Dendrobii Officinalis Caulis

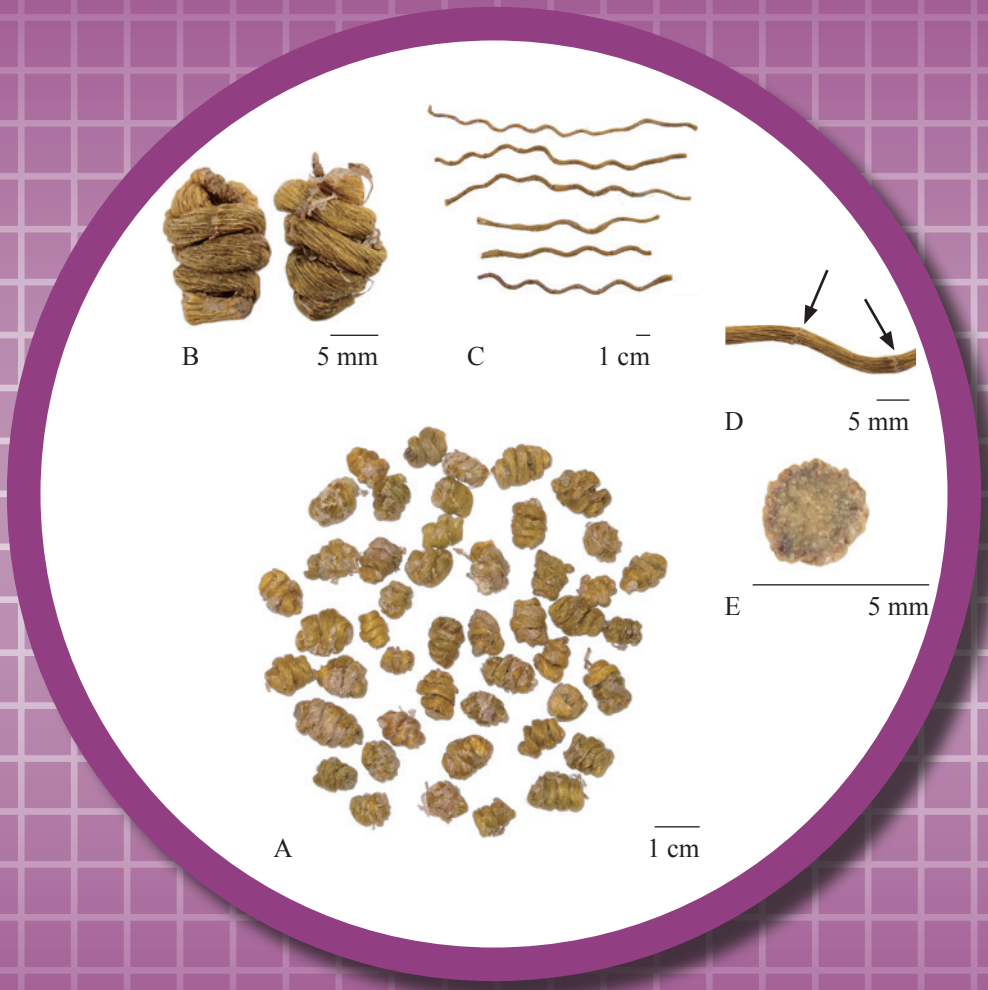

Figure 1 A photograph of Dendrobii Officinalis Caulis

A. Dendrobii Officinalis Caulis (Tiepifengdou)
B. Magnified image of stem (Tiepifengdou)
C. Dendrobii Officinalis Caulis (Tiepishihu)
D. Magnified image of node (Tiepishihu)
E. Magnified image of fracture

1. NAMES

Official Name: Dendrobii Officinalis Caulis

Chinese Name: 鐵皮石斛

Chinese Phonetic Name: Tiepishihu

2. SOURCE

Dendrobii Officinalis Caulis is the dried stem of *Dendrobium officinale* Kimura et Migo[1] (Orchidaceae). The stem is collected from November to the following March, fibrous roots and foreign matter removed, the stem is twisted into a spiral while heating, then baked to dryness (known as Tiepifengdou); or cut into sections, then dried or baked at below 60°C (known as Tiepishihu).

3. DESCRIPTION

'Tiepifengdou' spiral or spring-like, usually with 2-6 spires, 5-13 mm in diameter. 'Tiepishihu' cylindrical, slightly bended, 1-4.5 mm in diameter; externally yellowish-green to dark yellow, with fine longitudinal wrinkles; nodes distinct, sometimes with greyish-white residual leaf sheaths. Texture compact and hard. Fracture even, greyish-white, slightly corneous. Odour slight; taste bitter and viscous when chewed (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of cells, flattened, outer walls and lateral walls slightly thickened, covered with bright yellow cuticle. Parenchymatous cells polygonal, similar in size, scattered with numerous vascular bundles, the parenchymatous cells surrounding vascular bundles smaller in size. Mucilage cells scattered, containing raphides of calcium oxalate. Vascular bundles oblong, somewhat arranged in 4-5 rings. Bundle sheath fibres hat-shaped, consisting of 1-5 thick-walled fibre cells, arranged at the outer side of vascular bundles, some with parenchymatous cells containing subrounded silica bodies outside the fibre bundles (Fig. 2).

[1] *Dendrobium catenatum* Lindl.is the new accepted name of *Dendrobium officinale* Kimura et Migo.

Powder

Colour greyish-green. Bundle sheath fibres mostly arranged in bundles, 9-23 μm in diameter, walls thick, lumens relatively narrow, some surrounded by cells contain subrounded silica bodies of 6-23 μm in diameter; bright white or bright yellow under the polarized microscope. Raphides of calcium oxalate scattered or present in mucilage cells or parenchymatous cells, 25-120 μm long; polychromatic under the polarized microscope. Epidermal cells subpolygonal to elongated-polygonal in surface view, anticlinal walls beaded. Vessels mainly bordered-pitted, scalariform and spiral, 8-36 μm in diameter (Fig. 3).

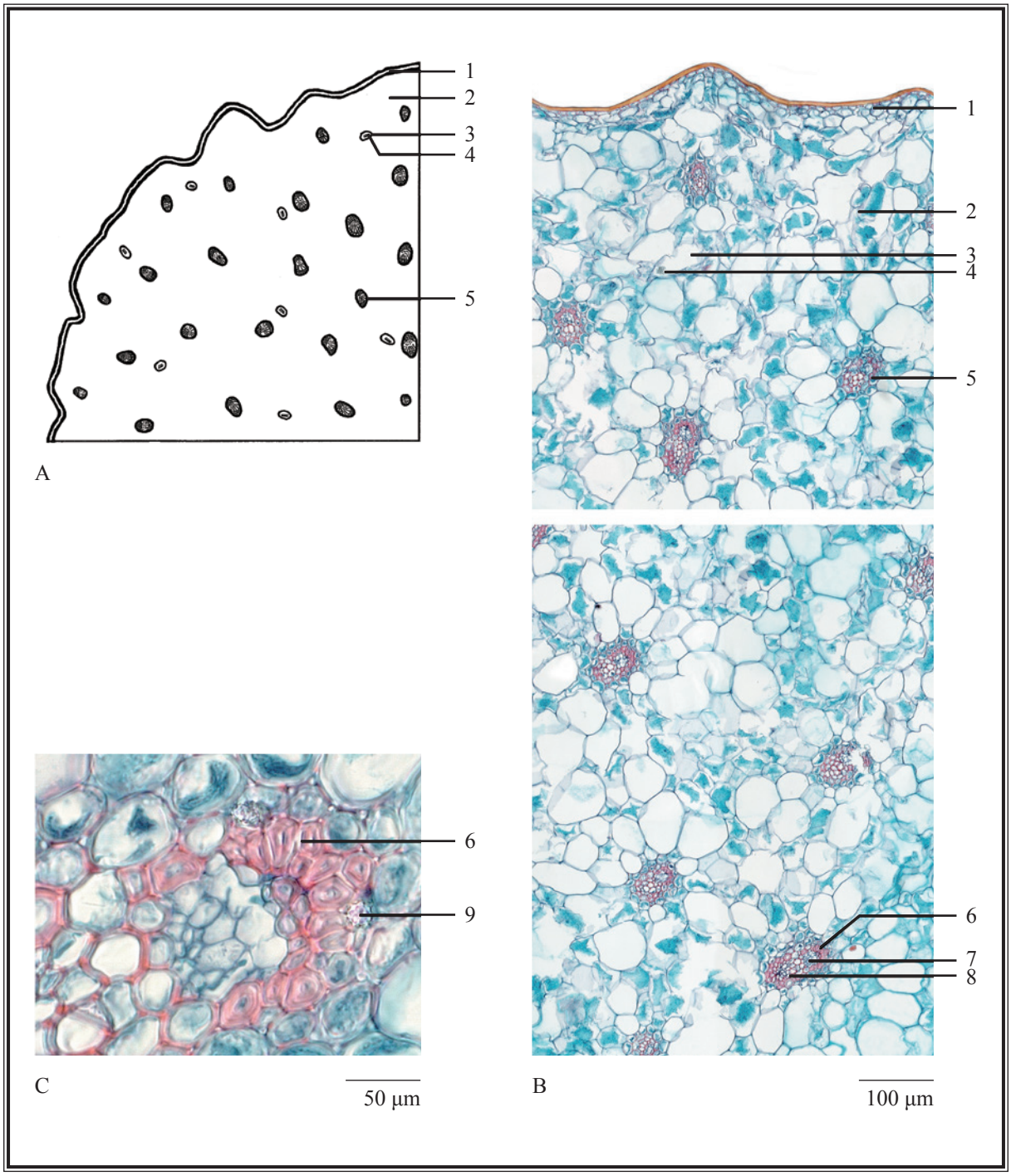

Figure 2 Microscopic features of transverse section of Dendrobii Officinalis Caulis

A. Sketch B. Section illustration C. Vascular bundle

1. Epidermis 2. Parenchyma 3. Mucilage cells 4. Raphides of calcium oxalate 5. Vascular bundle
6. Bundle sheath fibres 7. Phloem 8. Xylem 9. Silica body

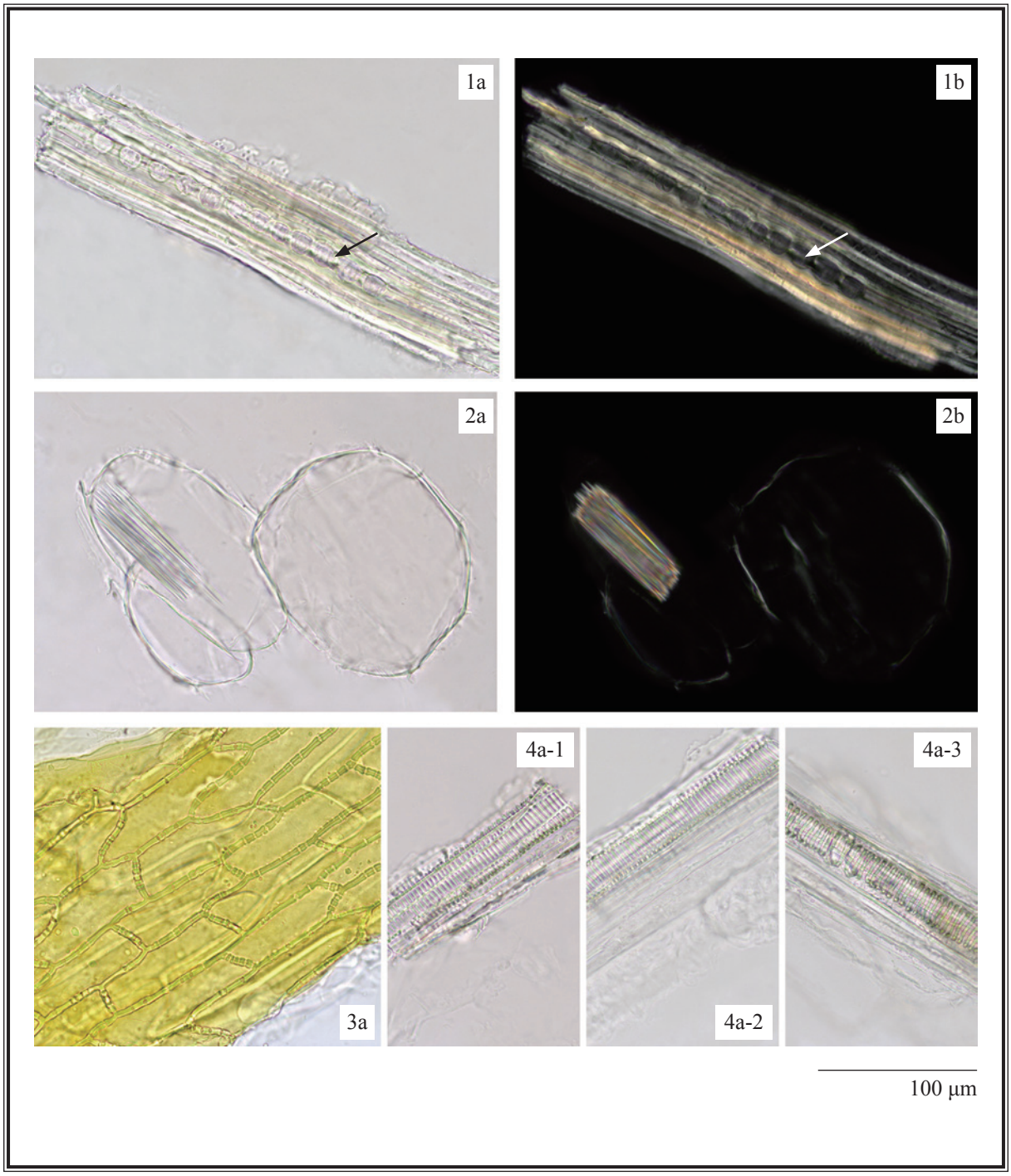

Figure 3 Microscopic features of powder of Dendrobii Officinalis Caulis

1. Bundle sheath fibres (silica body ⟶) 2. Raphides of calcium oxalate 3. Epidermal cells
4. Vessels (4-1 bordered-pitted vessel, 4-2 scalariform vessel, 4-3 spiral vessel)

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

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

Standard solution

Naringenin standard solution

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

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and formic acid (5:4:0.5, v/v).

Spray reagent

Weigh 2.5 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *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 1 mL of methanol. 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 naringenin standard solution (0.5 μL) and the test solution (15 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 85ºC (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

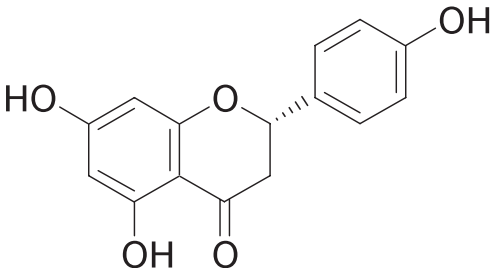

Figure 4 Chemical structure of naringenin

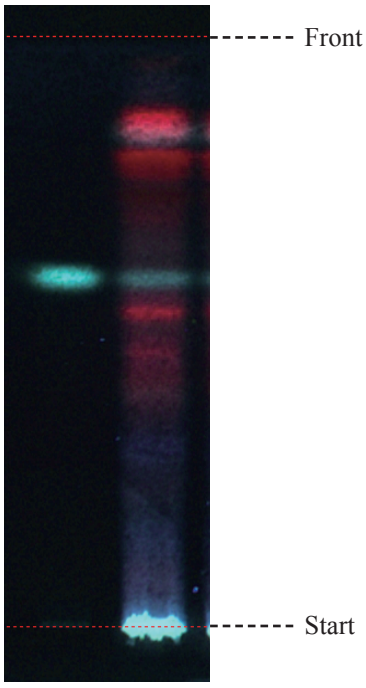

Figure 5 A reference HPTLC chromatogram of Dendrobii Officinalis Caulis extract observed under UV light (366 nm) after staining

1. Naringenin 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 naringenin (Fig. 5).

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

Standard solution

Naringenin standard solution for fingerprinting, Std-FP (7.5 mg/L)

Weigh 0.75 mg of naringenin CRS and dissolve in 100 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (226 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	75	25	isocratic
5 – 40	75 → 55	25 → 45	linear gradient
40 – 50	55 → 5	45 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of naringenin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of naringenin should not be more than 5.0%; the RSD of the retention time of naringenin peak should not be more than 2.0%; the column efficiency determined from naringenin peak should not be less than 20000 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 naringenin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of naringenin peak in the chromatogram of naringenin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify naringenin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of naringenin Std-FP. The retention times of naringenin 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 Dendrobii Officinalis Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Dendrobii Officinalis Caulis extract

Peak No.	RRT	Acceptable Range
1	0.78	± 0.03
2 (marker, naringenin)	1.00	-
3	1.37	± 0.03
4	1.46	± 0.03

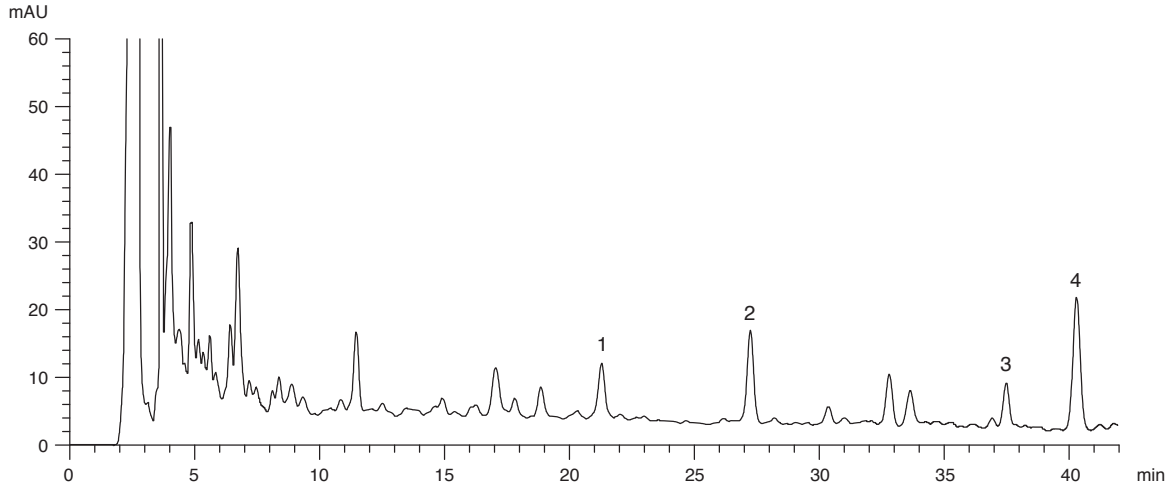

Figure 6 A reference fingerprint chromatogram of Dendrobii Officinalis Caulis extract

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).

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.5%.
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 (cold extraction method): not less than 26.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

Carry out the method as directed in Appendix XIV.

Reagent
Anthrone sulphuric acid solution
Weigh accurately 0.1 g of anthrone and dissolve in 100 mL of sulphuric acid (80%, v/v).

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 10, 30, 50, 70, 90 mg/L for anhydrous glucose.

Test solution
Weigh accurately 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of water. Place the mixture in a water bath for 1 h. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 100-mL of volumetric flask. Repeat the extraction for one more time. Wash the residue with water. Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and make up to the mark with water. Pipette 1 mL of the solution into a 50-mL centrifuge tube. Add 30 mL of ethanol. Place the mixture at 4ºC for 12 h. Centrifuge at about 1800 × *g* for 10 min. Discard the supernatant. Dissolve the residue in water. Transfer the solution to a 10-mL volumetric flask and make up to the mark with water. Make appropriate dilution where necessary.

Ultraviolet/ Visible spectrophotometric 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 sulphuric acid solution. Place the mixture in a water bath for 15 min. Cool the mixture in an ice water bath for 15 min. Using the corresponding anthrone sulphuric 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 (50 mg/L) by colourimetric method. The requirement of the system suitability parameter 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 as indicated in Appendix XIV.

Limits

The sample contains not less than 25% of polysaccharides [calculated as anhydrous glucose ($C_6H_{12}O_6$)], calculated with reference to the dried substance.