Radix Pseudostellariae

Figure 1　A photograph of Radix Pseudostellariae

1. NAMES

Official Name: Radix Pseudostellariae

Chinese Name: 太子參

Chinese Phonetic Name: Taizishen

2. SOURCE

Radix Pseudostellariae is the dried root tuber of *Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm. (Caryophyllaceae). The tuber is collected in the summer when most of the stems and leaves wither, washed clean, the rootlets and branch root removed; then put in boiling water for 1-3 min and dried thoroughly under the sun, or put it under the sun until dry to obtain Radix Pseudostellariae.

3. DESCRIPTION

Slender fusiform or slat-shaped, slightly curved; 1.0-9.8 cm long, 2-7 mm in diameter. The top end broader, bearing stem scars, becoming slender towards the distal end. Externally yellowish-white to yellowish-brown, relatively smooth, with light longitudinal wrinkles; the dented parts of the surface are marked with scars of rootlets. Texture hard and fragile, breaking easily; fracture smooth, either pale yellowish-white and cuticular or off-white and starchy. Odour slight; taste slightly sweet (Fig.1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 2-4 rows of subsquare and suberized cells. Cortex thin, consisting only of several rows of tangentially elongated parenchyma cells. Phloem narrow, rays broad. Cambium in a ring. The major portion of the root is occupied by xylem tissue, with vessels radially and distantly disposed, the primary xylem triarch or tetrarch. Parenchyma cells contain starch granules and cluster of calcium oxalate (Fig. 2).

Powder

Colour pale yellowish-white. Starch granules numerous, mostly of the simple type, subglobose; 2-29 (occasionally up to 35) μm in diameter; hilum asteroidal, Y-shaped, V-shaped or cleft-shaped, obscure in the small granules, but with indistinct striations in the large ones. Compound granules consist of 2-4 components, 5 components may be seen occasionally, 6-42 μm in diameter. Black cruciate shape observed under the polarized microscope. Cluster

of calcium oxalate 6-66 μm in diameter, brightly polychromatic observed under the polarized microscope. Vessels are mostly with spiral wall thickening, 4-39 μm in diameter; reticulate, scalariform and bordered-pitted vessels are also observed. Cork cells pale brownish-yellow, irregularly long-polygonal to subsquare, wall thin and slightly undulated and curved (Fig. 3).

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

Standard solution

Δ^7-Stigmastenol-3-O-β-D-glucoside standard solution

Weigh 1.0 mg of Δ^7-stigmastenol-3-O-β-D-glucoside CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (8:2:0.1, v/v).

Spray reagent

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

Test solution

Weigh 10.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of methanol. Sonicate (140 W) the mixture for 60 min. Centrifuge at about 1800 × *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 aqueous solution to a separatory funnel. Extract for three times each with 10 mL of petroleum ether (60-80°C) and discard the petroleum ether layer. Extract the aqueous layer for three times each with 10 mL of ethyl acetate. Combine the ethyl acetate extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol and then filter.

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 Δ^7-stigmastenol-3-O-β-D-glucoside standard solution and the test solution (4 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. Spray the plate evenly with the spray reagent and heat at about 120ºC until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. 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 Δ^7-stigmastenol-3-O-β-D-glucoside.

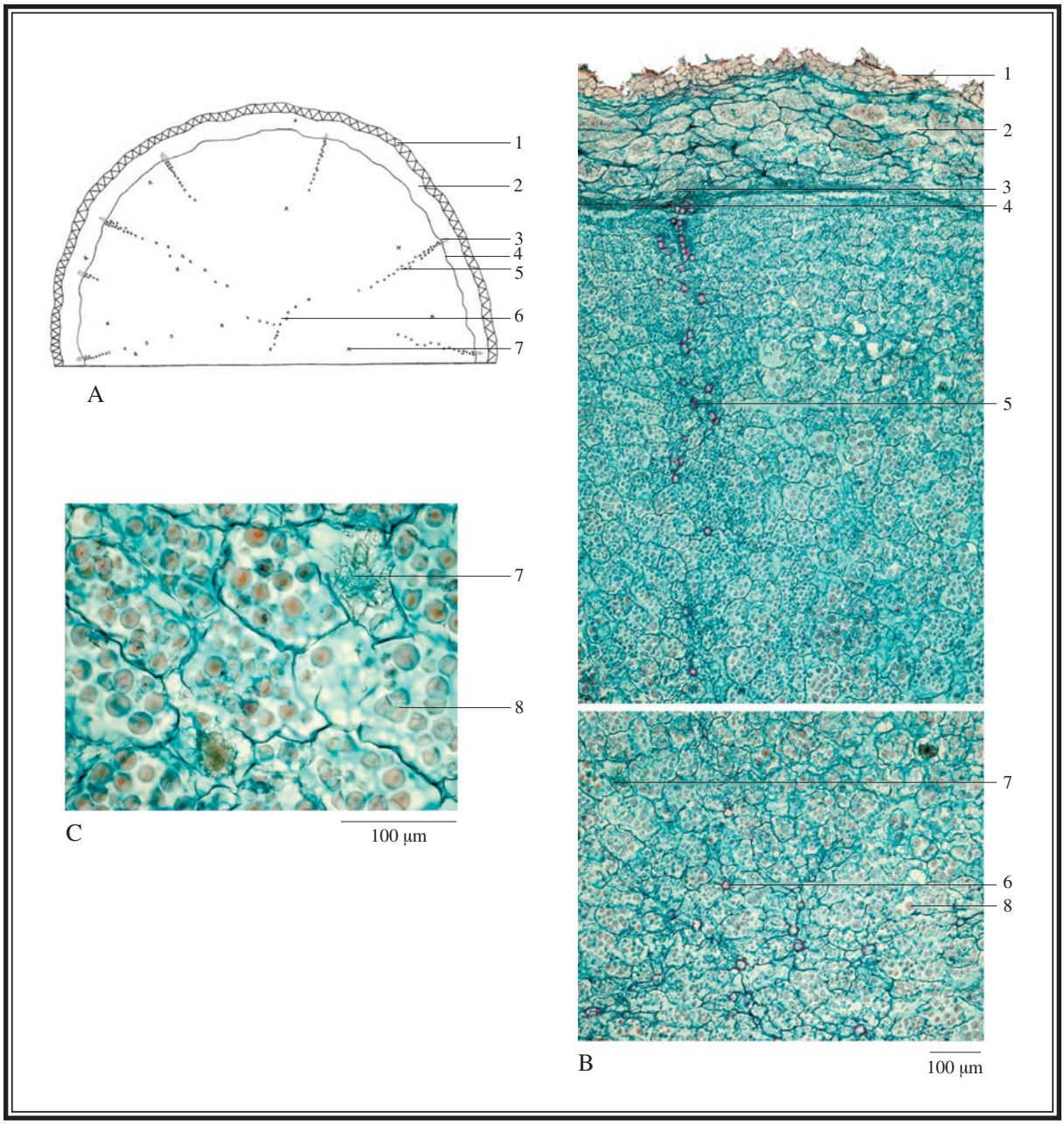

Figure 2 Microscopic features of transverse section of Radix Pseudostellariae

A. Sketch B. Section illustration

C. Parenchyma cells (containing starch granules and cluster of calcium oxalate)

1. Cork 2. Cortex 3. Phloem 4. Cambium 5. Xylem 6. Primary xylem

7. Cluster of calcium oxalate 8. Starch granules

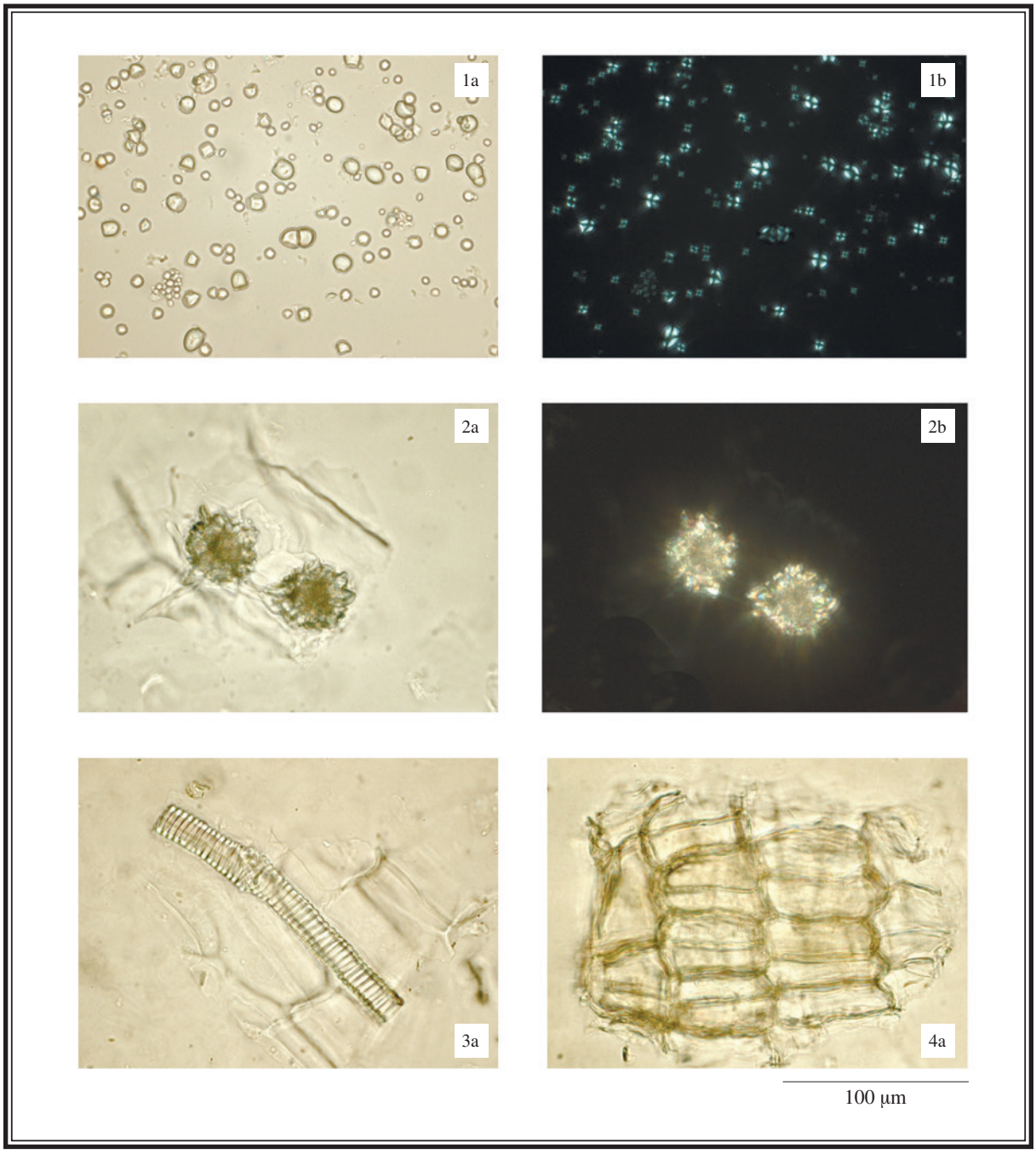

Figure 3 Microscopic features of powder of Radix Pseudostellariae

1. Starch granules 2. Clusters of calcium oxalate 3. Vessel 4. Cork cells

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

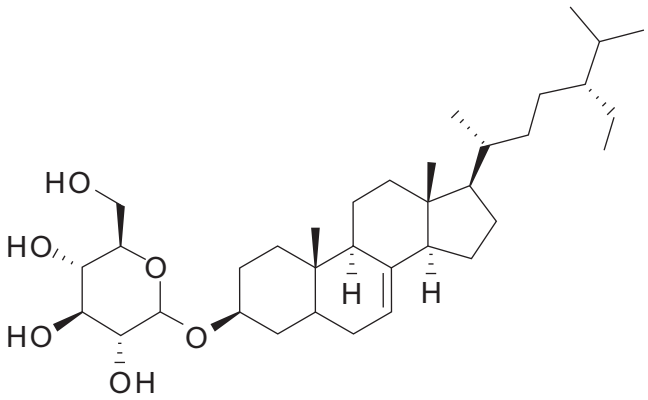

Figure 4 Chemical structure of Δ^7-stigmastenol-3-O-β-D-glucoside

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

Standard solution

Δ^7-Stigmastenol-3-O-β-D-glucoside standard solution for fingerprinting, Std-FP (500 mg/L)

Weigh 2.5 mg of Δ^7-stigmastenol-3-O-β-D-glucoside CRS and dissolve in 5 mL of methanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of ethanol (95%). Sonicate (270 W) the mixture for 60 min. Centrifuge at about 1800 × *g* for 5 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 PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35°C 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 60	8 → 0	92 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of Δ^7-stigmastenol-3-O-β-D-glucoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of Δ^7-stigmastenol-3-O-β-D-glucoside should not be more than 3.0%; the RSD of the retention time of Δ^7-stigmastenol-3-O-β-D-glucoside peak should not be more than 2.0%; the column efficiency determined from Δ^7-stigmastenol-3-O-β-D-glucoside peak should not be less than 12000 theoretical plates.

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. 5).

Procedure

Separately inject Δ^7-stigmastenol-3-O-β-D-glucoside Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of Δ^7-stigmastenol-3-O-β-D-glucoside peak in the chromatogram of Δ^7-stigmastenol-3-O-β-D-glucoside Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify Δ^7-stigmastenol-3-O-β-D-glucoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of Δ^7-stigmastenol-3-O-β-D-glucoside Std-FP. The retention times of Δ^7-stigmastenol-3-O-β-D-glucoside 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 five characteristic peaks of Radix Pseudostellariae extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Radix Pseudostellariae extract

Peak No.	RRT	Acceptable Range
1	0.61	±0.03
2	0.74	±0.03
3 (marker, Δ^7-stigmastenol-3-O-β-D-glucoside)	1.00	-
4	1.62	±0.03
5	1.74	±0.03

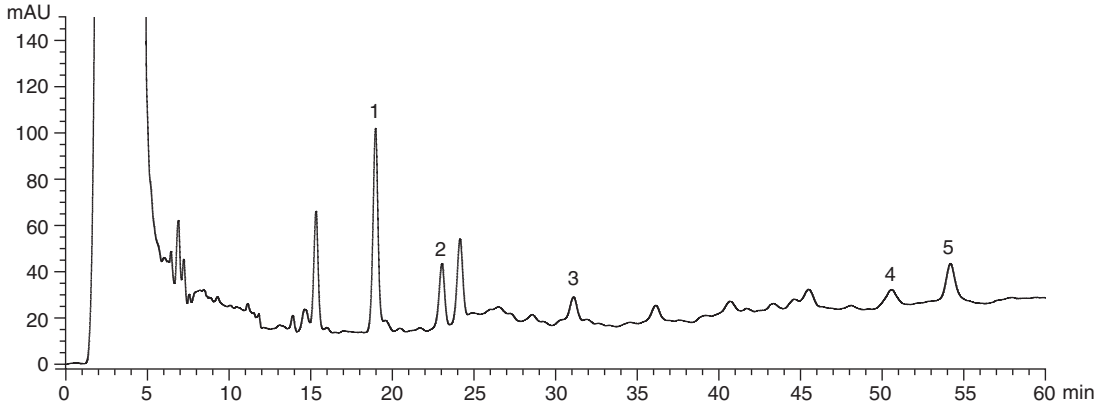

Figure 5 A reference fingerprint chromatogram of Radix Pseudostellariae extract

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. 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 XV)*: 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 1.0%.

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

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 28.0%.
Ethanol-soluble extractives (cold extraction method): not less than 18.0%.

7. ASSAY

Carry out the method as directed in Appendix XIII.

Reagent

Anthrone sulfuric acid solution

Weigh accurately 0.1 g of anthrone and dissolve in 100 mL of sulphuric acid (80%).

Standard solution

Anhydrous glucose standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 10.0 mg of anhydrous glucose CRS and dissolve in 50 mL of water.

Anhydrous glucose standard solution for assay, Std-AS

Measure accurately the volume of the anhydrous glucose Std-Stock, dilute with water to produce a series of solutions of 40, 50, 60, 70, 80 mg/L for anhydrous glucose.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of water. Place the mixture in a water bath for 60 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with 5 mL of water. Centrifuge at about 1800 × *g* for 10 min. Combine the extracts and make up to the mark with water. Pipette 3 mL of the solution into a 50-mL centrifuge tube. Add 30 mL of ethanol. Place the mixture at 4˚C for 12 h. Centrifuge at about 1800 × *g* for 10 min. Discard the supernatant. Dissolve the residue in water. Transfer the solution to a 25-mL volumetric flask and make up to the mark with water.

Ultraviolet/Visible spectrophotometeric system

The spectrophotometer is set at 625 nm.

Colourimetric method

Pipette 2 mL of the standard solution or test solution into a 10-mL test tube, then pipette 6 mL of anthrone sulfuric acid solution. Place the mixture in a water bath for 15 min. Cool the mixture in a ice water bath for 15 min. Using the corresponding anthrone sulfuric acid solution as the blank. Proceed to UV/Visible analysis at 625 nm.

System suitability requirements

Perform at least five replicate determinations, each using 2 mL of anhydrous glucose Std-AS (60 mg/L) by colourimetric method. The requirement of the system suitability parameters is as follows: the RSD of the absorbance of anhydrous glucose should not be more than 5.0%.

Calibration curve

Determine a series of anhydrous glucose Std-AS (2 mL each) in the ultraviolet/visible spectrophotometeric system and record the absorbance by colourimetric method. Plot the absorbances of anhydrous glucose against the corresponding concentrations of anhydrous glucose Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Measure the absorbance and calculate the concentration (in milligram per litre) of anhydrous glucose in the test solution, and calculate the percentage content of anhydrous glucose in the sample by using the equations indicated in Appendix XIII.

Limits

The sample contains not less than 6.0% of polysaccharides [calculated as anhydrous glucose ($C_6H_{12}O_6$)], calculated with reference to the dried substance.