Panacis Japonici Rhizoma

Figure 1 A photograph of Panacis Japonici Rhizoma

A. Panacis Japonici Rhizoma

B. Magnified image of dented stem scar

C. Magnified image of transverse section of rhizome

1. NAMES

Official Name: Panacis Japonici Rhizoma

Chinese Name: 竹節參

Chinese Phonetic Name: Zhujieshen

2. SOURCE

Panacis Japonici Rhizoma is the dried rhizome of *Panax japonicus* C. A. Mey. (Araliaceae). The rhizome is collected in autumn, main root and outer bark removed, then dried under the sun to obtain Panacis Japonici Rhizoma.

3. DESCRIPTION

Slightly cylindrical, somewhat curved, some with fleshy lateral roots. 4-28 cm long, 4-31 mm in diameter. Externally brownish-yellow or dark brown, rough, with dense longitudinal wrinkles and root scars. Nodes obvious, internode 0.5-2.5 cm long, each showing a dented stem scar, with obvious yellowish-brown dots of vascular bundle, arranged in a ring. Texture hard and fragile, easily broken. Fracture yellowish-white to pale yellow, with small dots of yellow vascular bundles, arranged in a ring. Odour slight; taste bitter with a slightly sweet aftertaste (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 2-10 layers of cells. Cortex relatively broad, containing few secretory canals. Vascular bundles collateral. Phloem distinct, secretory canals occasionally found. Cambium in a ring. Xylem bundles made up of 2-4 strands arranged radially or in a single row. Xylem fibres obvious, usually in several bundles. Pith visible, parenchymatous cells subrounded, scattered with numerous clusters of calcium oxalate (Fig. 2).

Powder

Colour yelowish-white to yellowish-brown. Clusters of calcium oxalate numerous, usually aggregated in rosette shape, 17-99 μm in diameter; polychromatic under the polarized microscope. Xylem fibres in bundles, walls slightly thickened, some crisscrossed to become V-shaped. Cork cells polygonal in surface view, narrow-rectangular in lateral view, walls thickened. Secretory ducts occasionally found, containing yellow masses. Vessels scalariform and reticulate, 10-86 μm in diameter; bordered-pitted vessels occasionally found. Starch granules numerous, mainly simple starch granules, subrounded, 4-15 μm in diameter; black and cruciate-shaped under the polarized microscope (Fig. 3).

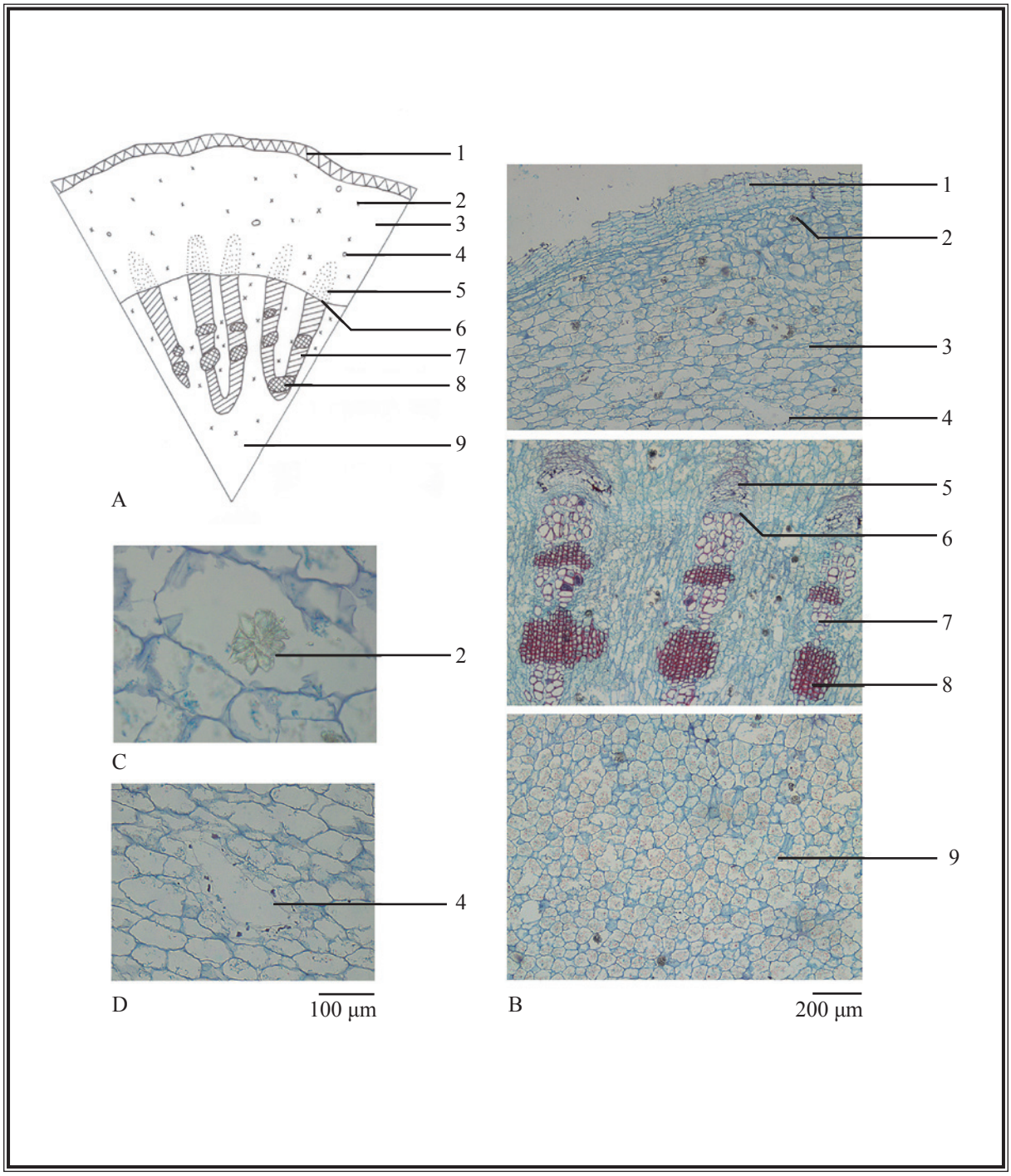


Figure 2 Microscopic features of the transverse section of Panacis Japonici Rhizoma

A. Sketch B. Section illustration C. Cluster of calcium oxalate
D. Secretory canal

1. Cork 2. Cluster of calcium oxalate 3. Cortex 4. Secretory canal
5. Phloem 6. Cambium 7. Xylem 8. Xylem fibre bundle 9. Pith

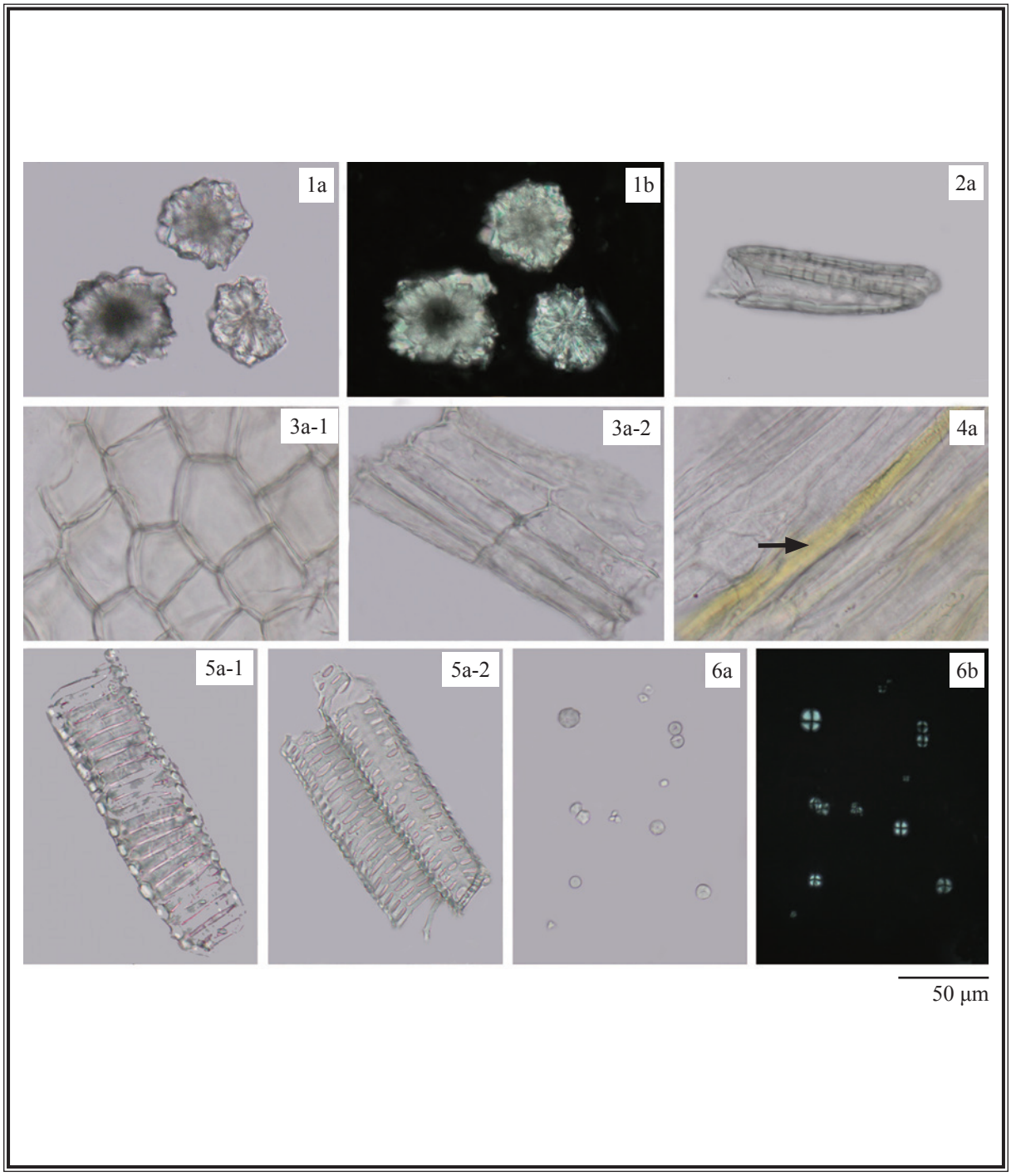


Figure 3 Microscopic features of powder of Panacis Japonici Rhizoma

1. Clusters of calcium oxalate 2. Xylem fibres
3. Cork cells (3-1 in surface view, 3-2 in lateral view) 4. Secretory canals (⟶)
5. Vessels (5-1 scalariform vessel, 5-2 reticulate vessels) 6. Starch granules

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

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

Standard solutions

Chikusetsusaponin IV standard solution

Weigh 2.0 mg of chikusetsusaponin IV CRS (Fig. 4) and dissolve in 1 mL of methanol (50%).

Chikusetsusaponin IVa standard solution

Weigh 2.0 mg of chikusetsusaponin IVa CRS (Fig. 4) and dissolve in 1 mL of methanol (50%).

Chikusetsusaponin V standard solution

Weigh 2.0 mg of chikusetsusaponin V CRS (Fig. 4) and dissolve in 1 mL of methanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-butanol, water, methanol and formic acid (10:5:3.5:2:0.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

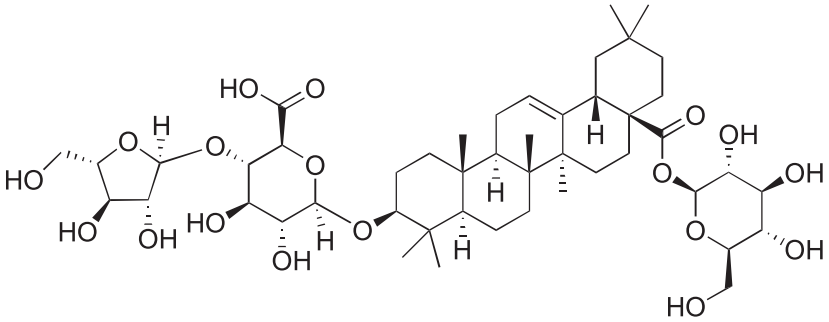
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (50%). Sonicate (350 W) the mixture for 15 min. Filter through a 0.45-μm nylon filter.

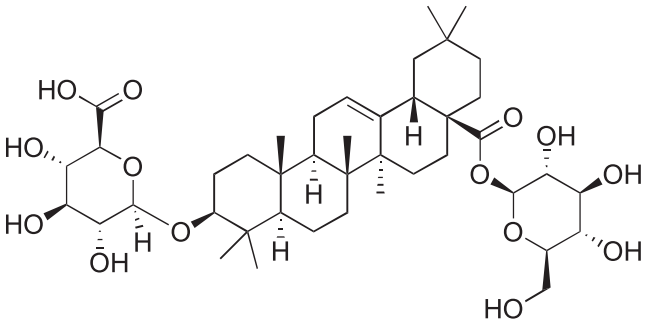
Procedure

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 chikusetsusaponin IV standard solution (1 μL), chikusetsusaponin IVa standard solution (1 μL), chikusetsusaponin V 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 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 105ºC (about 2 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)



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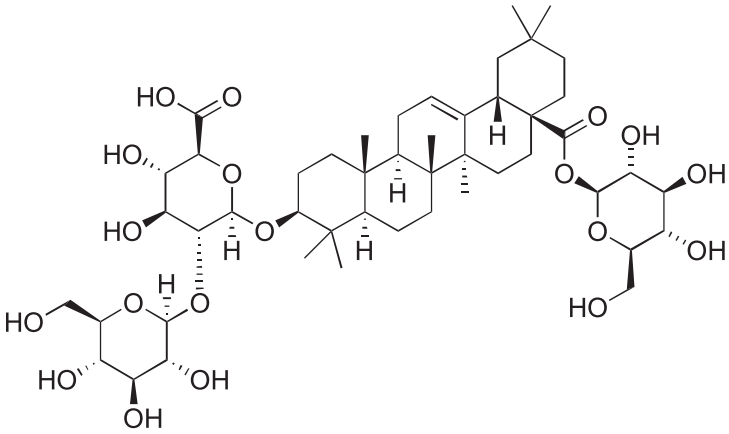

Figure 4 Chemical structures of (i) chikusetsusaponin IV (ii) chikusetsusaponin IVa and (iii) chikusetsusaponin V

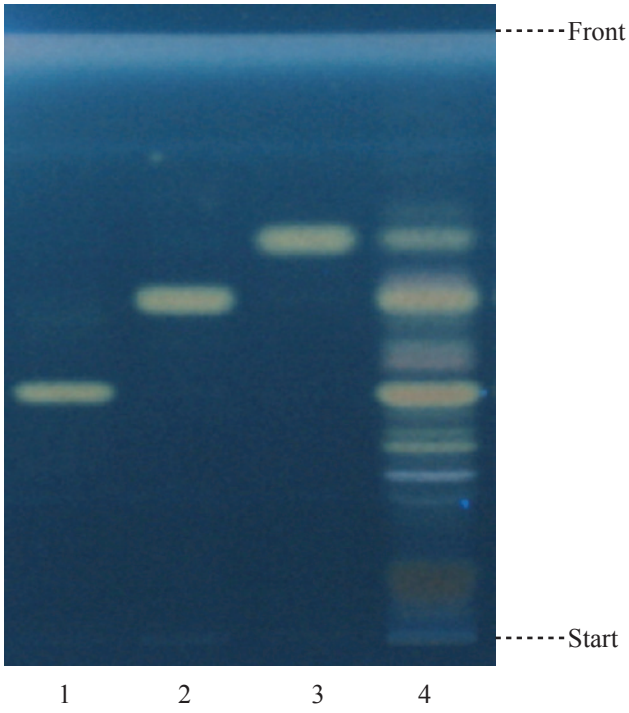

Figure 5 A reference HPTLC chromatogram of Panacis Japonici Rhizoma extract observed under UV light (366 nm) after staining

1. Chikusetsusaponin V standard solution 2. Chikusetsusaponin IV standard solution
3. Chikusetsusaponin IVa standard solution 4. Test solution

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 chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V (Fig. 5).

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

Standard solutions

Chikusetsusaponin IV standard solution for fingerprinting, Std-FP (180 mg/L)

Weigh 1.8 mg of chikusetsusaponin IV CRS and dissolve in 10 mL of methanol (50%).

Chikusetsusaponin IVa standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 0.6 mg of chikusetsusaponin IVa CRS and dissolve in 10 mL of methanol (50%).

Chikusetsusaponin V standard solution for fingerprinting, Std-FP (350 mg/L)

Weigh 3.5 mg of chikusetsusaponin V CRS and dissolve in 10 mL of methanol (50%).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (203 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.05% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	70	30	isocratic
20 – 30	70 → 66	30 → 34	linear gradient
30 – 45	66 → 55	34 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of chikusetsusaponin IV Std-FP, chikusetsusaponin IVa Std-FP and chikusetsusaponin V Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V should not be more than 5.0%; the RSD of the retention times of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V peaks should not be more than 2.0%; the column efficiencies determined from chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V peaks should not be less than 30000, 60000 and 6000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; the *R* value between peak 2 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject chikusetsusaponin IV Std-FP, chikusetsusaponin IVa Std-FP, chikusetsusaponin V Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V

peaks in the chromatograms of chikusetsusaponin IV Std-FP, chikusetsusaponin IVa Std-FP, chikusetsusaponin V Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chikusetsusaponin IV Std-FP, chikusetsusaponin IVa Std-FP and chikusetsusaponin V Std-FP. The retention times of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V 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 three characteristic peaks of Panacis Japonici Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Panacis Japonici Rhizoma extract

Peak No.	RRT	Acceptable Range
1 (marker, chikusetsusaponin V)	1.00	-
2 (chikusetsusaponin IV)	1.39	± 0.03
3 (chikusetsusaponin IVa)	1.55	± 0.03

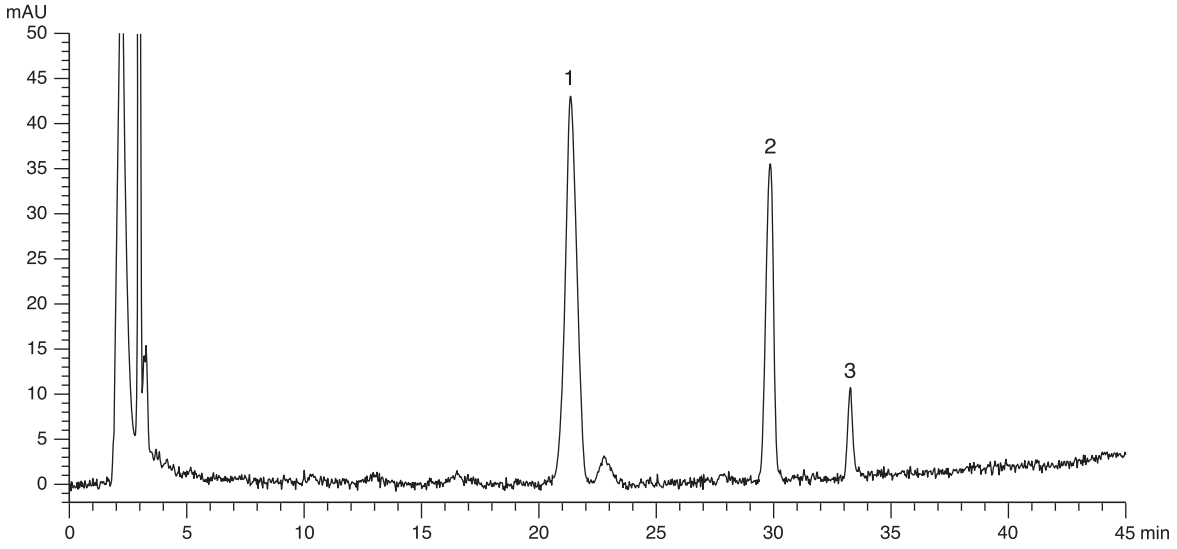

Figure 6 A reference fingerprint chromatogram of Panacis Japonici Rhizoma extract

For positive identification, the sample must give the above three 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 7.5%.
Acid-insoluble ash: not more than 1.0%.

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 31.0%.
Ethanol-soluble extractives (cold extraction method): not less than 29.0%.

7. ASSAY

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

Standard solution
Mixed chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V standard stock solution, Std-Stock (1050 mg/L for chikusetsusaponin IV, 800 mg/L for chikusetsusaponin IVa and 1050 mg/L for chikusetsusaponin V)
Weigh accurately 10.5 mg of chikusetsusaponin IV CRS, 8.0 mg of chikusetsusaponin IVa CRS and 10.5 mg of chikusetsusaponin V CRS, and dissolve in 10 mL of methanol (50%).

Mixed chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V standard solution for assay, Std-AS

Measure accurately the volume of the mixed chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V Std-Stock, dilute with methanol (50%) to produce a series of solutions of 52.5, 105, 210, 420, 525 mg/L for chikusetsusaponin IV, 16, 24, 80, 160, 240 mg/L for chikusetsusaponin IVa and 52.5, 210, 315, 420, 630 mg/L for chikusetsusaponin V.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (50%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time with 8 mL of methanol (50%). Wash the residue with methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (203 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.05% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	70	30	isocratic
20 – 30	70 → 66	30 → 34	linear gradient
30 – 45	66 → 55	34 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V Std-AS (210 mg/L for chikusetsusaponin IV, 80 mg/L for chikusetsusaponin IVa and 315 mg/L for chikusetsusaponin V). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V should not be more than 5.0%; the RSD of the retention times of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V peaks should not be more than 2.0%; the column efficiencies determined from chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V peaks should not be less than 30000, 60000 and 6000 theoretical plates respectively.

The *R* value between chikusetsusaponin IV peak and the closest peak; the *R* value between chikusetsusaponin IVa peak and the closest peak; and the *R* value between chikusetsusaponin V 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 chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V against the corresponding concentrations of the mixed chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V 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 chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V Std-AS. The retention times of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V 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 chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V in the test solution, and calculate the percentage contents of chikusetsusaponin IV, chikusetsusaponin IVa and chikusetsusaponin V in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 10% of the total content of chikusetsusaponin IV ($C_{47}H_{74}O_{18}$), chikusetsusaponin IVa ($C_{42}H_{66}O_{14}$) and chikusetsusaponin V ($C_{48}H_{76}O_{19}$), calculated with reference to the dried substance.