Radix Saposhnikoviae

Figure 1 A photograph of Radix Saposhnikoviae

1. NAMES

Official Name: Radix Saposhnikoviae

Chinese Name: 防風

Chinese Phonetic Name: Fangfeng

2. SOURCE

Radix Saposhnikoviae is the dried root of *Saposhnikovia divaricata* (Turcz.) Schischk. (Apiaceae/Umbelliferae). The root is collected in the spring and autumn before the growth of flowering stem. After removal of the rootlets and soil, the root is dried under the sun to obtain Radix Saposhnikoviae.

3. DESCRIPTION

Long-conical or long-cylindrical, gradually tapering towards the lower part, some slightly tortuous, 5-30 cm long, 4-20 mm in diameter. Externally greyish-brown or yellowish-brown, rugged, with longitudinal wrinkles, numerous transverse, elongated lenticels, and dotted with raised rootlet scars. Rootstock with obvious dense annulations, some annulations marked by brown, hair-like remains of leaf bases. Texture light, easily broken; fracture uneven, bark brownish and cracked, wood yellowish. Odour characteristic; taste sweetish (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The cork consisting of several rows of cells. Cortex parenchyma cells arrange in rows tangentially, and show large elliptical vittae. Phloem scattered with numerous subround vittae surrounded by 4-8 secretory cells; phloem rays, mostly curved and becoming cleft in the outer part. Cambium distinct. Xylem vessels fairly abundant, arranged radially. Pith present at the root stock. Stone cells scattered in the parenchyma occasionally (Fig. 2).

Powder

Colour brownish-yellow to pale brown. Vittae 13-74 μm in diameter, filled with golden yellow or orangish-brown secretion. Reticulate and bordered-pitted vessels 5-98 μm in diameter. Fibres mostly in bundles, elongated. Stone cells infrequent, yellowish-brown or yellowish-green in colour, subrectangular or irregular in shape, with thick wall, showing polychrome when examined under the polarized microscope (Fig. 3).

4.2 Physicochemical Identification

Procedure

Weigh 0.2 g of the powdered sample and put into a 15-mL centrifugal tube, then add 2 mL of ethanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000×*g* for 5 min. Apply 10 μL of the supernatant onto a filter paper. Spray the filter paper evenly with aluminum (III) chloride solution (1%, w/v) and heat at about 80 ˚C for 5 min. A blue fluorescent spot is observed under UV light (365 nm).

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

Standard solutions

Prim-O-glucosylcimifugin standard solution

Weigh 1.0 mg of prim-*O*-glucosylcimifugin CRS (Fig. 4) and dissolve in 1 mL of methanol.

4'-O-β-D-glucosyl-5-O-methylvisamminol standard solution

Weigh 1.0 mg of 4'-*O*-β-D-glucosyl-5-*O*-methylvisamminol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane and methanol (4:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

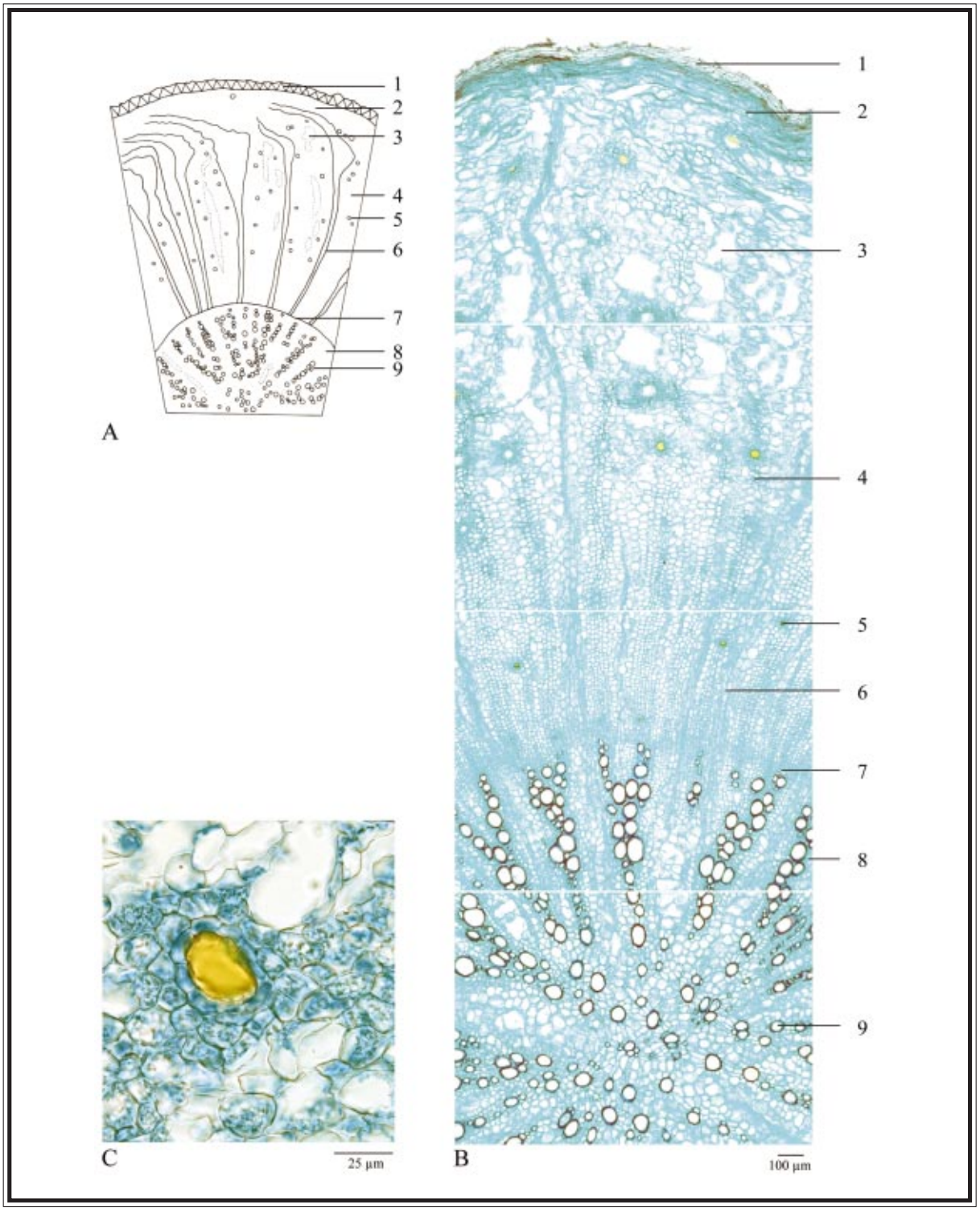


Figure 2 Microscopic features of transverse section of Radix Saposhnikoviae

A. Sketch B. Section illustration C. Vitta

1. Cork 2. Cortex 3. Cleft 4. Phloem 5. Vitta 6. Ray 7. Cambium 8. Xylem 9. Vessels

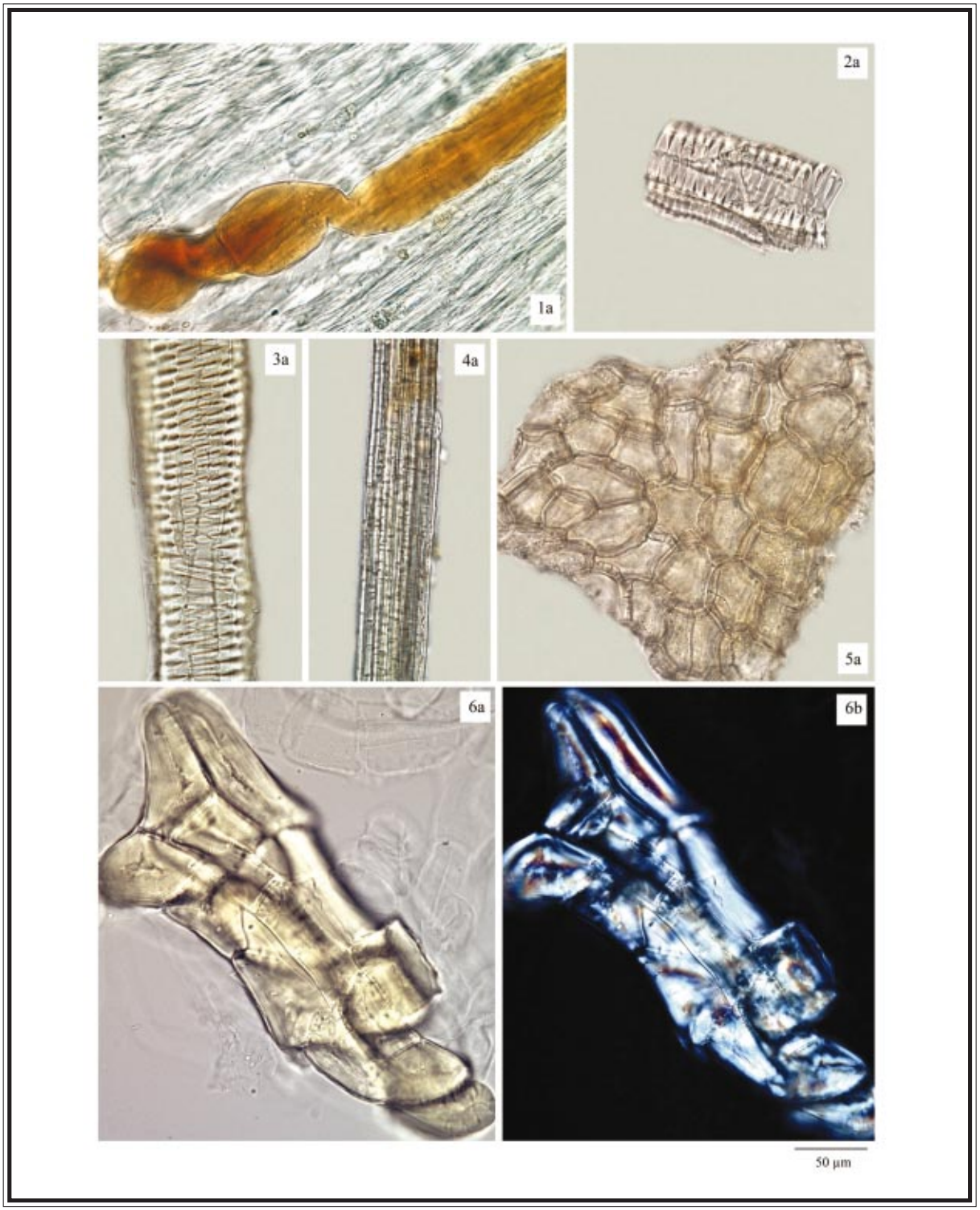


Figure 3 Microscopic features of powder of Radix Saposhnikoviae

1. Vitta 2. Reticulate vessel 3. Bordered-pitted vessel 4. Fibre bundle 5. Cork cells 6. Stone cells

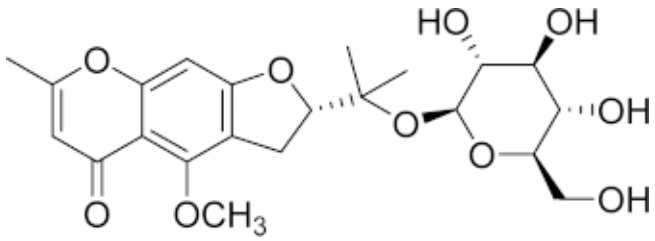
a. Features under the light microscope b. Features under the polarized microscope

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 prim-*O*-glucosylcimifugin standard solution, 4'-*O*-*β*-D-glucosyl-5-*O*- methylvisamminol standard solution and the test solution (2 µL each) to the plate. Develop over a path of about 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 (254 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 prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol.

(i)



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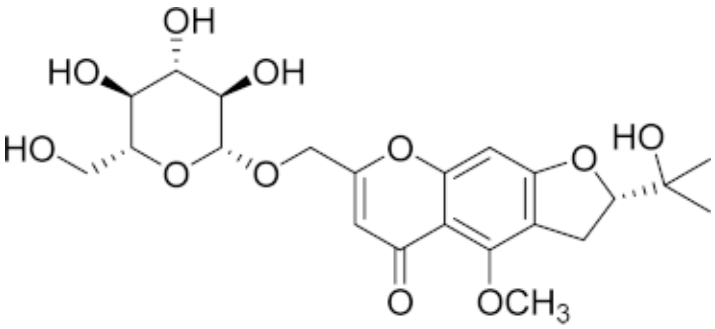

Figure 4 Chemical structures of (i) 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol and (ii) prim-*O*-glucosylcimifugi

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

Standard solution

4'-O-β-D-glucosyl-5-O-methylvisamminol standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and put into a 50-mL test tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (210 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	100 → 40	0 → 60	linear gradient
30 – 60	40 → 0	60 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol should not be more than 5.0%; the RSD of the retention time of 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peak should not be more than 2.0%; the column efficiency determined from 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peak should not be less than 20000 theoretical plates.

The *R* value between peak 2 and peak 3 in the chromatogram of the test solution should not be less than 0.95 (Fig. 5).

Procedure

Separately inject 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peak in the chromatogram of 4'-*O*-*β*-D-glucosyl -5-*O*-methylvisamminol Std-FP and the retention times of the seven characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol Std-FP. The retention times of 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol 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 seven characteristic peaks of Radix Saposhnikoviae extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the seven characteristic peaks of Radix Saposhnikoviae extract

Peak No.	RRT	Acceptable Range
1 (prim-*O*-glucosylcimifugin)	0.85 (vs peak 2)	±0.03
2 (marker, 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol)	1.00	-
3	1.01 (vs peak 2)	±0.03
4	1.22 (vs peak 2)	±0.03
5	0.84 (vs peak 6)	±0.04
6	2.70 (vs peak 2)	±0.09
7	1.17 (vs peak 6)	±0.07

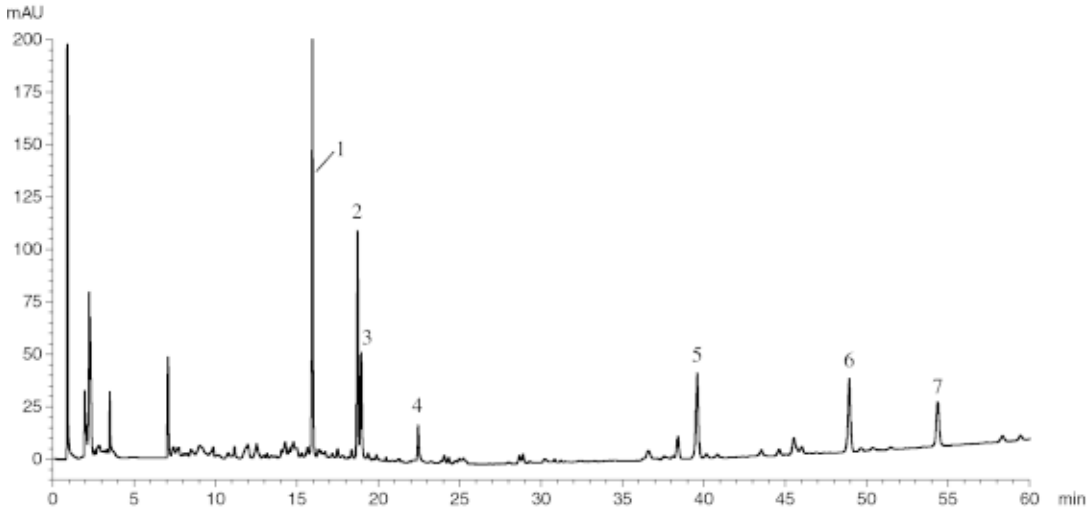

Figure 5 A reference fingerprint chromatogram of Radix Saposhnikoviae extract

For positive identification, the sample must give the above seven 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.0%.
Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 22.0%.
Ethanol-soluble extractives (hot extraction method): not less than 19.0%.

7. ASSAY

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

Standard solution

Mixed prim-O-glucosylcimifugin and 4'-O-β-D-glucosyl-5-O-methylvisamminol standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 10.0 mg of prim-*O*-glucosylcimifugin CRS and 10.0 mg of 4'-*O*-β-D-glucosyl-5-*O*-methylvisamminol CRS and dissolve in 20 mL of methanol.

Mixed prim-O-glucosylcimifugin and 4'-O-β-D-glucosyl-5-O-methylvisamminol standard solution for assay, Std-AS

Measure accurately the volume of the mixed prim-*O*-glucosylcimifugin and 4'-*O*-β-D-glucosyl-5-*O*-methylvisamminol Std-Stock, dilute with methanol to produce a series of solutions of 1, 5, 25, 50, 100 mg/L for both prim-*O*-glucosylcimifugin and 4'-*O*-β-D-glucosyl-5-*O*-methylvisamminol.

Test solution

Weigh accurately 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter. Repeat the extraction twice. Combine the filtrate. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (254 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	90	10	isocratic
10 – 20	90 → 40	10 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections each with 10 μL of the mixed prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol Std-AS (50 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol should not be more than 5.0%; the RSD of the retention times of prim-*O*-glucosylcimifugin peak and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peak should not be more than 2.0%; the column efficiencies determined from prim-*O*-glucosylcimifugin peak and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peak should not be less than 300000 theoretical plates.

The *R* value between 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol 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 prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol against the corresponding concentrations of the mixed prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol 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 prim-*O*-glucosylcimifugin peak and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol Std-AS. The retention times of prim-*O*-glucosylcimifugin peaks and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol peaks from the two chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol in the test solution, and calculate the percentage contents of prim-*O*-glucosylcimifugin and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.24% of the total content of prim-*O*-glucosylcimifugin ($C_{22}H_{28}O_{11}$) and 4'-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol ($C_{22}H_{28}O_{10}$), calculated with reference to the dried substance.