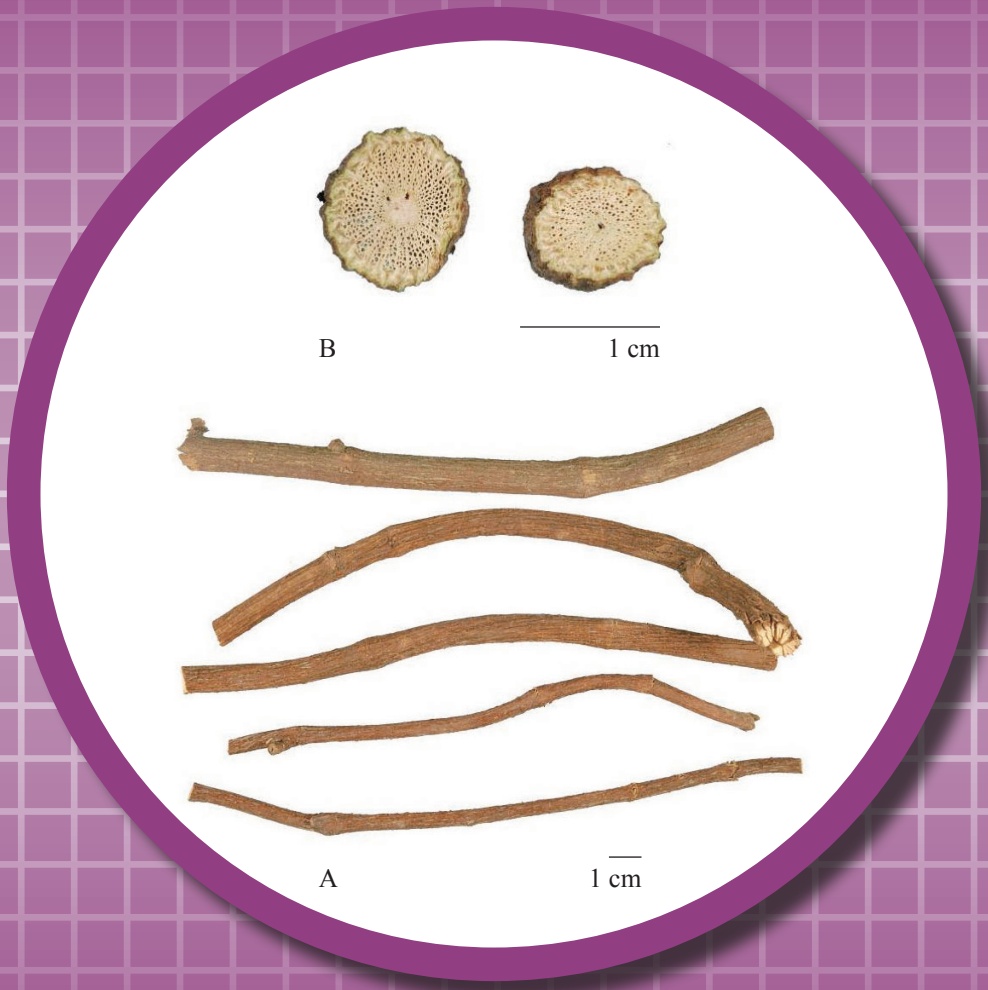


Figure 1 A photograph of Polygoni Multiflori Caulis

A. Polygoni Multiflori Caulis

B. Magnified image of transverse sections of lianoid stem

1. NAMES

Official Name: Polygoni Multiflori Caulis

Chinese Name: 首烏藤

Chinese Phonetic Name: Shouwuteng

2. SOURCE

Polygoni Multiflori Caulis is the dried lianoid stem of *Polygonum multiflorum* Thunb. (Polygonaceae). The stem is collected in autumn and winter, remnants of leaves removed, bundled up or cut into sections when fresh, then dried under the sun or baked at 45-50ºC to dryness to obtain Polygoni Multiflori Caulis.

3. DESCRIPTION

Long-cylindrical, slightly twisted, varying in length, 2-10 mm in diameter. Externally purplish-red or purplish-brown, rough, with twisted longitudinal wrinkles. Nodes slightly swollen, with scars of lateral branches. The outer bark thin and easily stripped. Texture fragile, easily broken. In sectional view, bark purplish-red, wood yellowish-white or pale brown, vessel pores distinct, pith lax and whitish. Odour slight; taste astringent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Fragments of remnant epidermal cells sometimes visible. Cork consists of 3-4 layers of cells, containing brown pigment. Cortex relatively narrow. Pericycle fibre bundles arranged in an interrupted ring, with walls markedly thickened and lignified. Groups of stone cells occasionally found, interspersed among fibre bundles. Phloem relatively broad. Cambium in a ring. Xylem vessels subrounded, singly scattered or several in groups. Rays consist of 1-3 rows of cells, containing clusters of calcium oxalate. Pith relatively small. Parenchymatous cells contain clusters of calcium oxalate (Fig. 2).

Powder

Colour yellowish-brown to purplish-brown. Cork cells reddish-brown, subsquare or irregular in shape, walls relatively thickened and slightly undulate in surface view. Stone cells usually in groups, pale yellow, rectangular, subsquare, subtriangular or irregular in shape, 16-72 μm in diameter, walls thin, lumens large, pits and pit canals distinct. Vessels mostly bordered-pitted, up to 213 μm in diameter, pits arranged densely, pit apertures distinct. Clusters of calcium oxalate numerous, scattered singly or arranged in a row in parenchyma, 12-89 μm in diameter, angles blunt; polychromatic under the polarized microscope. Pericyclic fibres mostly in bundles, pale yellow, 7-32 μm in diameter, relatively long, walls thickened, lumens narrow and pit canals distinct; bright white or yellowish-white under the polarized microscope (Fig. 3).

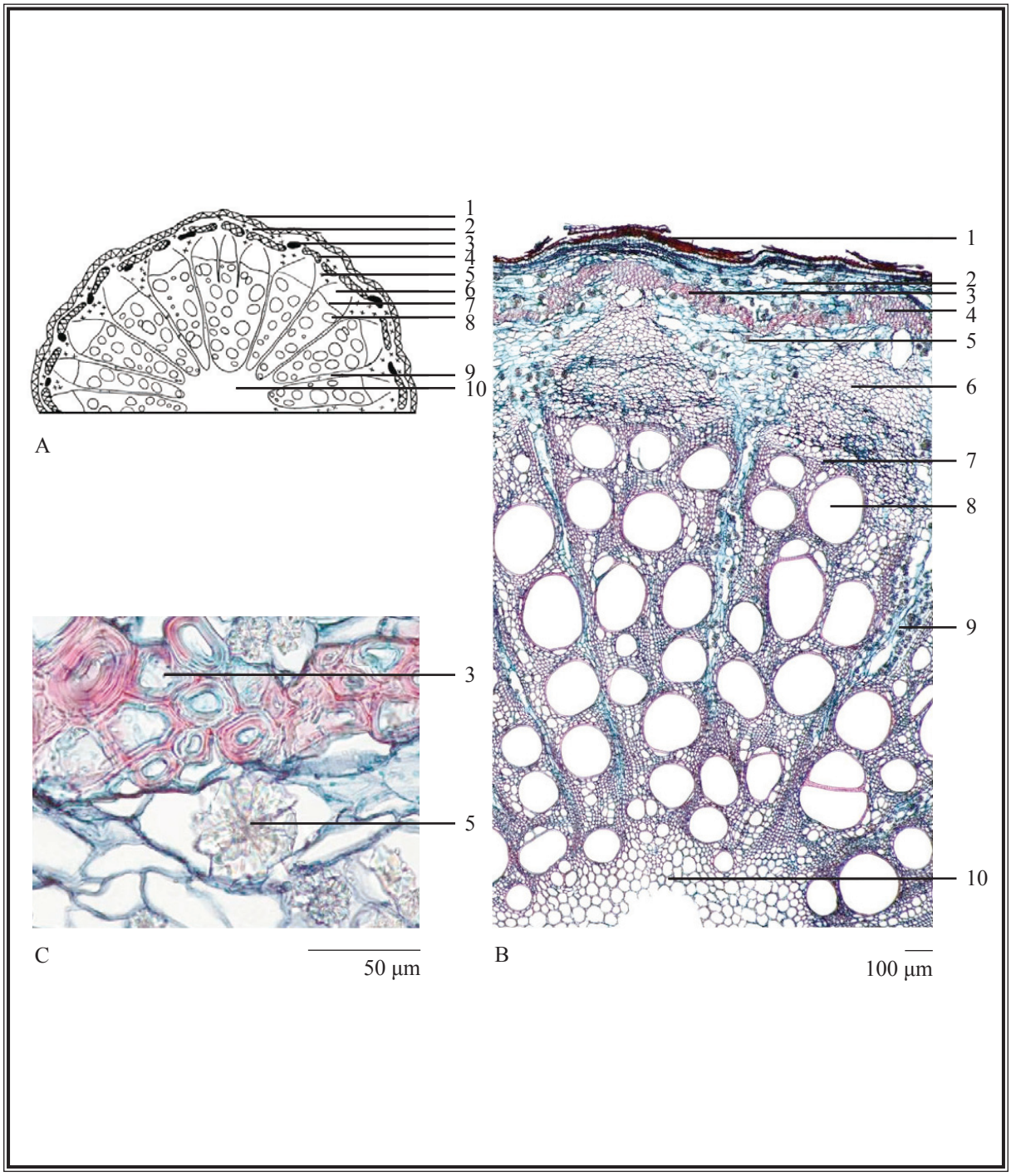


Figure 2 Microscopic features of transverse section of Polygoni Multiflori Caulis

A. Sketch B. Section illustration C. Clusters of calcium oxalate and stone cells

1. Cork 2. Cortex 3. Stone cell 4. Pericycle fibre bundles 5. Clusters of calcium oxalate
6. Phloem 7. Cambium 8. Xylem 9. Ray 10. Pith

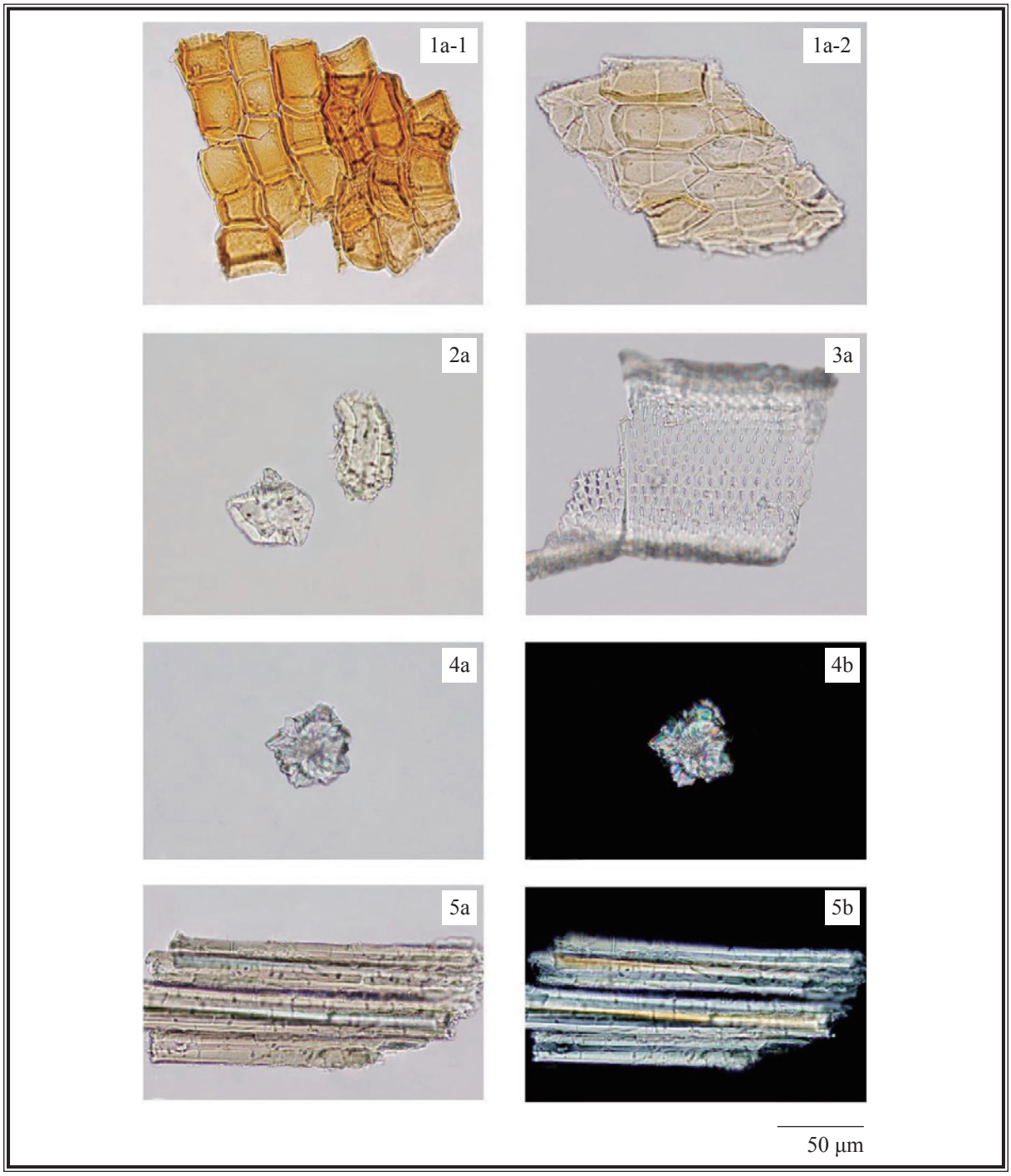


Figure 3 Microscopic features of powder of Polygoni Multiflori Caulis

1. Cork cells 2. Stone cells 3. Vessels (in lateral view) 4. Cluster of calcium oxalate 5. Fibre bundle

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

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

Standard solution

2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside standard solution

Weigh 1.0 mg of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside CRS (Fig. 4) and dissolve in 1 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of dichloromethane, ethanol and glacial acetic acid (10:5:0.4, v/v).

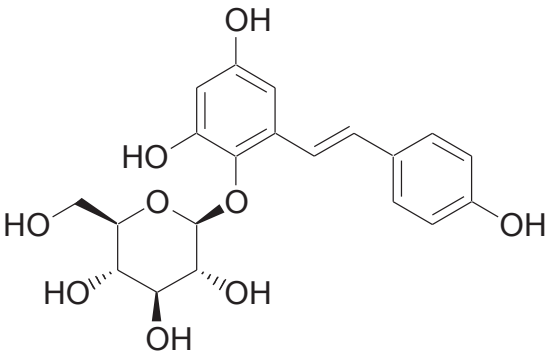
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (95%). Sonicate (400 W) the mixture for 30 min. 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 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside standard solution (1 μL) and the test solution (2 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 8 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).

(i)



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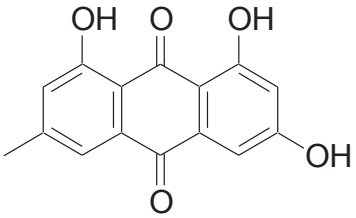

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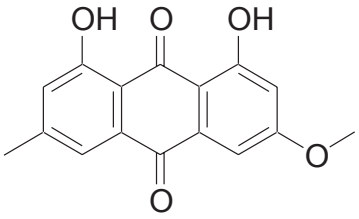

Figure 4 Chemical structures of (i) 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside (ii) emodin and (iii) physcion

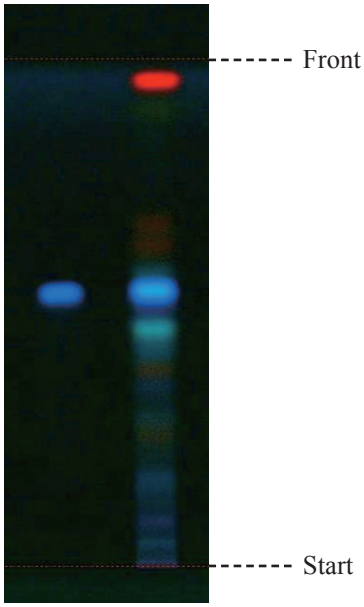

Figure 5 A reference HPTLC chromatogram of Polygoni Multiflori Caulis extract observed under UV light (366 nm)

1. 2,3,5,4'-Tetrahydroxystilbene-2-*O*-β-D-glucoside 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 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside (Fig. 5).

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

Standard solutions

2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside standard solution for fingerprinting, Std-FP (45 mg/L)

Weigh 0.45 mg of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside CRS and dissolve in 10 mL of methanol.

Emodin standard solution for fingerprinting, Std-FP (4 mg/L)

Weigh 0.2 mg of emodin CRS (Fig. 4) and dissolve in 50 mL of methanol.

Physcion standard solution for fingerprinting, Std-FP (4 mg/L)

Weigh 0.2 mg of physcion CRS (Fig. 4) and dissolve in 50 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (75%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC 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)	0.5% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 18	83	17	isocratic
18 – 30	83 → 65	17 → 35	linear gradient
30 – 40	65	35	isocratic
40 – 50	65 → 5	35 → 95	linear gradient
50 – 60	5	95	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside Std-FP, emodin Std-FP and physcion Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion should not be more than 5.0%; the RSD of the retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion peaks should not be more than 2.0%; the column efficiencies determined from 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion peaks should not be less than 8000, 400000 and 400000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; the *R* value between peak 4 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 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside Std-FP, emodin Std-FP, physcion Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion peaks in the chromatograms of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside Std-FP, emodin Std-FP, physcion Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside Std-FP, emodin Std-FP and physcion Std-FP. The retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion 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 Polygoni Multiflori Caulis extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside)	0.30	± 0.03
2	0.66	± 0.03
3	0.69	± 0.03
4 (marker, emodin)	1.00	-
5 (physcion)	1.07	± 0.03

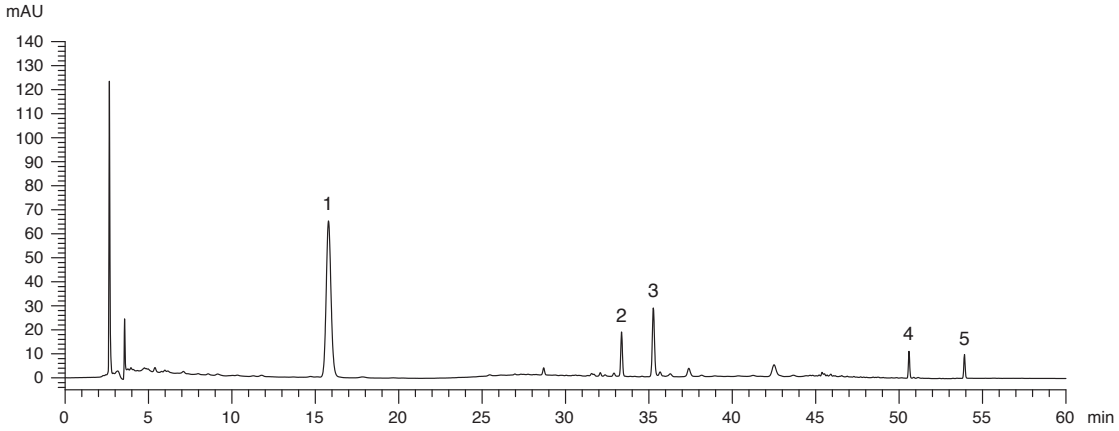

Figure 6 A reference fingerprint chromatogram of Polygoni Multiflori Caulis extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 5.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 9.0%.
Ethanol-soluble extractives (hot extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

Mixed 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, emodin and physcion standard stock solution, Std-Stock (180 mg/L for 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside and 16 mg/L for both emodin and physcion)

Weigh accurately 1.8 mg of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside CRS, 0.16 mg of emodin CRS and 0.16 mg of physcion CRS, and dissolve in 10 mL of methanol.

Mixed 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, emodin and physcion standard solution for assay, Std-AS

Measure accurately the volume of the mixed 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion Std-Stock, dilute with methanol to produce a series of solutions of 3, 6, 12, 24, 45 mg/L for 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside and 0.2, 0.4, 1, 2, 4 mg/L for both emodin and physcion.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (75%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC 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)	0.5% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 18	83	17	isocratic
18 – 30	83 → 65	17 → 35	linear gradient
30 – 40	65	35	isocratic
40 – 50	65 → 5	35 → 95	linear gradient
50 – 60	5	95	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion Std-AS (12 mg/L for 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside and 1 mg/L for both emodin and physcion). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion should not be more than 5.0%; the RSD of the retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion peaks should not be more than 2.0%; the column efficiencies determined from 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion peaks should not be less than 8000, 400000 and 400000 theoretical plates respectively.

The *R* value between 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside peak and the closest peak; the *R* value between emodin peak and the closest peak; and the *R* value between physcion 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 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion against the corresponding concentrations of the mixed 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion Std-AS. The retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion 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 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion in the test solution, and calculate the percentage contents of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside, emodin and physcion in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.20% of 2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucoside ($C_{20}H_{22}O_9$) and not less than 0.034% of the total content of emodin ($C_{15}H_{10}O_5$) and physcion ($C_{16}H_{12}O_5$), calculated with reference to the dried substance.