Astragali Complanati Semen

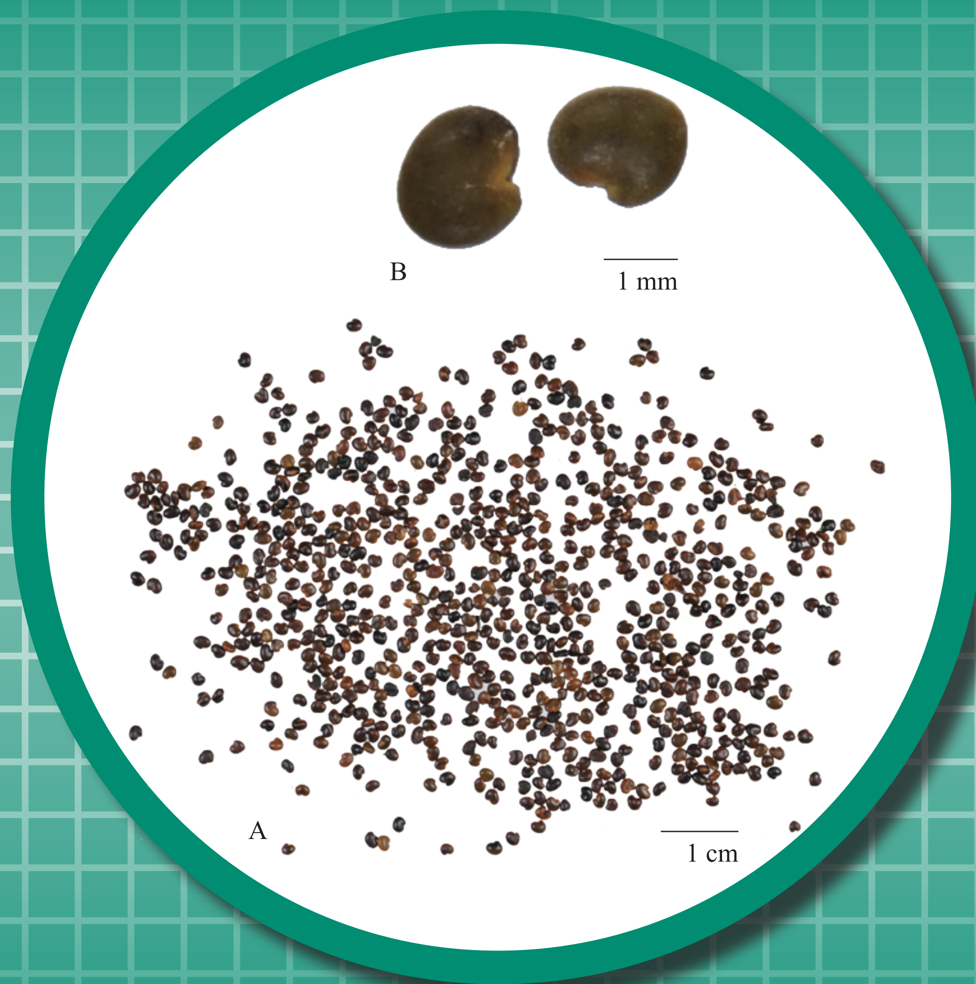


Figure 1 A photograph of Astragali Complanati Semen

A. Astragali Complanati Semen B. Magnified seeds

1. NAMES

Official Name: Astragali Complanati Semen

Chinese Name: 沙苑子

Chinese Phonetic Name: Shayuanzi

2. SOURCE

Astragali Complanati Semen is the dried ripe seed of *Astragalus complanatus* R. Br. (Fabaceae). The fruit with stalk is collected in late autumn and early winter when ripe but not dehiscent; dried under the sun; the seed is tapped out; foreign matter removed, then dried under the sun to obtain Astragali Complanati Semen.

3. DESCRIPTION

Reniform but slightly flattened, 2-2.5 mm long, 1.5-2 mm wide, about 1 mm thick. Externally smooth, brownish-green or greyish-brown, the rounded hilum located on the slightly dented edge. Texture hard, uneasily broken. Cotyledons 2, pale yellow, radicle curved, about 1 mm long. Odour slight; taste bland and bean-like flavor when chewed (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Longitudinal section

Epidermal palisade tissue consists of 1 layer of cells, 2 layers of cells in hilum region, covered with cuticle. Palisade cells radially elongated, 15-67 μm in radial length, with gradually thickened wall outward, with longitudinal striation in the upper part, colourless or containing yellowish-brown contents; 1/6 part close to surface bearing a light line. 1 layer brace cells dumbbell-shaped, with longitudinally striated-thickened wall, beneath palisade cells, colourless or containing yellow contents. Endosperm consists of 5-9 layers of parenchymatous cells or depressed degenerate cells, most crimpled, colourless. Cotyledons contain abundant oil droplets (Fig. 2).

Powder

Colour yellowish-brown. Palisade cells arrange in 1 layer, colourless or pale yellow, narrow rectangular in lateral view, subpolygonal in surface view, 15-67 µm long. Brace cells dumbbell-shaped in lateral view, subrounded or oval in surface view, with 3 concentric circles, 7-43 µm in diameter. Parenchymatous cells of cotyledon contain abundant oil droplets (Fig. 3).

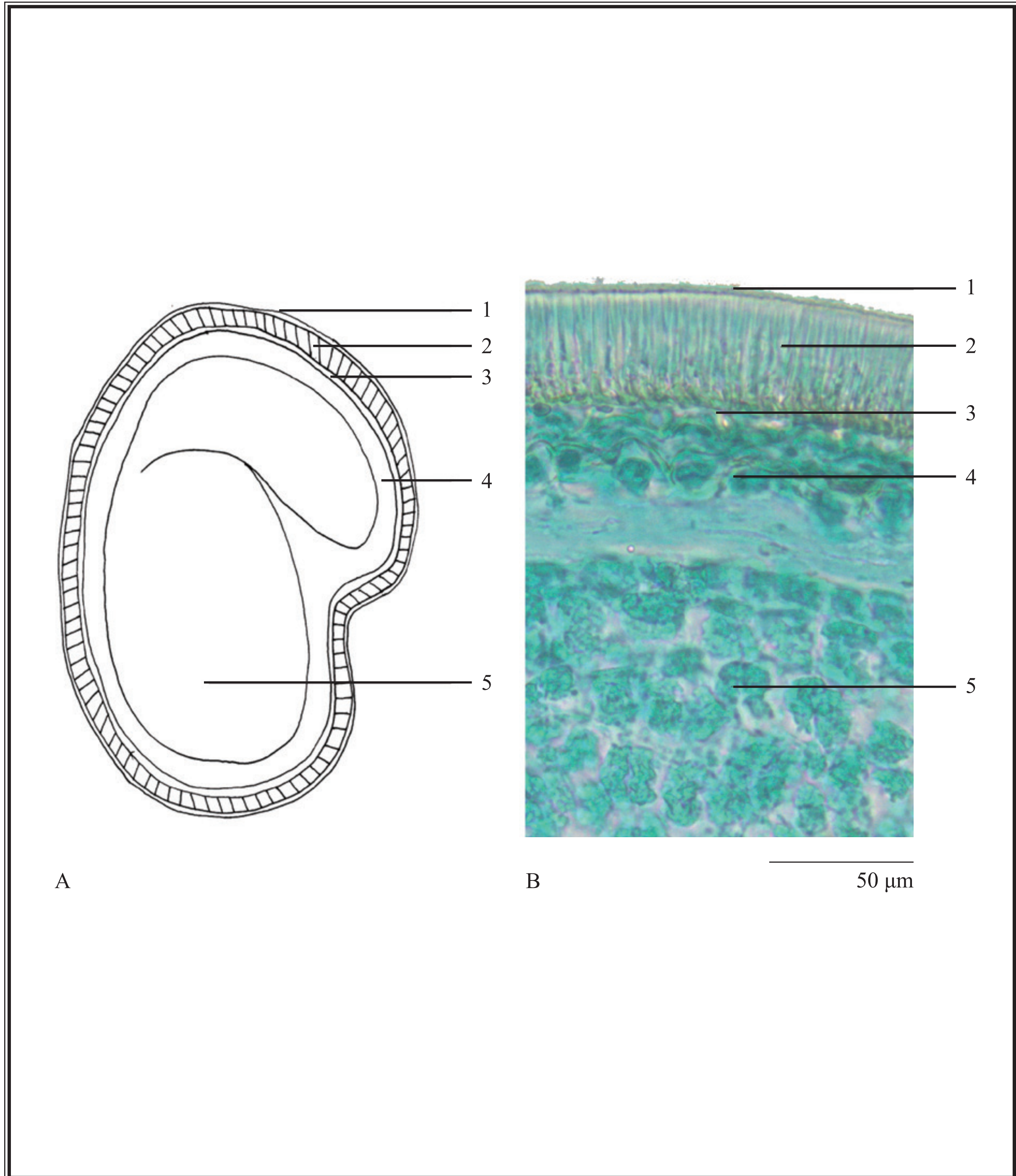
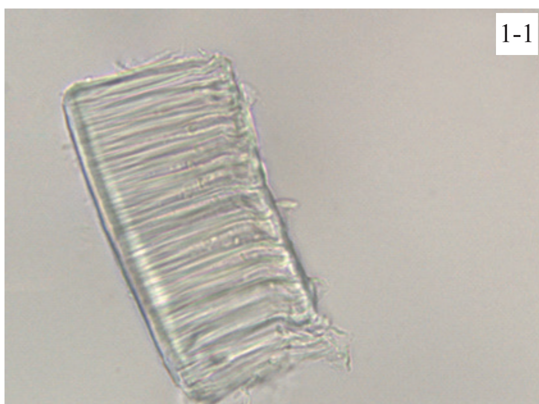
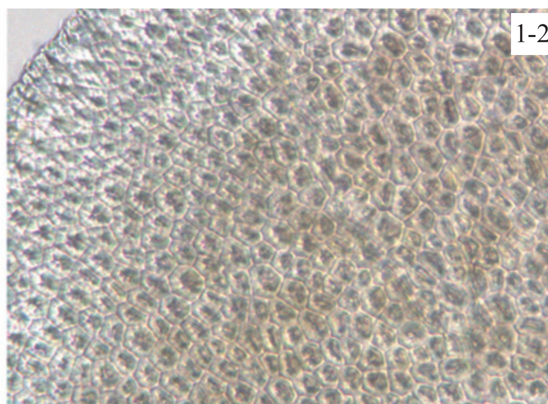


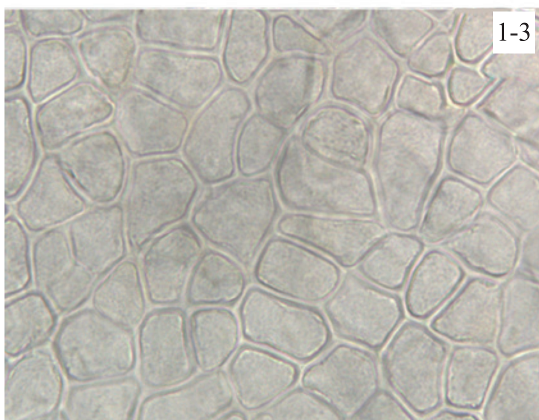
Figure 2 Microscopic features of longitudinal section of Astragali Complanati Semen

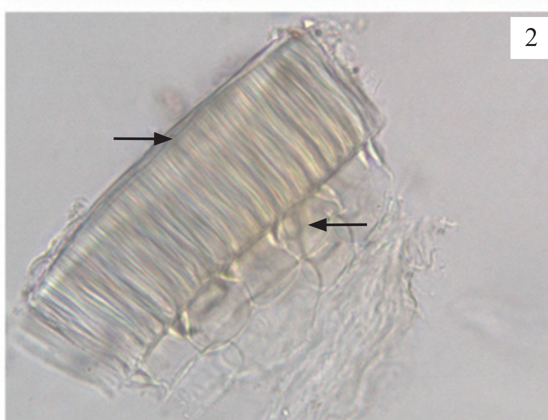
A. Sketch B. Section illustration

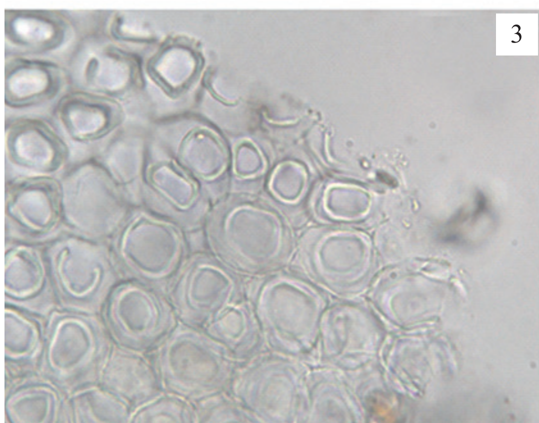
1. Cuticle 2. Palisade tissue 3. Brace cells 4. Endosperm 5. Cotyledon

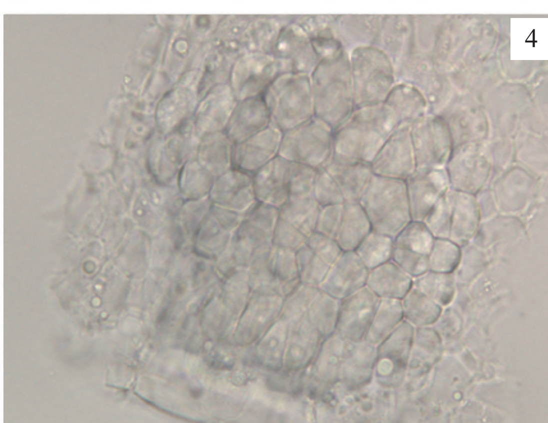












50 μm

Figure 3 Microscopic features of powder of Astragali Complanati Semen (under the light microscope)

1. Palisade cells (1-1 in lateral view, 1-2 in upper surface view, 1-3 in under surface view)
2. Palisade cells and brace cells (in lateral view)
3. Brace cells (in surface view) 4. Fragment of cotyledon

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

Standard solution

Complanatoside A standard solution

Weigh 2.5 mg of complanatoside A CRS (Fig. 4) and dissolve in 5 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol, formic acid and water (5:1:1:0.5, v/v).

Spray reagent

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

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of methanol. Sonicate (150 W) the mixture for 30 min. Filter the mixture.

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 complanatoside A standard solution (0.5 µL) and the test solution (10 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. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 1-3 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

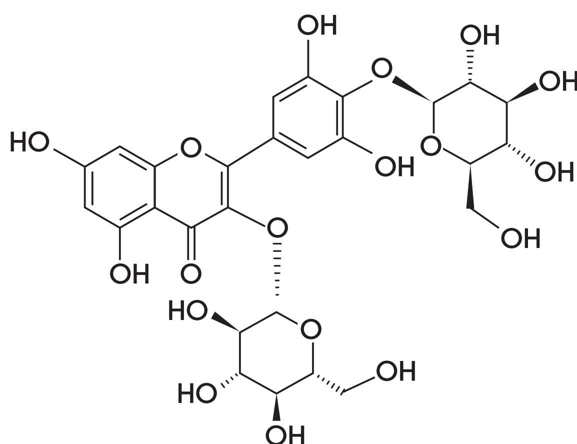


Figure 4 Chemical structure of complanatoside A

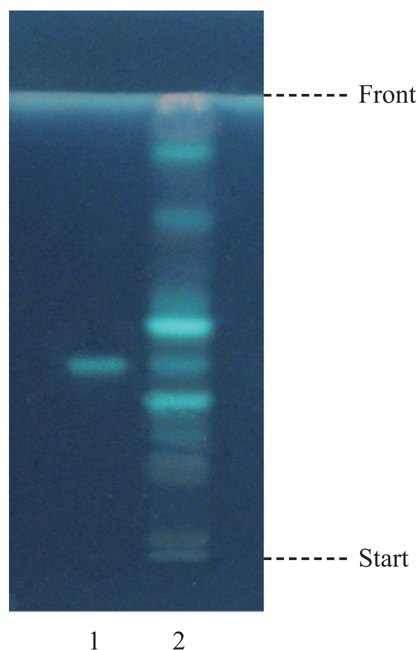


Figure 5 A reference HPTLC chromatogram of Astragali Complanati Semen extract observed under UV light (366 nm) after staining

1. Complanatoside A 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 complanatoside A (Fig. 5).

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

Standard solution

Complanatoside A standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of complanatoside A CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 25 mL of ethanol (50%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (267 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 (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.2% Formic acid (%, v/v)	Elution
0 – 15	13 → 15	87 → 85	linear gradient
15 – 25	15	85	isocratic
25 – 60	15 → 25	85 → 75	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of complanatoside A Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of complanatoside A should not be more than 5.0%; the RSD of the retention time of complanatoside A peak should not be more than 2.0%; the column efficiency determined from complanatoside A peak should not be less than 85000 theoretical plates.

The *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject complanatoside A Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of complanatoside A peak in the chromatogram of complanatoside A Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify complanatoside A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of complanatoside A Std-FP. The retention times of complanatoside A 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 Astragali Complanati Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Astragali Complanati Semen extract

Peak No.	RRT	Acceptable Range
1	0.28	± 0.03
2	0.38	± 0.04
3	0.71	± 0.06
4	0.96	± 0.03
5 (marker, complanatoside A)	1.00	-

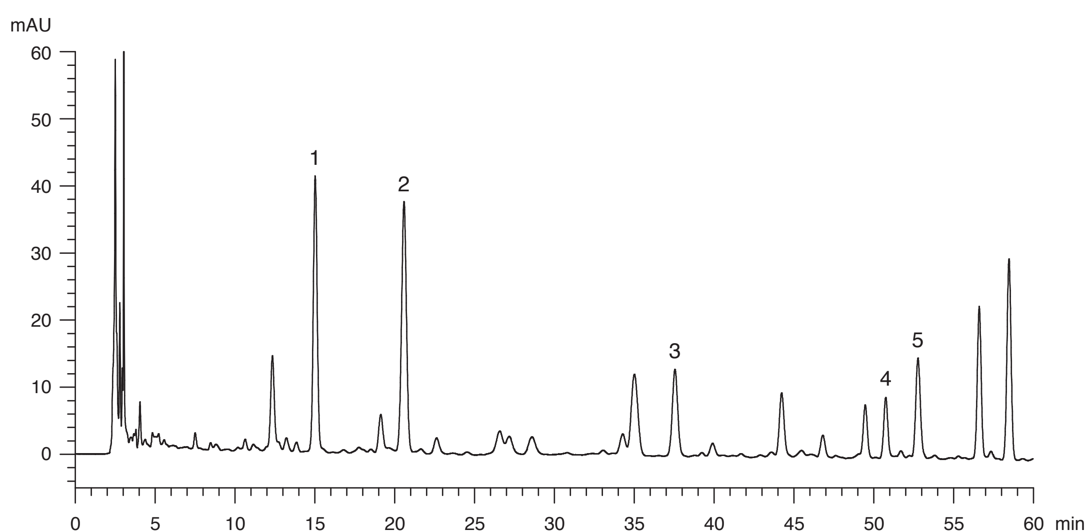


Figure 6 A reference fingerprint chromatogram of Astragali Complanati Semen extract

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 24.0%.
Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

7. ASSAY

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

Standard solution
Complanatoside A standard stock solution, Std-Stock (200 mg/L)
Weigh accurately 2.0 mg of complanatoside A CRS and dissolve in 10 mL of ethanol (50%).
Complanatoside A standard solution for assay, Std-AS
Measure accurately the volume of the complanatoside A Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 2.5, 5, 10, 30, 50 mg/L for complanatoside A.

Test solution
Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of ethanol (50%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 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 ethanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system
The liquid chromatograph is equipped with a DAD (267 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 (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.2% Formic acid (%, v/v)	Elution
0 – 20	20.5	79.5	isocratic
20 – 25	20.5 → 25	79.5 → 75	linear gradient
25 – 30	25	75	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of complanatoside A Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of complanatoside A should not be more than 5.0%; the RSD of the retention time of complanatoside A peak should not be more than 2.0%; the column efficiency determined from complanatoside A peak should not be less than 8000 theoretical plates.

The *R* value between complanatoside A 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 complanatoside A Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of complanatoside A against the corresponding concentrations of complanatoside A Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify complanatoside A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of complanatoside A Std-AS. The retention times of complanatoside A peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of complanatoside A in the test solution, and calculate the percentage content of complanatoside A in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.060% of complanatoside A ($C_{27}H_{30}O_{18}$), calculated with reference to the dried substance.