Bolbostemmatis Rhizoma

Figure 1 A photograph of Bolbostemmatis Rhizoma

A. Bolbostemmatis Rhizoma B. Magnified image of tuber
C. Magnified image of fracture of tuber

1. NAMES

Official Name: Bolbostemmatis Rhizoma

Chinese Name: 土貝母

Chinese Phonetic Name: Tubeimu

2. SOURCE

Bolbostemmatis Rhizoma is the dried tuber of *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae). The tuber is collected in autumn, washed clean, broken off, boiled until the tuber is devoid of the white core, taken out, then dried under the sun to obtain Bolbostemmatis Rhizoma.

3. DESCRIPTION

Irregular masses, varying in size. Externally pale brown, pale reddish-brown to dark brown, bumpy. Texture hard and uneasily broken. Fracture corneous. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of small rectangular cells, arranged orderly, usually suberized. Several layers of parenchymatous cells located beneath epidermis often suberized. Vascular bundles small, scattered irregularly in a haphazard manner in the stele parenchyma. Parenchymatous cells of the stele contain gelatinous starch masses (Fig. 2).

Powder

Colour yellowish-brown. Gelatinous starch masses fairly abundant. Vessels sometimes visible, spiral, annular or reticulate, 5-30 μm in diameter. Epidermal cells rectangular, neatly arranged in lateral view; subpolygonal in surface view, anticlinal walls beaded or verrucose thickened, some cell walls suberized (Fig. 3).

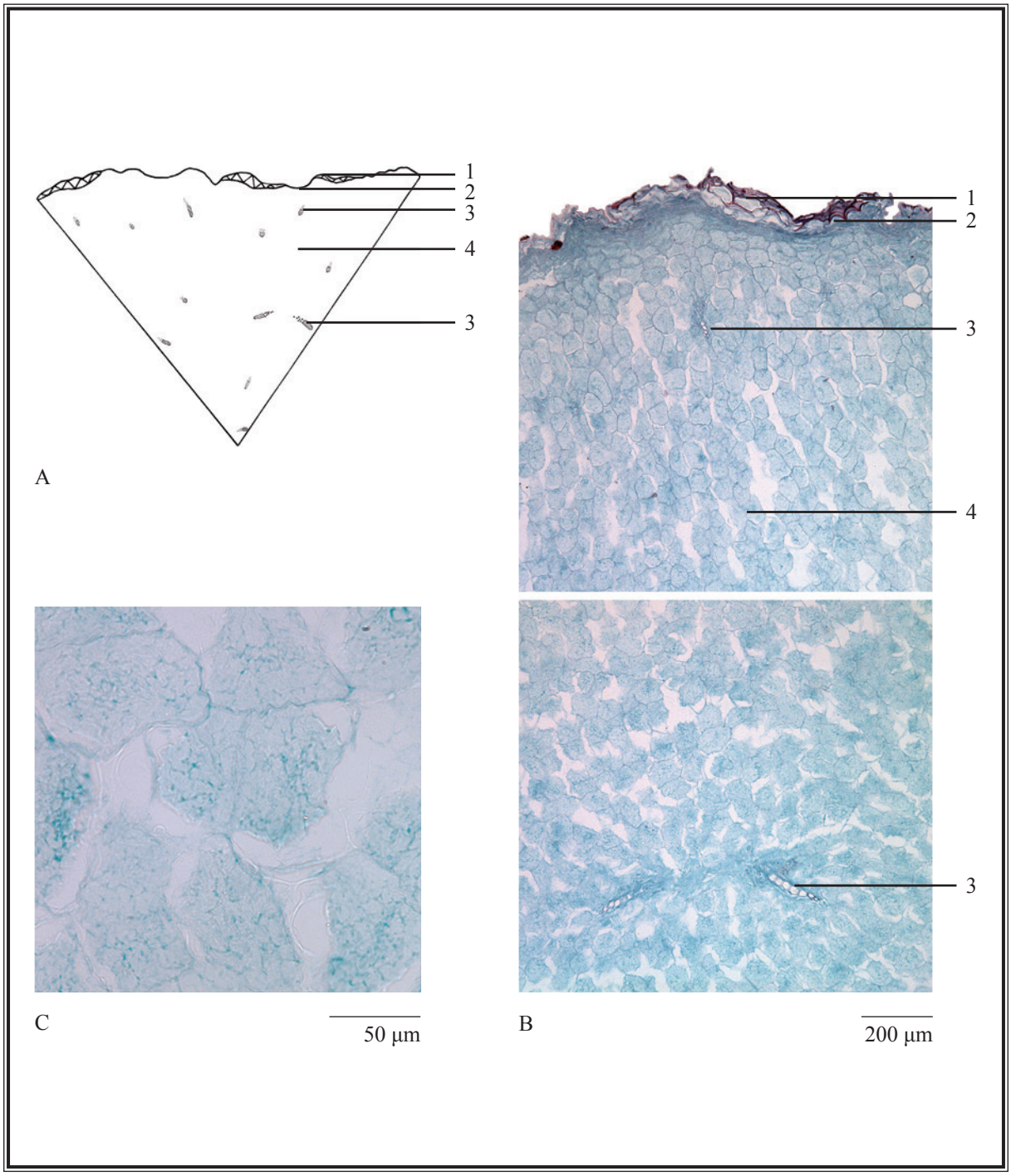

Figure 2 Microscopic features of transverse section of Bolbostemmatis Rhizoma

A. Sketch B. Section illustration

C. Stele parenchymatous cells containing gelatinous starch masses

1. Epidermis 2. Suberized cells 3. Vascular bundles

4. Stele parenchyma containing gelatinous starch masses

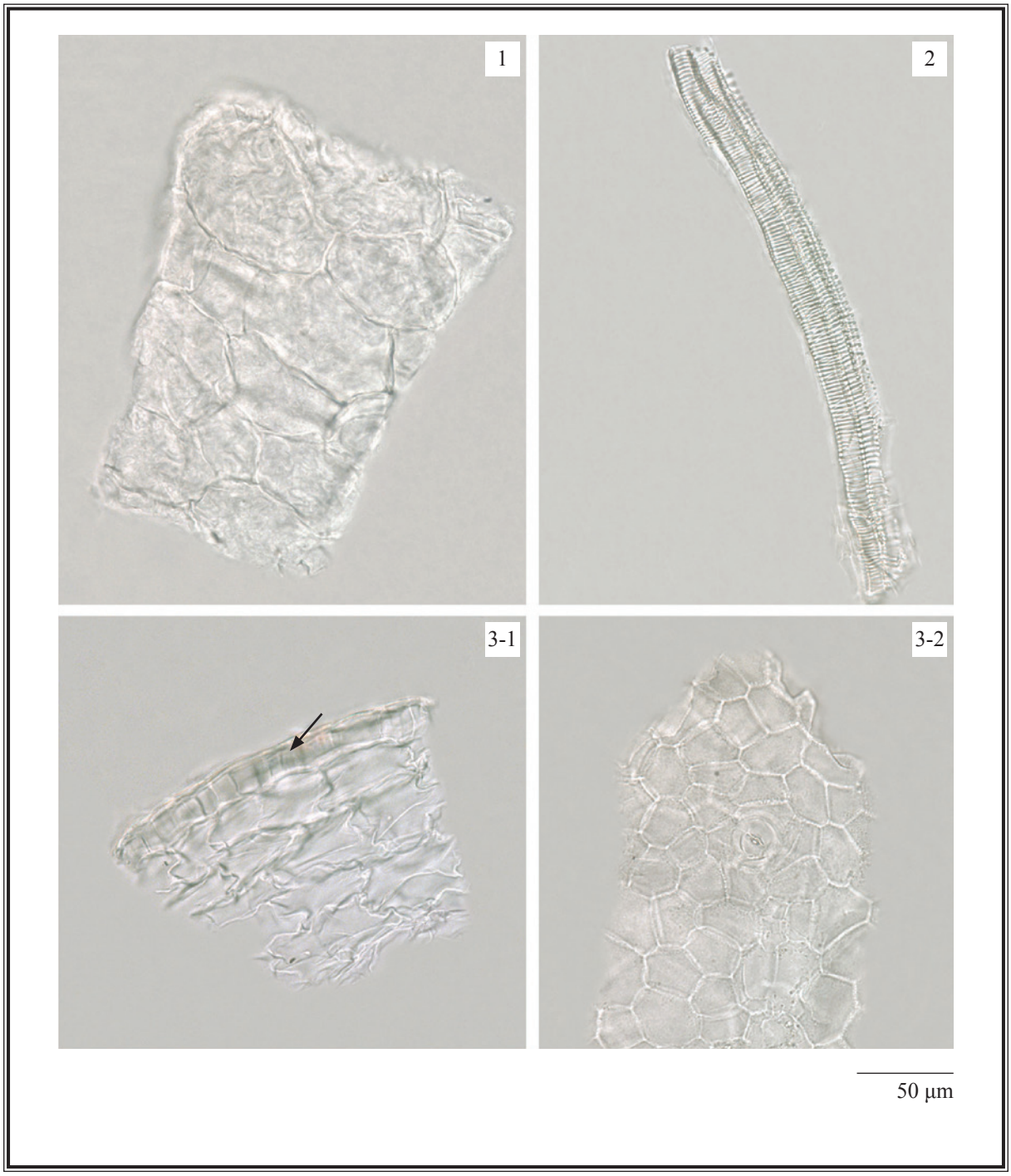

Figure 3 Microscopic features of powder of Bolbostemmatis Rhizoma (under the light microscope)

1. Gelatinous starch masses 2. Vessels 3. Epidermal cells (3-1 in lateral view ⟶, 3-2 in surface view)

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

Standard solution

Tubeimoside I standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, dichloromethane, methanol and water (4:3:3:1, v/v).

Spray reagent

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

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (70%). Sonicate (270 W) the mixture for 20 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (70%).

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 tubeimoside I standard solution and the test solution (1 μL each) 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 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 105°C (about 10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

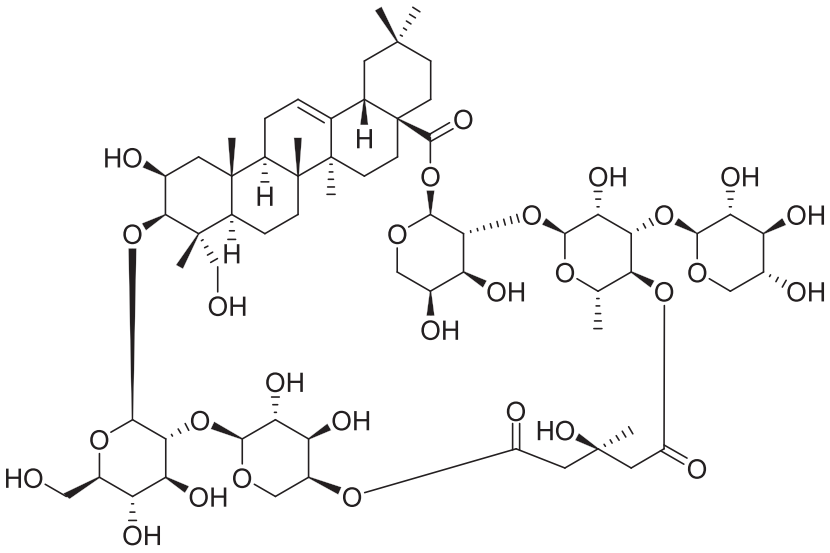

Figure 4 Chemical structure of tubeimoside I

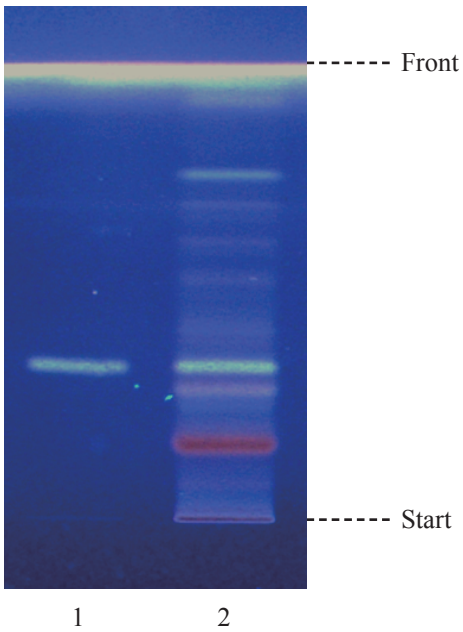

Figure 5 A reference HPTLC chromatogram of Bolbostemmatis Rhizoma extract observed under UV light (366 nm) after staining

1. Tubeimoside I 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 tubeimoside I (Fig. 5).

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

Standard solution

Tubeimoside I standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 5.0 mg of tubeimoside I CRS and dissolve in 25 mL of methanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (70%). Pipette 1 mL of the solution to a 5-mL volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (214 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)	Water (%, v/v)	Elution
0 – 20	10 → 40	90 → 60	linear gradient
20 – 30	40 → 70	60 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of tubeimoside I Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of tubeimoside I should not be more than 5.0%; the RSD of the retention time of tubeimoside I peak should not be more than 2.0%; the column efficiency determined from tubeimoside I peak should not be less than 200000 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. 6).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.91	± 0.03
2	0.92	± 0.03
3 (marker, tubeimoside I)	1.00	-

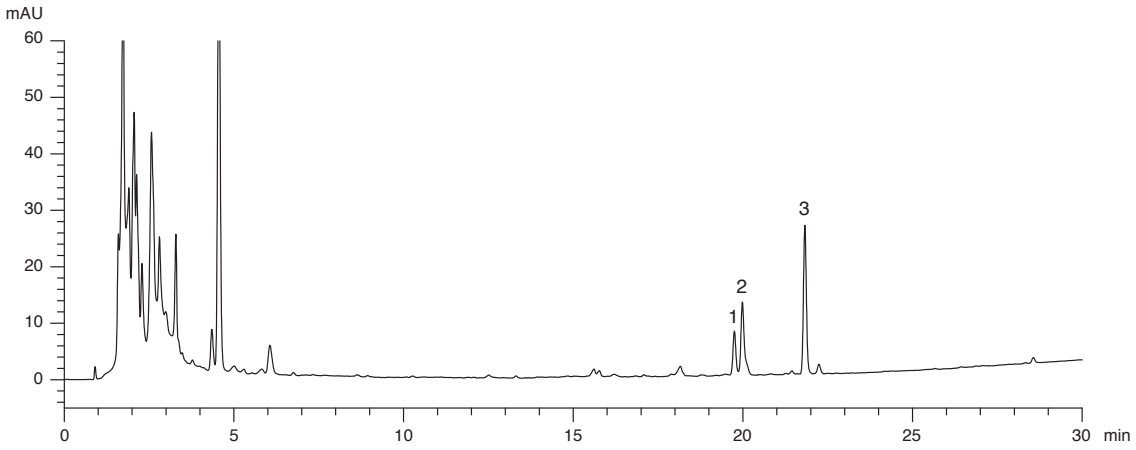

Figure 6 A reference fingerprint chromatogram of Bolbostemmatis Rhizoma extract

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 – Aflatoxins *(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 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 49.0%.
Ethanol-soluble extractives (cold extraction method): not less than 28.0%.

7. ASSAY

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

Standard solution
Tubeimoside I standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 5.0 mg of tubeimoside I CRS and dissolve in 5 mL of methanol (70%).
Tubeimoside I standard solution for assay, Std-AS
Measure accurately the volume of the tubeimoside I Std-Stock, dilute with methanol (70%) to produce a series of solutions of 25, 100, 200, 300, 500 mg/L for tubeimoside I.

Test solution
Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (70%). Pipette 1 mL of the

solution to a 5-mL volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (214 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)	Water (%, v/v)	Elution
0 – 20	10 → 40	90 → 60	linear gradient
20 – 30	40 → 70	60 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of tubeimoside I Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of tubeimoside I should not be more than 5.0%; the RSD of the retention time of tubeimoside I peak should not be more than 2.0%; the column efficiency determined from tubeimoside I peak should not be less than 200000 theoretical plates.

The *R* value between tubeimoside I 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 tubeimoside I Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of tubeimoside I against the corresponding concentrations of tubeimoside I 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 tubeimoside I peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of tubeimoside I Std-AS. The retention times of tubeimoside I 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 tubeimoside I in the test solution, and calculate the percentage content of tubeimoside I in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.9% of tubeimoside I ($C_{63}H_{98}O_{29}$), calculated with reference to the dried substance.