

Radix Polygoni Multiflori

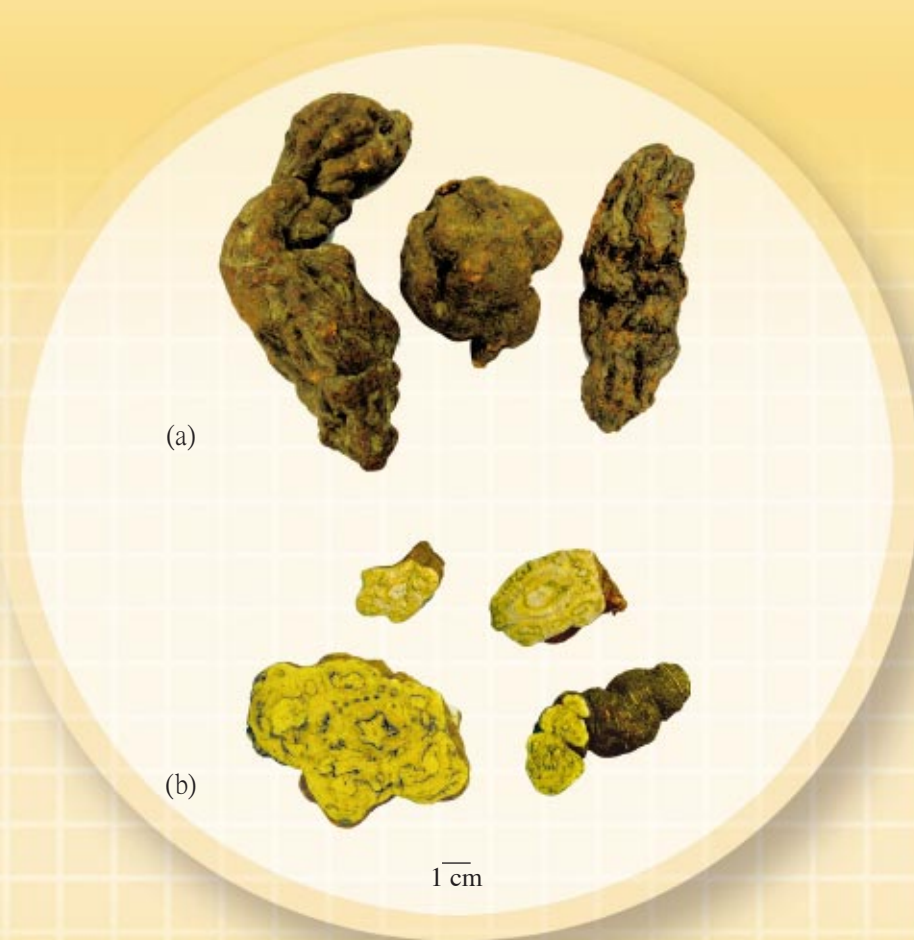


Figure 1 (a) A photograph of Radix Polygoni Multiflori
(b) A cross-section of Radix Polygoni Multiflori

1. NAMES

Official Name: Radix Polygoni Multiflori

Chinese Name: 何首烏

Chinese Phonetic Name: Heshouwu

2. SOURCE

Radix Polygoni Multiflori is the dried tuberous root of *Polygonum multiflorum* Thunb. (Polygonaceae). The tuberous root is collected in autumn and winter when the leaves wither. Both pointed ends are cut off, washed clean, and the large ones are cut into pieces, then dried to obtain Radix Polygoni Multiflori.

3. DESCRIPTION

The tuberous root is lump-shaped or irregularly fusiform in shape, 7-17 cm long, 2.5-11 cm in diameter, externally reddish-brown, shrunken and uneven, shallowly grooved, with transverse elongated lenticels and fine rootlet scars. The texture is heavy, compact, not easily broken, with the fracture pale yellowish-brown or pale reddish-brown, and starchy. The phloem exhibits 4-11 subround allotype vascular bundles arranged in a ring, forming brocaded patterns, the xylem in the central part relatively large, some having a woody core. Odourless; the taste bitterish, sweetish and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

The cork consists of several layers of cells filled with brown contents. Phloem relatively broad, scattered with 4-11 subround allotype vascular bundles of collateral type, vessels few. The central cambium of the tuberous root is in the form of a ring, few vessels in the xylem, surrounded by some tracheids and a few xylem fibres. The parenchyma cells contain cluster crystals of calcium oxalate and starch grains (Fig. 2).

Powder

Colour yellowish-brown. Simple starch grains numerous, subround, 3-81 μm in diameter, hilum V-shaped, stellate or Y-shaped, striations of large ones fairly distinct; compound grains consist of 2-9 units, black and cruciate in shape when observed under the polarized microscope. Cluster crystals of calcium oxalate 14-129 μm in diameter, jointed with prisms occasionally found. Colour bright orange when observed under the polarized microscope. Surface view of cork cells polygonal, filled with yellowish-brown contents in the lumina. Bordered pitted vessels 12-118 μm in diameter (Fig. 3).

4.2 Physicochemical Identification**Procedure**

Weigh 0.1 g of the powdered sample and put into a test tube, then add 10 mL of sodium hydroxide solution (10%, w/v). Cautiously heat the mixture in a hot water bath for 3 min. Cool down to room temperature. Filter and transfer 5 mL of the filtrate to another test tube, acidify the filtrate with hydrochloric acid (6.2%, v/v) until a pale pink colour solution is observed (about 8 mL). Add 5 mL of diethyl ether. Vortex. Transfer 4 mL of the upper layer to another test tube. Add 2 mL of ammonia solution. Vortex. A red or reddish-brown coloration is observed in the lower layer.

4.3 Thin-Layer Chromatographic Identification [Appendix IV(A)]**Standard solutions***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 methanol.

Emodin standard solution

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

Physcion standard solution

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

Developing solvent system*Developing solvent system 1*

Prepare a mixture of toluene, ethanol and glacial acetic acid (4:3:0.5, v/v).

Developing solvent system 2

Prepare a mixture of toluene and ethanol (4:1, v/v).

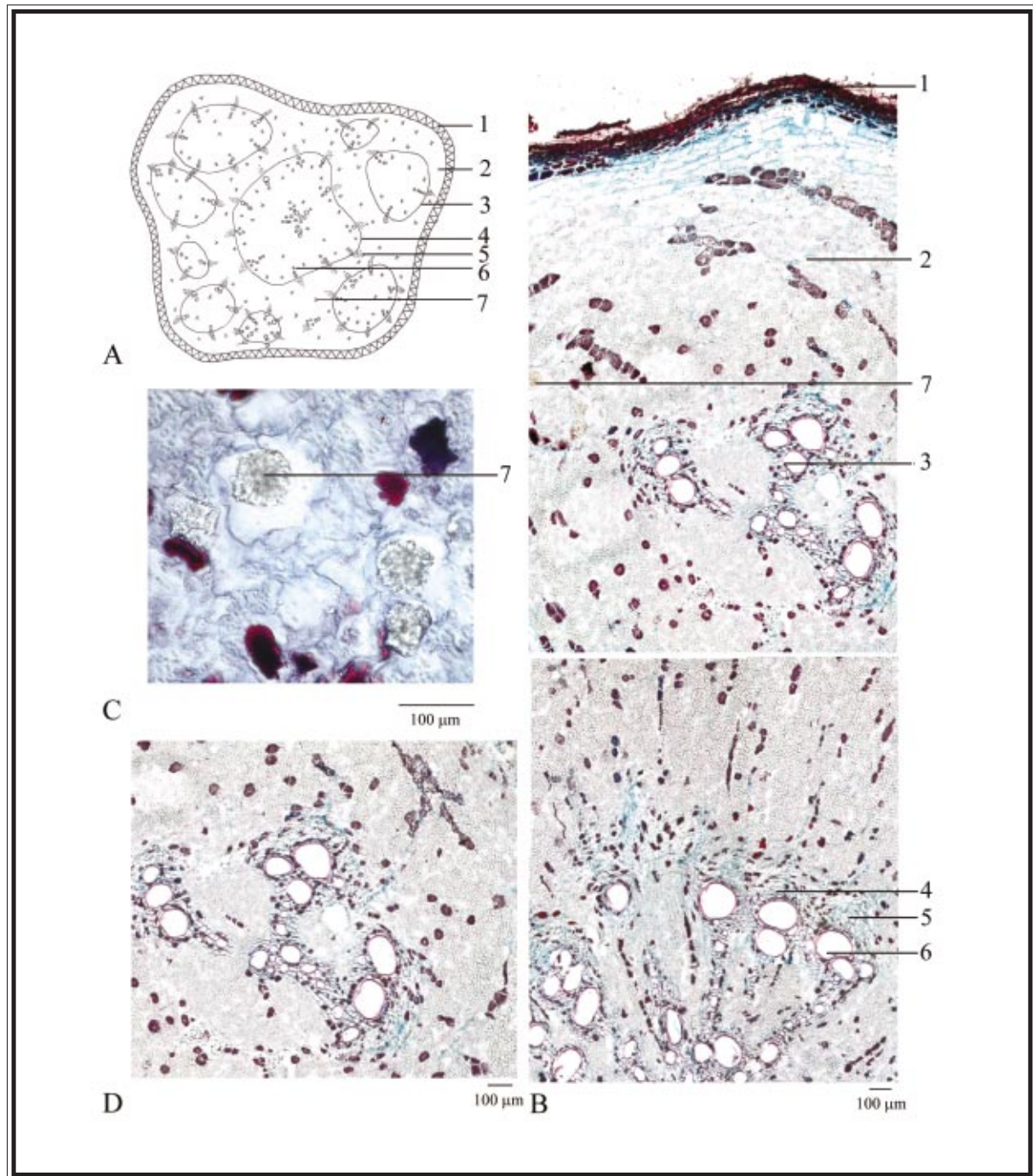


Figure 2 Microscopic features of transverse section of Radix Polygami Multiflori

A. Sketch B. Transverse section illustration C. Cluster crystals of calcium oxalate

D. Allotype vascular bundles

1. Cork 2. Parenchyma cells 3. Allotype vascular bundles 4. Central cambium of the tuberous root

5. Phloem 6. Xylem 7. Cluster crystals of calcium oxalate

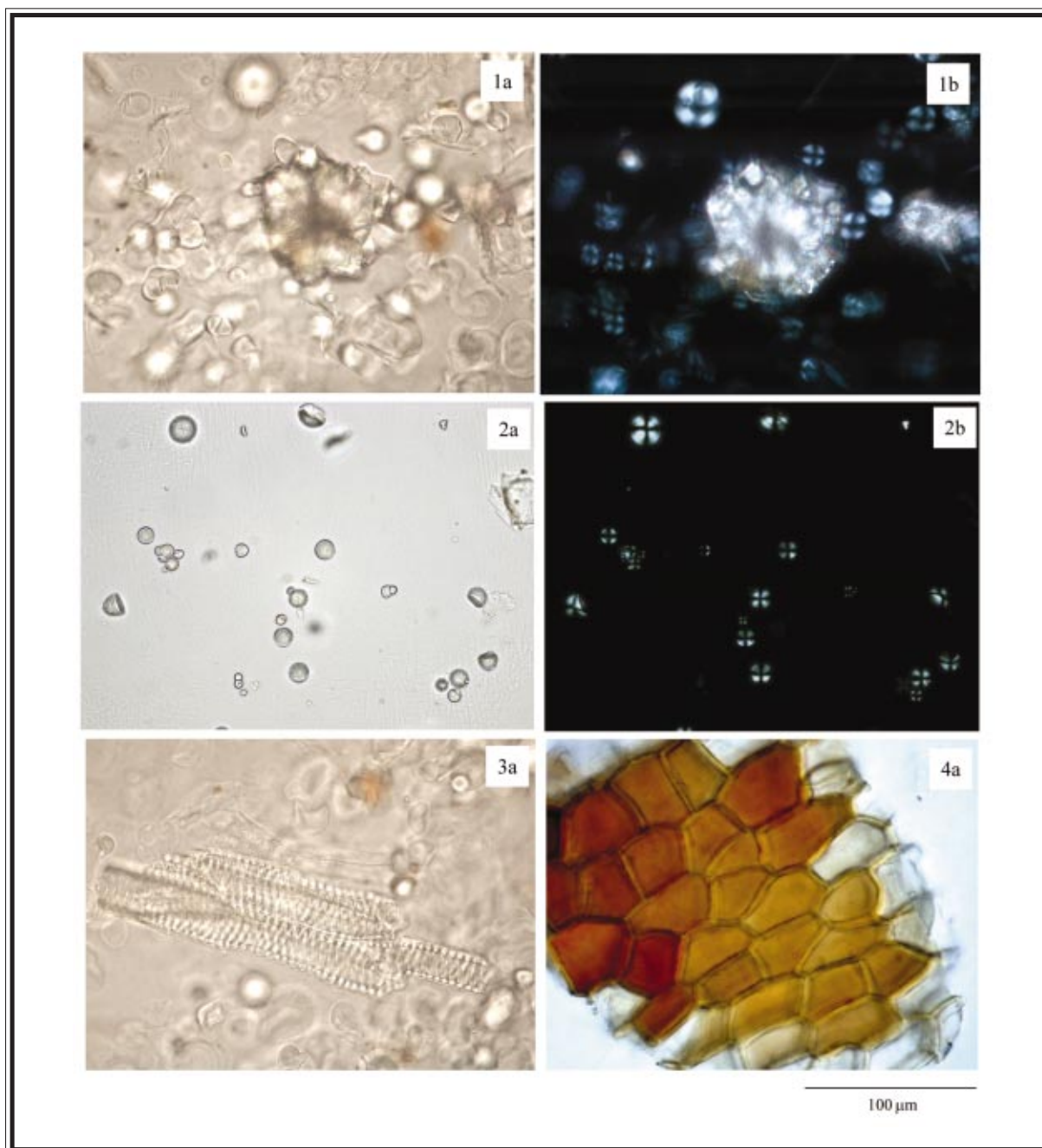


Figure 3 Microscopic features of powder of *Radix Polygoni Multiflori*

1. Cluster crystals of calcium oxalate 2. Starch grains 3. Vessels 4. Cork cells

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

Test solution

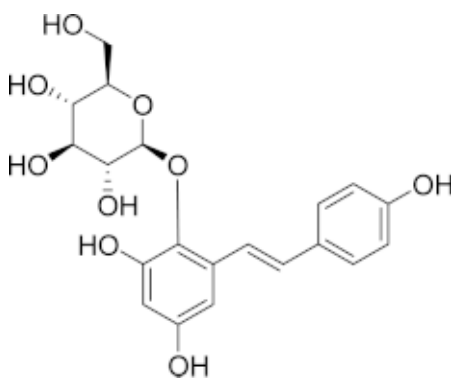
Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about $1800\times g$ for 10 min and then filter.

Procedure

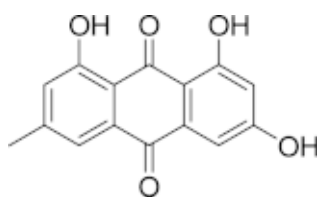
Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent systems as described above. Apply separately 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside standard solution (1 μ L), emodin standard solution (0.5 μ L), physcion standard solution (1 μ L) and the test solution (1.5 μ L) to the plate. Develop over a path of about 3.5 cm in developing solvent system 1, after the development, remove the plate from the chamber and dry in air. Redevelop over a path of 6 cm in developing solvent system 2. 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).

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 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside, emodin and physcion.

(i)



(ii)



(iii)

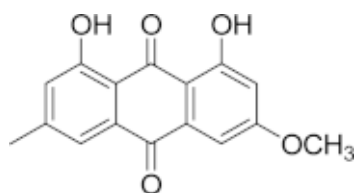


Figure 4 Chemical structures of (i) 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside
 (ii) emodin and (iii) physcion

4.4 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 (50 mg/L)

Weigh 5.0 mg of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside CRS and dissolve in 100 mL of methanol.

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

Weigh 1.0 mg of emodin CRS and dissolve in 100 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of acetone. Sonicate (490 W) the mixture for 60 min. Centrifuge at about $1800 \times g$ for 10 min. Transfer the supernatant to a 25-mL volumetric flask and make up to the mark with acetone. Transfer 5 mL of the solution to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (290 nm) and a column (4.6 × 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 –

Time (min)	0.1% Phosphoric acid (% , v/v)	Acetonitrile (% , v/v)	Elution
0 – 35	90 → 60	10 → 40	linear gradient
35 – 55	60 → 0	40 → 100	linear gradient
55 – 70	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 10 μ L of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside should not be more than 3.0%; the RSD of the retention time of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside peak should not be more than 2.0%; the column efficiency determined from 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside peak should not be less than 50000 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. 5).

Procedure

Separately inject 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside Std-FP, emodin Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside peak and emodin peak in the chromatograms of the corresponding Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside peak and emodin peak in the chromatogram of the test solution by comparing their retention times with those in the chromatograms of the corresponding Std-FP. The retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside peaks and emodin peaks in the chromatograms of the test solution and the corresponding Std-FP should not differ from their counterparts 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 Radix Polygoni Multiflori extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Radix Polygoni Multiflori extract

Peak No.	RRT	Acceptable Range
1 (marker 1, 2,3,5,4'-tetrahydroxystilbene-2- <i>O</i> - β -D-glucoside)	1.00	-
2	1.56 (vs peak 1)	± 0.04
3 (marker 2, emodin)	1.00	-
4 (physcion)	1.12 (vs peak 3)	± 0.02

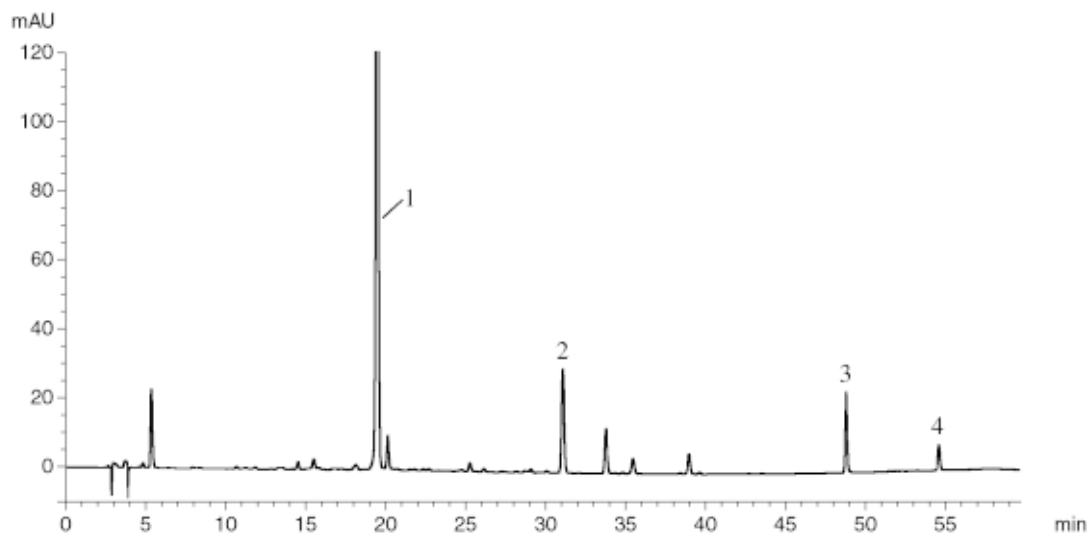


Figure 5 A reference fingerprint chromatogram of Radix Polygoni Multiflori extract

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. 5).

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 5.0%.
 - Acid-insoluble ash: not more than 0.5%.
- 5.7 **Water Content** (*Appendix X*): not more than 12.0%.

6. EXTRACTIVES (Appendix XI)

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

Ethanol-soluble extractives (cold extraction method): not less than 15.0%.

7. ASSAY

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

Standard solution

2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 10.0 mg of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside CRS and dissolve in 10 mL of methanol.

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

Measure accurately the volume of the 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside Std-Stock, dilute with methanol to produce a series of solutions of 10, 50, 100, 200, 400 mg/L for 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of ethanol (50%). Sonicate (490 W) the mixture for 30 min. Centrifuge at about $1800 \times g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction twice with 15 mL and 10 mL of ethanol (50%), respectively. Wash the residue with 3 mL of ethanol (50%). Centrifuge at about $1800 \times g$ for 10 min. Combine the extracts and make up to the mark with ethanol (50%). Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (320 nm) and a column (4.6 × 150 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of acetonitrile and water (17:83, v/v). The elution time is about 27 min.

System suitability requirements

Perform at least five replicate injections each with 10 μL of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside should not be more than 3.0%; the RSD of the retention time of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside peak should

not be more than 3.0%; the column efficiency determined from 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside peak should not be less than 3000 theoretical plates.

The *R* value between 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside 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 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside Std-AS (10 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside against the corresponding concentrations of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 μ L of the test solution into the HPLC system and record the chromatogram. Identify 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside Std-AS. The retention times of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside 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 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside in the test solution, and calculate the percentage content of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 2.2% of 2,3,5,4'-tetrahydroxystilbene-2-*O*- β -D-glucoside ($C_{20}H_{22}O_9$), calculated with reference to the dried substance.