Lycii Cortex

Figure 1　A photograph of Lycii Cortex

難冠花 Celosiae Cristatae Flos

Dryopteridis Crassirhizomatis Rhizoma

Cinnamomi Cortex
肉桂

路路通
Liquidambaris Fructus

Allii Tuberosi Semen
韭菜子

綿馬貫眾

益智

Genkwa Flos
芫花

Acanthopanacis Cortex

五加皮

Bistortae Rhizoma

拳參

胡黃連

Polygoni Orientalis Fructus
水紅花子

Alpiniae Oxyphyllae Fructus

Picrorhizae Rhizoma

Centellae Herba
積雪草

Lycii Cortex

1. NAMES

Official Name: Lycii Cortex

Chinese Name: 地骨皮

Chinese Phonetic Name: Digupi

2. SOURCE

Lycii Cortex is the dried root bark of *Lycium chinense* Mill. (Solanaceae). The root is collected in early spring or late autumn, washed clean, stripped off the root bark, then dried under the sun to obtain Lycii Cortex.

3. DESCRIPTION

Quilled, channeled or irregular, 3-12 cm long, 5-30 mm in diameter, 1-5 mm thick. Externally greyish-yellow to brownish-yellow, rough and lax, with irregular longitudinal fissures and the cork easily exfoliated; inner surface yellowish-white to greyish-yellow, relatively even, with fine longitudinal wrinkles. Texture fragile and light in weight, easily broken. Fracture uneven, outer layer yellowish-brown and inner layer greyish-white. Odour slight; taste slightly sweet, followed by bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section
Rhytidome relatively thick. Cork consists of 4-10 or even more layers of flattened cells, tangential elongated. Cortex narrow, cells rounded-elongated, with clefts. Phloem broad, phloem rays mostly 1 row of cells wide. Fibres occasionally found, mostly scattered singly, wall thickened and lignified. Parenchymatous cells contain abundant microcrystals of calcium oxalate (Fig. 2).

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Gentianae Macrophyllae Radix
秦艽

覆盆子
Rubi Fructus

Celosiae Cristatae Flos
雞冠花

Sennae Folium
番瀉葉

鬱金 Curcumae Radix

沙苑子 Astragali Complanati Semen

川楝子
Toosendan Fructus

Solidaginis Herba
一枝黃花

Cyathulae Radix
川牛膝

Buddlejae Flos
密蒙花

Drynariae Rhizoma
骨碎補

皂角刺 Gleditsiae Spina

豬牙皂

Gleditsiae Fructus Abnormalis

Lycii Cortex

Powder

Colour greyish-white. Starch granules abundant, simple starch granules subrounded or elliptical, 2-10 μm in diameter; black and cruciate-shaped under the polarized microscope; compound starch granules usually composed of 2 or more units. Microcrystals of calcium oxalate abundant, scattered or present in parenchymatous cells, slightly arrow head-like, extremely minute; blue or bright white under the polarized microscope. Fibres scattered singly or in bundles, pale yellow or pale brown, fusiform or slender fusiform, 5-29 μm in diameter. Cork cells pale brown or brown, polygonal or square (Fig. 3).

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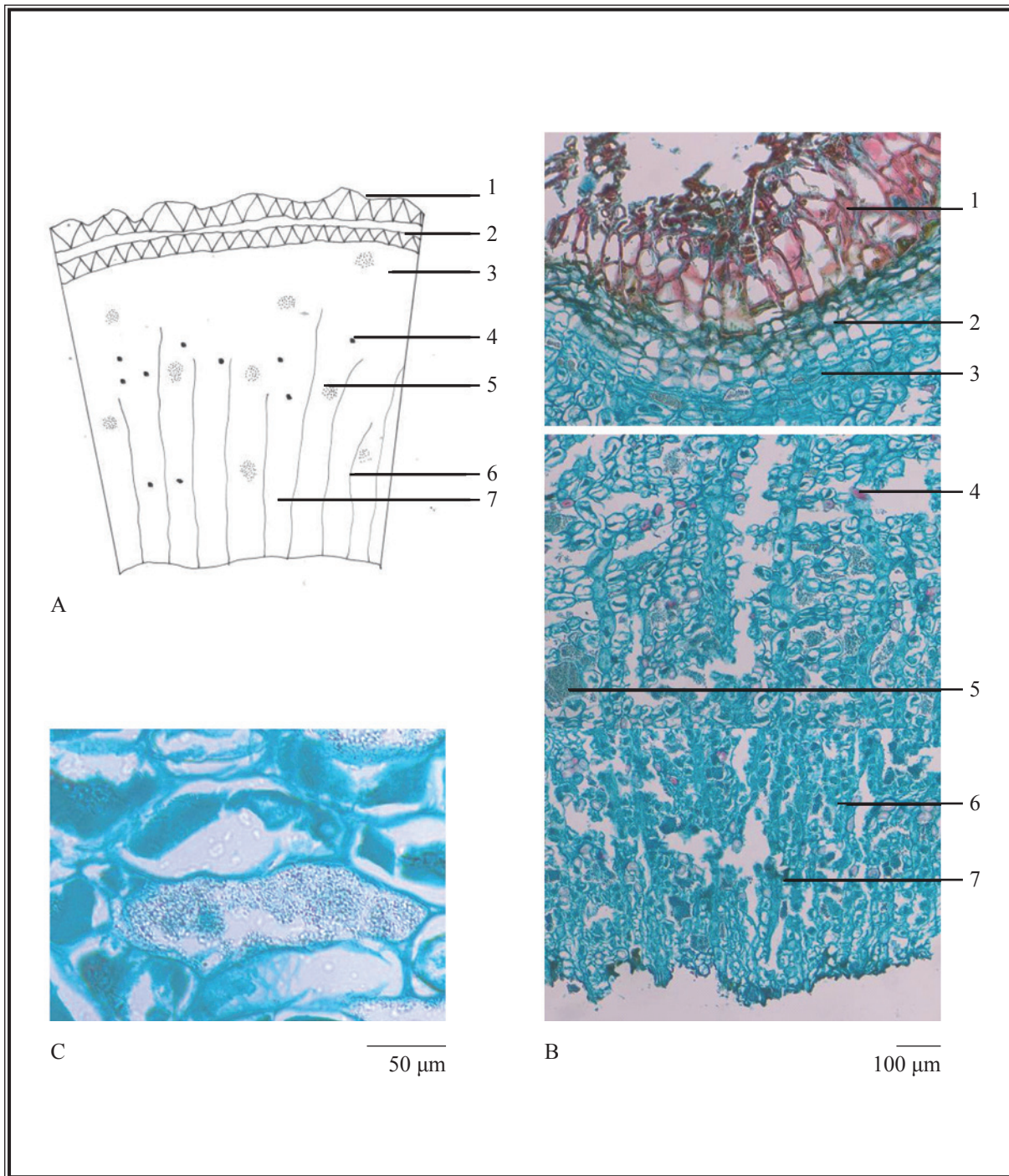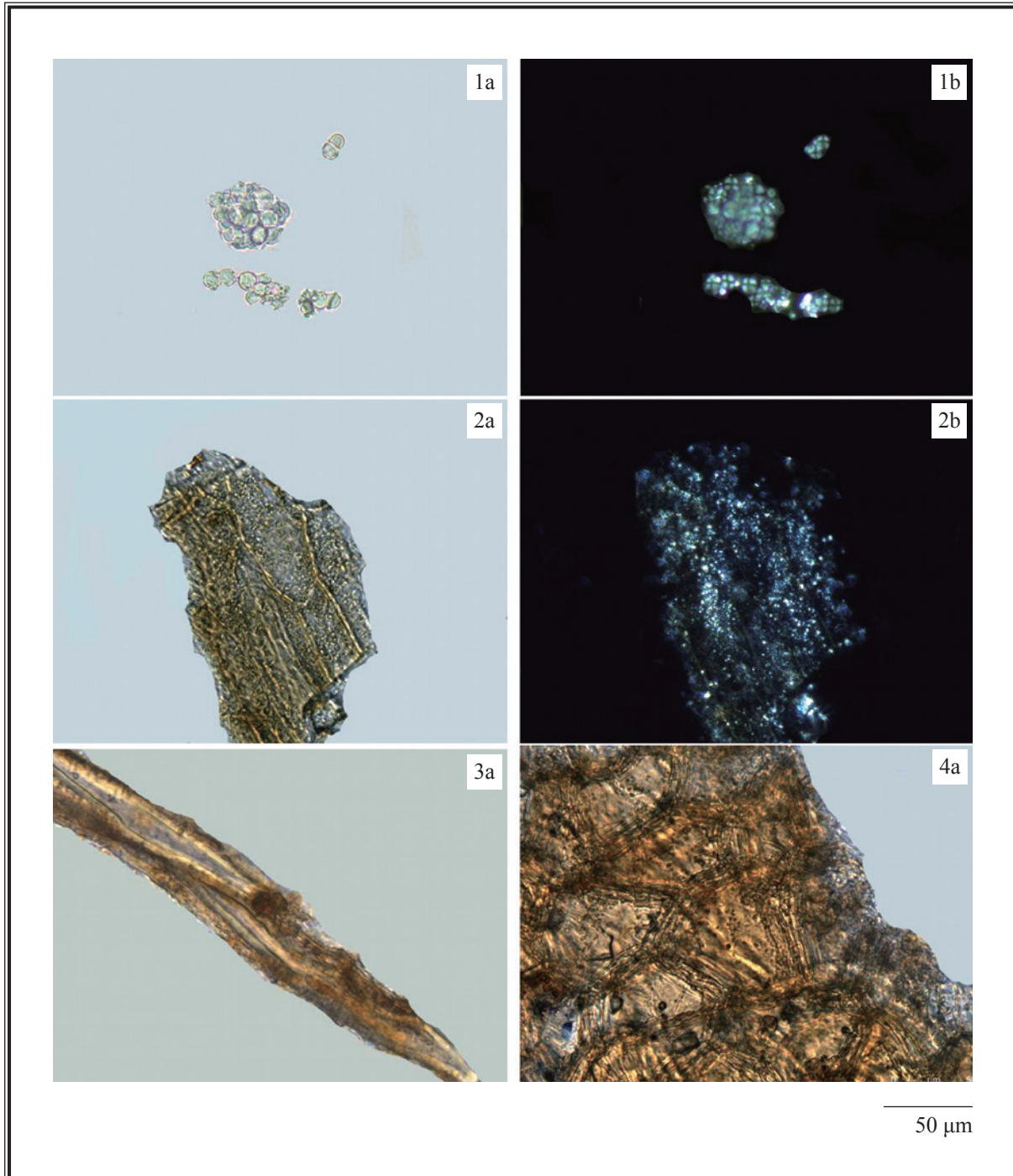
Bistortae Rhizoma
拳參
Centellae Herba
積雪草

Lycii Cortex

Figure 2　Microscopic features of transverse section of Lycii Cortex

A. Sketch　　B. Section illustration　　C. Microcrystals of calcium oxalate

1. Rhytidome　2. Cork　3. Cortex　4. Fibres　5. Microcrystals of calcium oxalate
6. Phloem ray　7. Phloem

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Figure 3 Microscopic features of powder of Lycii Cortex

1. Starch granules   2. Microcrystals of calcium oxalate   3. Fibres   4. Cork cells

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

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

Standard solution

Scopoletin standard solution

Weigh 0.5 mg of scopoletin CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, cyclohexane and glacial acetic acid (6:5:0.2, v/v).
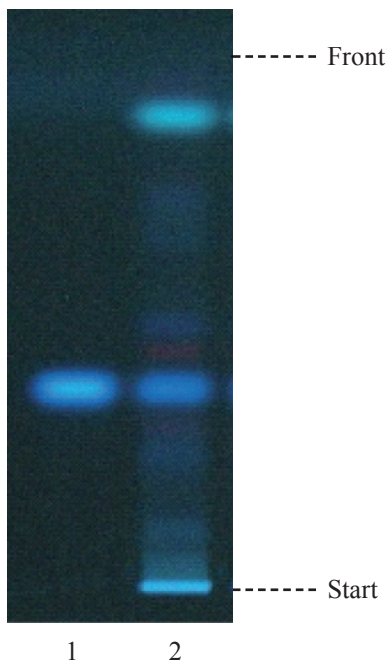
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (350 W) the mixture for 30 min. Filter and transfer the filtrate 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 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 scopoletin standard solution (2 μL) and the test solution (5 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 8.5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

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(i)

(ii)
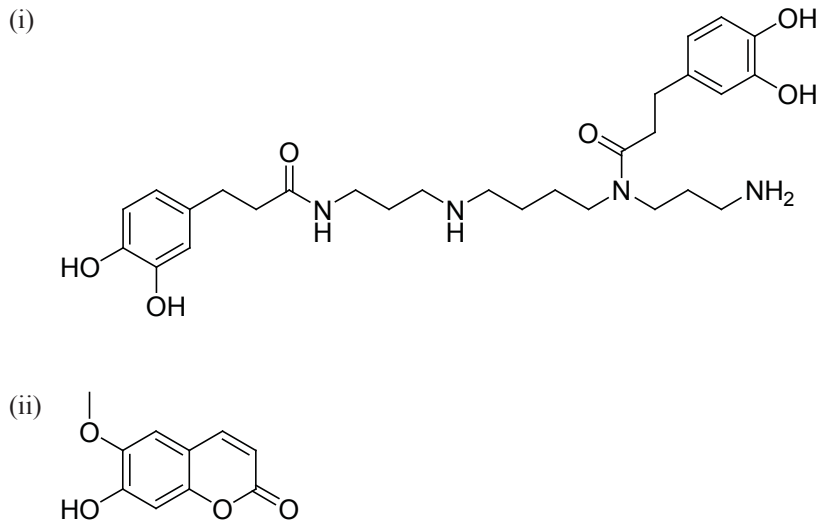
Figure 4   Chemical structures of (i) kukoamine B and (ii) scopoletin

Figure 5   A reference HPTLC chromatogram of Lycii Cortex extract observed under UV light (366 nm)

1. Scopoletin 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 scopoletin (Fig. 5).

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

Standard solution

Kukoamine B standard solution for fingerprinting, Std-FP (370 mg/L)
Weigh 3.7 mg of kukoamine B CRS (Fig. 4) and dissolve in 10 mL of methanol (50%) containing 0.5% acetic acid.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%) containing 0.5% acetic acid. Sonicate (350 W) the mixture for 30 min. Centrifuge at about $4000 \times g$ for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 40ºC 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)	0.15% Trifluoroacetic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	88	12	isocratic
10 – 20	88 → 84	12 → 16	linear gradient
20 – 40	84	16	isocratic
40 – 60	84 → 78	16 → 22	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of kukoamine B Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of kukoamine B should not be more than 5.0%; the RSD of the retention time of kukoamine B peak should not be more than 2.0%; the column efficiency determined from kukoamine B peak should not be less than 10000 theoretical plates.

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

Procedure

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

Table 2   The RRTs and acceptable ranges of the four characteristic peaks of Lycii Cortex extract

Peak No.	RRT	Acceptable Range
1	0.47	± 0.03
2 (marker, kukoamine B)	1.00	-
3 (kukoamine A)	1.11	± 0.03
4	1.26	± 0.03
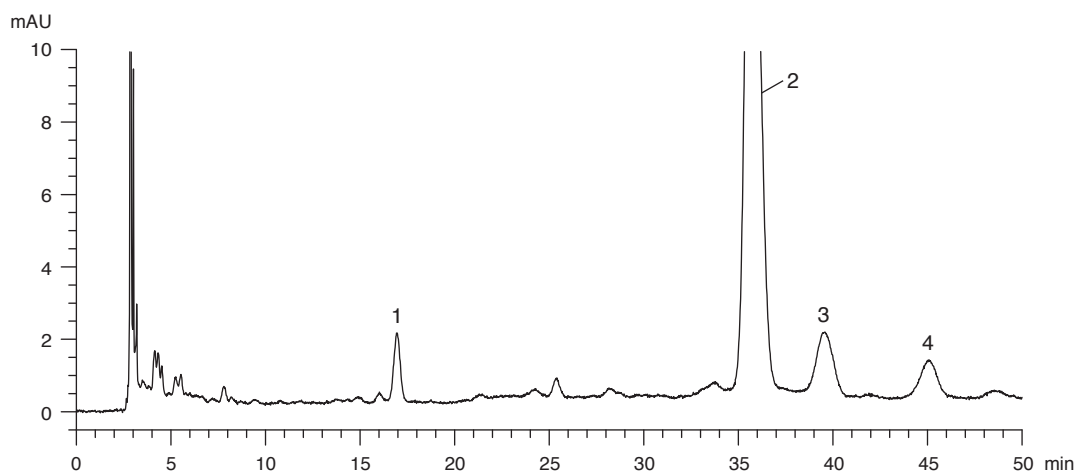
Figure 6   A reference fingerprint chromatogram of Lycii Cortex extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 6).

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Picrorhizae Rhizoma

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 19.0%.
Acid-insoluble ash: not more than 4.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 9.0%.
Ethanol-soluble extractives (hot extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Kukoamine B standard stock solution, Std-Stock (2000 mg/L)
Weigh accurately 2.0 mg of kukoamine B CRS and dissolve in 1 mL of methanol (50%) containing 0.5% acetic acid.

Kukoamine B standard solution for assay, Std-AS
Measure accurately the volume of the kukoamine B Std-Stock, dilute with methanol (50%) containing 0.5% acetic acid to produce a series of solutions of 40, 60, 80, 180, 360 mg/L for kukoamine B.

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Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 7 mL of methanol (50%) containing 0.5% acetic acid. Sonicate (350 W) the mixture for 30 min. Centrifuge at about $4000 \times g$ for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with methanol (50%) containing 0.5% acetic acid. Combine the solutions and make up to the mark with methanol (50%) containing 0.5% acetic acid. Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3   Chromatographic system conditions

Time (min)	0.15% Trifluoroacetic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	88	12	isocratic
10 – 20	88 → 84	12 → 16	linear gradient
20 – 40	84	16	isocratic
40 – 60	84 → 78	16 → 22	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of kukoamine B Std-AS (80 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of kukoamine B should not be more than 5.0%; the RSD of the retention time of kukoamine B peak should not be more than 2.0%; the column efficiency determined from kukoamine B peak should not be less than 10000 theoretical plates.

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

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Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify kukoamine B peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of kukoamine B Std-AS. The retention times of kukoamine B 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 kukoamine B in the test solution, and calculate the percentage content of kukoamine B in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.0% of kukoamine B ($C_{28}H_{42}N_4O_6$), calculated with reference to the dried substance.