Dendrobii Caulis

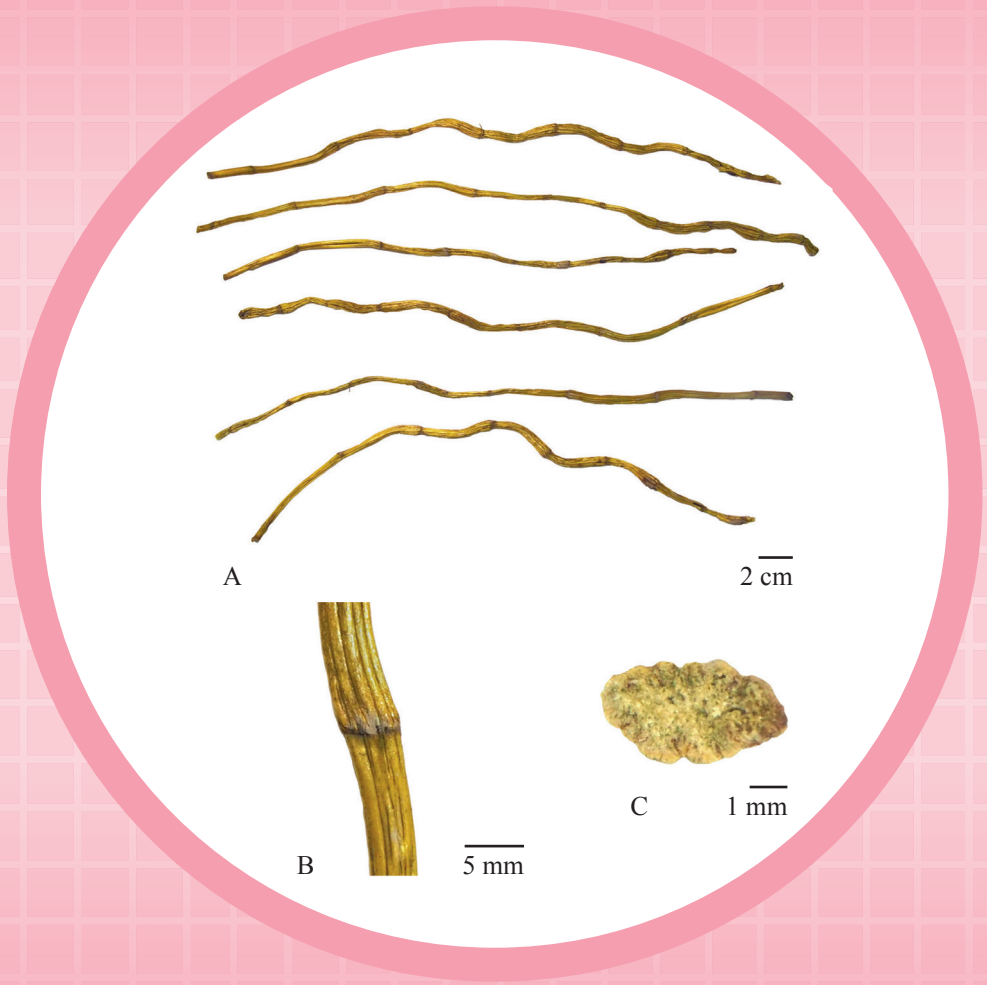

Figure 1 A photograph of Dendrobii Caulis

A. Dendrobii Caulis B. Magnified image of node
C. Magnified image of fracture

1. NAMES

Official name: Dendrobii Caulis

Chinese name: 石斛

Chinese phonetic name: Shihu

2. SOURCE

Dendrobii Caulis is the dried stem of cultivated *Dendrobium nobile* Lindl. (Orchidaceae). The stem is collected all year round, foreign matter removed, softened by dipping into boiling water or baking, then dried and rubbed by baking or under the sun until all leaf sheaths are removed and dried to obtain Dendrobii Caulis.

3. DESCRIPTION

Flat-cylindrical or cylindrical, slightly zigzag bended, 2-10 mm in diameter, internode 1.8-7.5 cm long; surface golden yellow to dark yellow, lustrous, with deep longitudinal furrows and wrinkles; nodes distinct, brown, some with residual grey leaf sheaths. Light in weight and brittle, easily broken, fracture yellowish-white to greyish-yellow, loose, flat or slightly fibrous. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of flat cells, covered with thick cuticle, 2-3 layers of cells with slightly thickened wall located underneath the epidermis. Parenchyma scattered with numerous collateral vascular bundles, some cells contain raphides of calcium oxalate. Bundle sheath fibres several layers, located at the outer side of the vascular bundles, wall thick, lumen relatively small, with parenchymatous cells containing subrounded silica bodies outside the fibre bundles; always with several layers of fibres with relatively large lumen located on the inner side of vascular bundles (Fig. 2).

Powder

Colour brownish-yellow. Bundle sheath fibres mostly in bundles, wall thick, lumen relatively narrow, with sparse pits and pit canals, 10-65 μm in diameter, some surrounded by cells containing subrounded silica bodies of 4-21 μm in diameter; bright white or bright yellow under the polarized microscope. Raphides of calcium oxalate scattered or present in parenchymatous cells, 12-122 μm long; polychromatic under the polarized microscope. Epidermal cells subpolygonal or long-polygonal in surface view, anticlinal walls beaded, always accompanied by bright yellow cuticle. Fibres mostly in bundles, wall slightly thickened, lumen relatively large, with numerous pits and pit canals, pits dotted or slit-shaped, 9-42 μm in diameter. Vessels mainly scalariform and spiral vessels, 9-58 μm in diameter (Fig. 3).

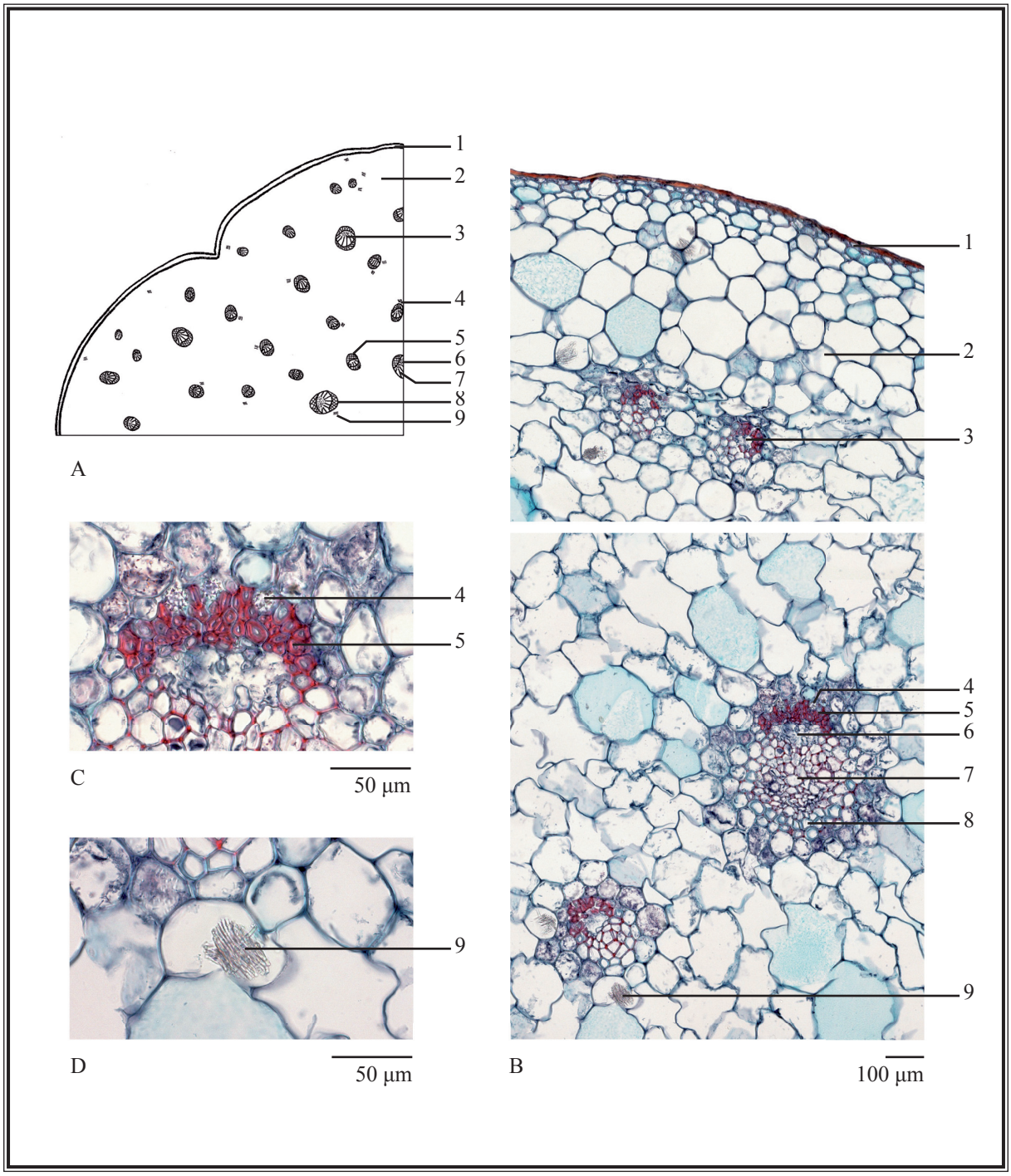

Figure 2 Microscopic features of transverse section of Dendrobii Caulis

A. Sketch B. Section illustration
C. Bundle sheath fibres and silica bodies
D. Raphides of calcium oxalate

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

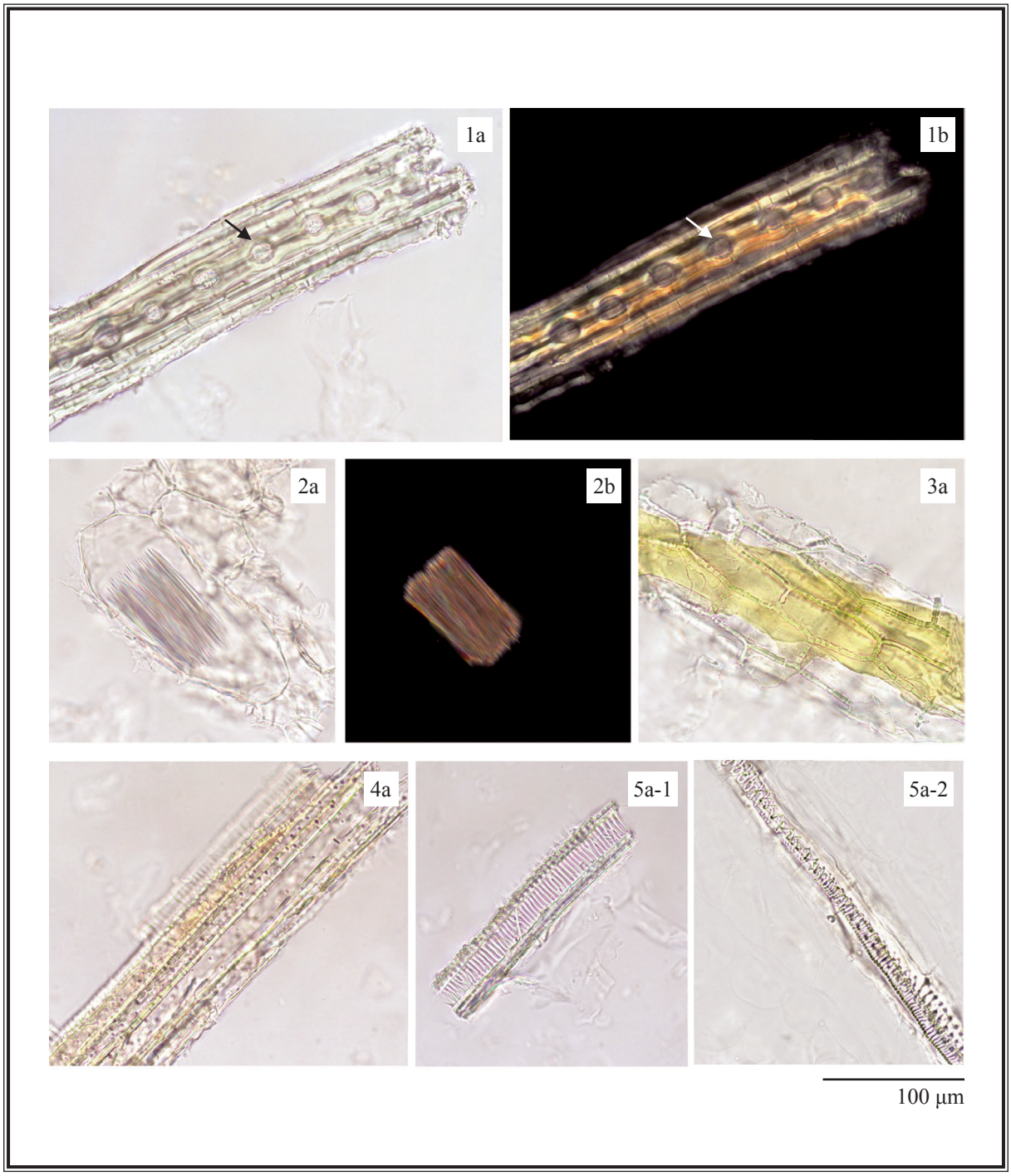

Figure 3 Microscopic features of powder of Dendrobii Caulis

1. Bundle sheath fibres (silica body ⟶) 2. Raphides of calcium oxalate
3. Epidermal cells 4. Fibres 5. Vessels (5-1 scalariform vessel, 5-2 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

Dendrobine standard solution

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

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), isopropanol and ethyl acetate (0.4: 2: 15, v/v).

Staining reagent

Iodine.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the supernatant to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 4 mL of methanol. Filter through a 0.45-μm PTFE 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 dendrobine standard solution (4 μL) and the test solution (8 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. Expose the plate to iodine vapour in a chamber for about 20 min until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

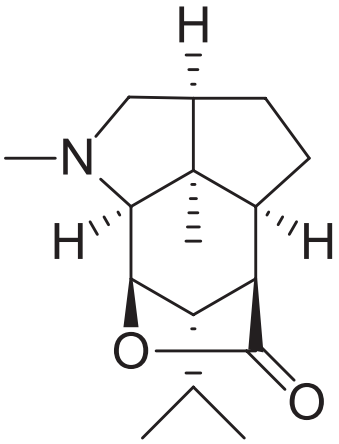

Figure 4 Chemical structure of dendrobine

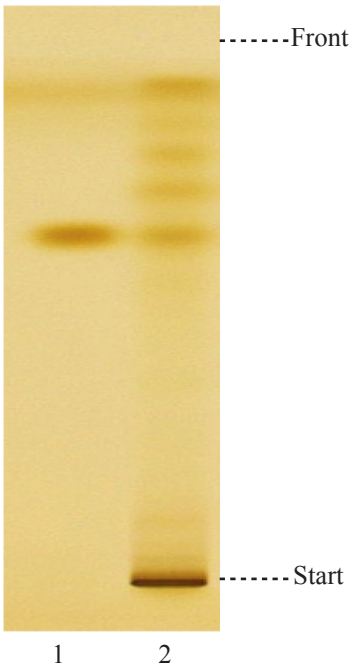

Figure 5 A reference HPTLC chromatogram of Dendrobii Caulis extract observed under visible light after staining

1. Dendrobine 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 dendrobine (Fig. 5).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

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

Weigh 2.0 mg of dendrobine CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with 10 mL of methanol. Repeat the extraction one more time. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the extract 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 gas chromatograph is equipped with a FID and a capillary column (DB-5 MS, 0.25 mm × 30 m) of which the internal wall is covered with phenyl arylene polymer in a layer about 0.25 μm thick. Helium is used as the carrier gas at 1.3 mL/min. The injection temperature is at 280ºC. The detector temperature is at 280ºC. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 16	130 → 210	5
16 – 21	210	-
21 – 30	210 → 280	8
30 – 45	280	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of dendrobine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of dendrobine should not be more than 5.0%; the RSD of the retention time of dendrobine peak should not be more than 2.0%; the column efficiency determined from dendrobine peak should not be less than 100000 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 dendrobine Std-FP and the test solution (1 μL each) into the GC system and record the chromatograms. Measure the retention time of dendrobine peak in the chromatogram of dendrobine Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify dendrobine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dendrobine Std-FP. The retention times of dendrobine 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 Dendrobii Caulis extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.71	± 0.03
2	0.97	± 0.03
3 (marker, dendrobine)	1.00	-
4	1.01	± 0.03
5	1.54	± 0.03

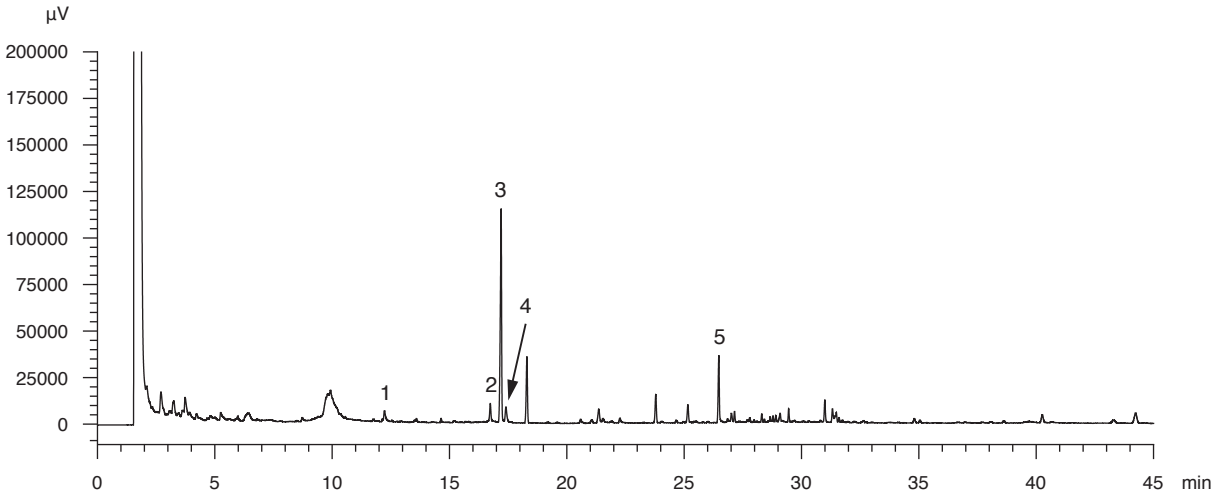

Figure 6 A reference GC fingerprint chromatogram of Dendrobii Caulis extract

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 GC 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 XVI)*: 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 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution

Dendrobine standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 2.0 mg of dendrobine CRS and dissolve in 2 mL of methanol.

Dendrobine standard solution for assay, Std-AS

Measure accurately the volume of the dendrobine Std-Stock, dilute with methanol to produce a series of solutions of 10, 100, 200, 500, 1000 mg/L for dendrobine.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with 10 mL of methanol. Repeat the extraction one more time. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the extract 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 gas chromatograph is equipped with a FID and a capillary column (DB-5 MS, 0.25 mm × 30 m) of which the internal wall is covered with phenyl arylene polymer in a layer about 0.25 µm thick. Helium is used as the carrier gas at 1.3 mL/min. The injection temperature is at 280ºC. The detector temperature is at 280ºC. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 16	130 → 210	5
16 – 21	210	-
21 – 30	210 → 280	8
30 – 45	280	-

System suitability requirements

Perform at least five replicate injections, each using 1 µL of dendrobine Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of dendrobine should not be more than 5.0%; the RSD of the retention time of dendrobine peak should not be more than 2.0%; the column efficiency determined from dendrobine peak should not be less than 100000 theoretical plates.

The *R* value between dendrobine peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of dendrobine Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of dendrobine against the corresponding concentrations of dendrobine Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify dendrobine peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dendrobine Std-AS. The retention times of dendrobine 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 dendrobine in the test solution, and calculate the percentage content of dendrobine in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.24% of dendrobine ($C_{16}H_{25}NO_2$), calculated with reference to the dried substance.

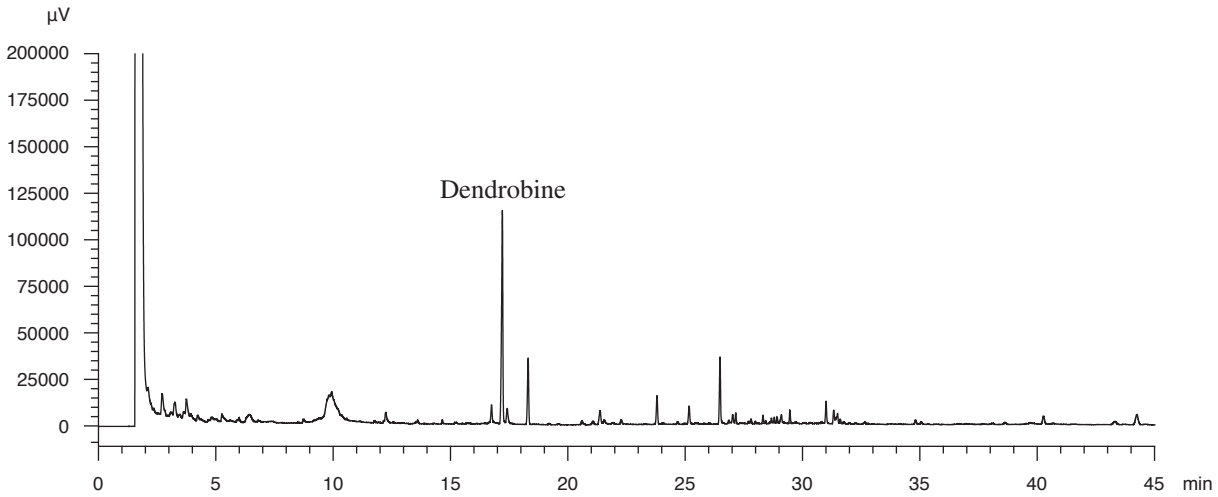

Figure 7 A reference GC assay chromatogram of Dendrobii Caulis extract