Dryopteridis Crassirhizomatis Rhizoma

Figure 1 A photograph of Dryopteridis Crassirhizomatis Rhizoma

A. Dryopteridis Crassirhizomatis Rhizoma

B. Transverse section (1. rhizome 2. frond bases)

1. NAMES

Official Name: Dryopteridis Crassirhizomatis Rhizoma

Chinese Name: 綿馬貫眾

Chinese Phonetic Name: Mianmaguanzhong

2. SOURCE

Dryopteridis Crassirhizomatis Rhizoma is the dried rhizome and frond bases of *Dryopteris crassirhizoma* Nakai (Dryopteridaceae). The rhizome and frond bases are collected in autumn, fronds and fibrous roots removed, then dried under the sun to obtain Dryopteridis Crassirhizomatis Rhizoma.

3. DESCRIPTION

Elongated obovoid, slightly curved, 3-17.8 cm long, 22-95 mm in diameter. Externally yellowish-brown to dark brown, densely covered with regularly arranged frond bases and ramenta, with curved rootlets, and ramenta easily fallen off. Texture hard, fracture slightly even, yellowish-green to yellowish-brown, with 5-13 yellowish-white meristeles prominently visible, arranged in a ring, and with numerous leaf trace vascular bundles scattered on the outer part. The frond bases oblate, 2-9 mm in diameter. Texture hard and fragile, fracture slightly even, yellowish-green to yellowish-brown, with 5-13 yellowish-white meristeles arranged in a ring. Odour characteristic; taste bland and astringent, followed by bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Rhizome: Sclerenchyma consists of several layers of sclerenchymatous cells, brown to dark brown. Parenchymatous cells in basal tissue loosely arranged, containing yellowish-brown contents and starch guanules. Leaf trace vascular bundle scattered at the outer part of the basal tissue. Meristele amphicribral, 5-13 arranged in a ring; each surrounded by a layer of endodermis cells, casparian dots distinct. Xylem consists of polygonal tracheids. Intercellular glandular hairs present in the intercellar space, mostly broken [Fig. 2 (i)].

Frond base: Sclerenchyma consists of several layers of sclerenchymatous cells, brown to dark brown. Parenchymatous cells in basal tissue loosely arranged, containing yellowish-brown contents and starch guanules. Meristele amphicribral, 5-13 arranged in a ring; each surrounded by a layer of endodermis cells, casparian dots distinct. Xylem consists of polygonal tracheids. Intercellular glandular hairs present in the intercellar space, mostly broken [Fig. 2 (ii)].

Powder

Colour greyish-brown to yellowish-brown. Starch granules abundant, simple starch granules subrounded, elliptical or ovate, 2-14 μm in diameter, hilum and striations indistinct; black and cruciate-shaped under the polarized microscope. Sclerenchymatous cells scattered singly or in bundles, yellowish-brown or brown, fibre-like, 6-42 μm in diameter; pale yellowish-brown under the polarized microscope. Intercellular glandular hairs unicellular, mostly broken, occasionally intact, elliptical or long-ovate, base elongated, some contain yellowish-brown secretions. Tracheids mainly scalariform, few reticulate tracheids also found, 7-43 μm in diameter (Fig. 3).

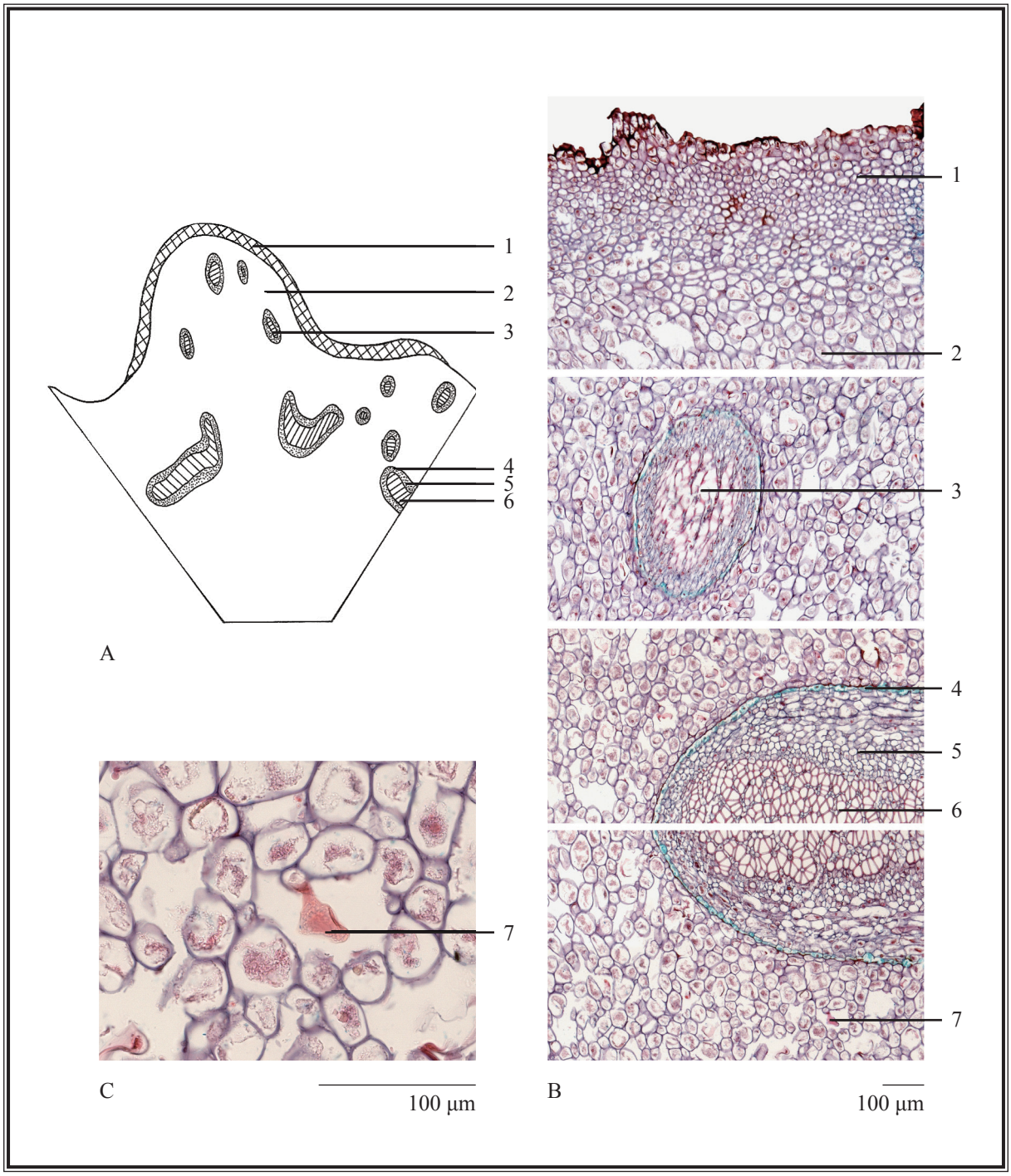

Figure 2 (i) Microscopic features of transverse section of rhizome of Dryopteridis Crassirhizomatis Rhizoma

A. Sketch B. Section illustration C. Intercellular glandular hair

1. Sclerenchyma 2. Basal tissue 3. Leaf trace vascular bundle 4. Endodermis
5. Phloem 6. Xylem 7. Intercellular glandular hair

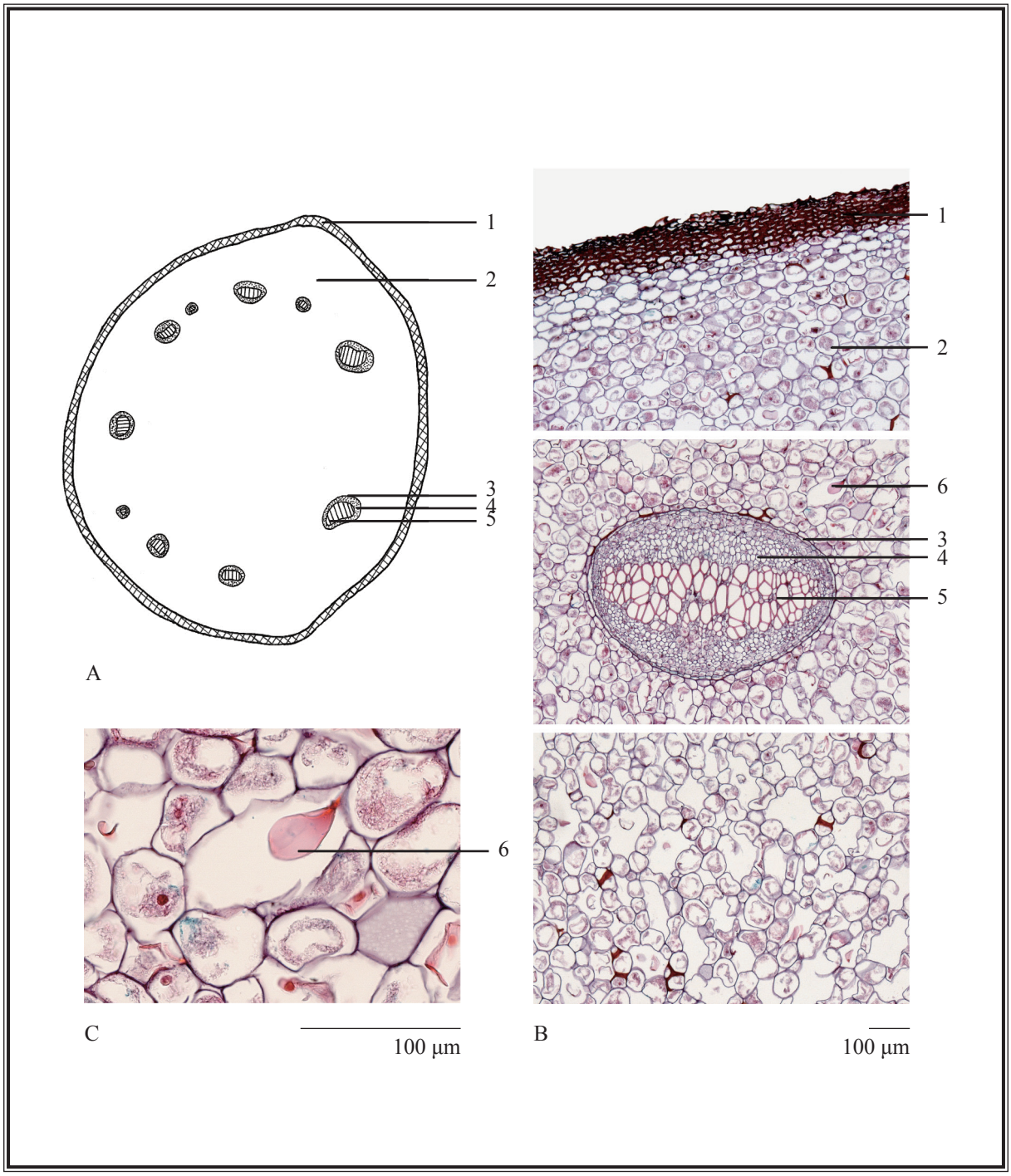

Figure 2 (ii) Microscopic features of transverse section of frond base of Dryopteridis Crassirhizomatis Rhizoma

A. Sketch B. Section illustration C. Intercellular glandular hair

1. Sclerenchyma 2. Basal tissue 3. Endodermis 4. Phloem 5. Xylem
6. Intercellular glandular hair

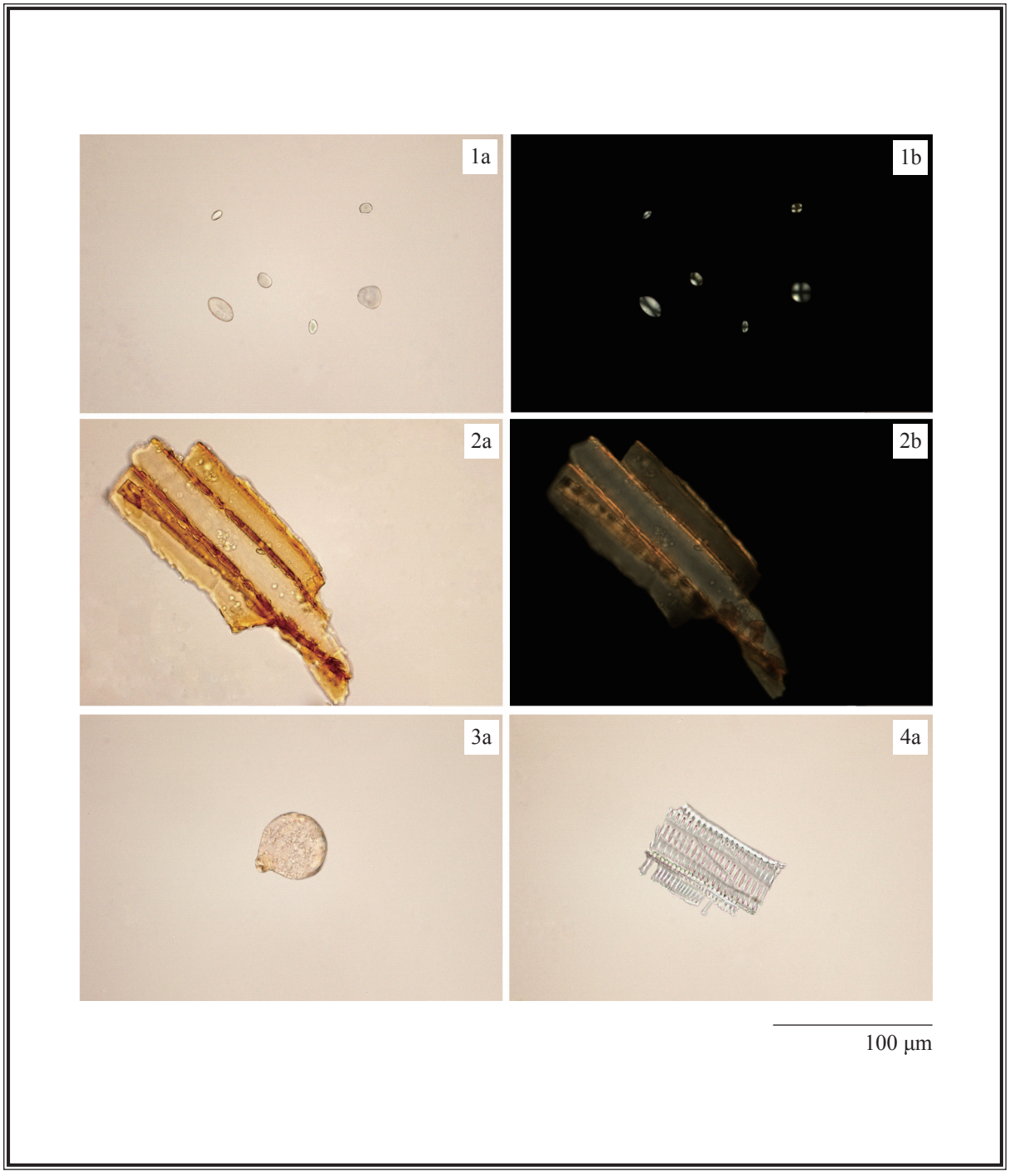

Figure 3 Microscopic features of powder of Dryopteridis Crassirhizomatis Rhizoma

1. Starch granules 2. Sclerenchymatous cells 3. Intercellular glandular hair
4. Tracheids

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

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

Standard solution

Dryocrassin standard solution

Weigh 1.0 mg of dryocrassin CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and glacial acetic acid (20:5:0.5, v/v).

Spray reagent

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

Test solution

Weigh 0.5 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of ethyl acetate. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. 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 dryocrassin standard solution (4 μL) and the test solution (2 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 105ºC until the spots or bands become visible (about 3-5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

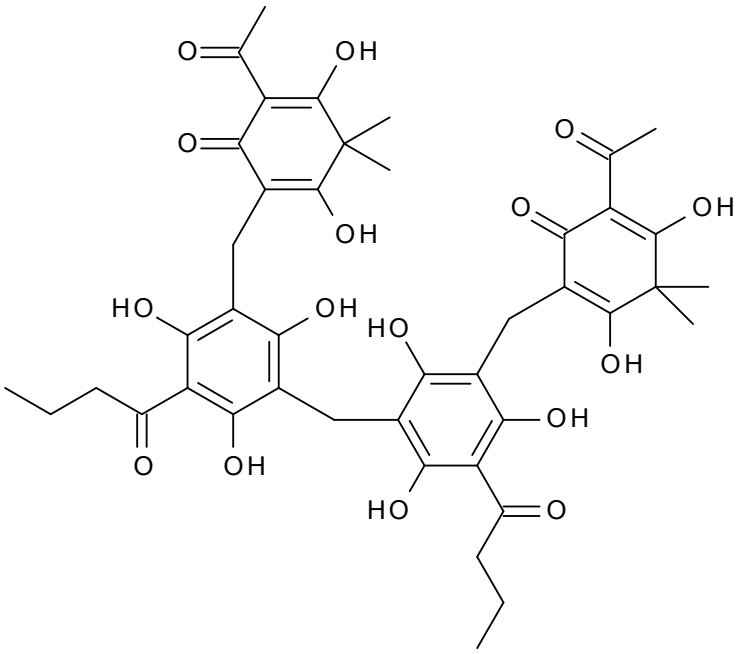

Figure 4 Chemical structure of dryocrassin

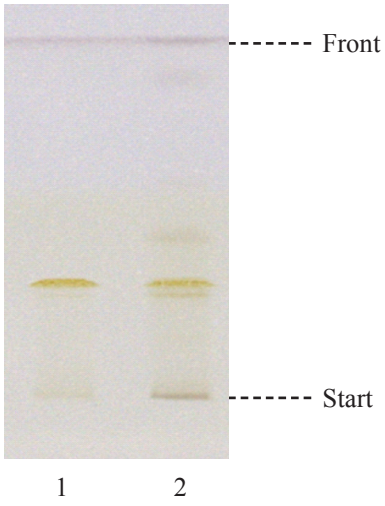

Figure 5 A reference HPTLC chromatogram of Dryopteridis Crassirhizomatis Rhizoma extract observed under visible light after staining

1. Dryocrassin standard solution 2. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f value, corresponding to those of dryocrassin (Fig. 5).

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

Reagents

0.1 M Citric acid solution

Weigh 9.6 g of citric acid and dissolve in 500 mL of water.

0.1 M Disodium hydrogen phosphate solution

Weigh 17.8 g of disodium hydrogen phosphate and dissolve in 500 mL of water.

Disodium hydrogen phosphate-citric acid buffer solution (pH 5.0)

Pipette 50 mL of 0.1 M disodium hydrogen phosphate solution, 50 mL of 0.1 M citric acid solution and 900 mL of water to a 1500-mL beaker. Adjust the pH value to 5.0 with 0.1 M citric acid solution.

Extraction solution

Weigh 2.5 g of L-ascorbic acid and dissolve it in a mixture of 100 mL of dimethyl sulphoxide and 400 mL of methanol. Freshly prepare the solution.

Standard solution

Dryocrassin standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 0.5 mg of dryocrassin CRS and place it in a 5-mL volumetric flask, then dissolve in 1 mL of dimethyl sulphoxide. Make up to the mark with extraction solution. Keep at about -10ºC.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of extraction solution. Sonicate (270 W) the mixture for 20 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with extraction solution. Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and make up to the mark with extraction solution. Filter through a 0.45-μm PTFE filter. Freshly prepare the test solution.

Chromatographic system

The liquid chromatograph is equipped with a DAD (302 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC 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)	Methanol (%, v/v)	Disodium hydrogen phosphate-citric acid buffer solution (pH 5.0) (%, v/v)	Elution
0 – 60	70 → 100	30 → 0	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of dryocrassin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of dryocrassin should not be more than 5.0%; the RSD of the retention time of dryocrassin peak should not be more than 2.0%; the column efficiency determined from dryocrassin peak should not be less than 15000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Dryopteridis Crassirhizomatis Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.73	± 0.03
2	0.84	± 0.03
3	0.91	± 0.03
4 (marker, dryocrassin)	1.00	-
5	1.15	± 0.03
6	1.68	± 0.03

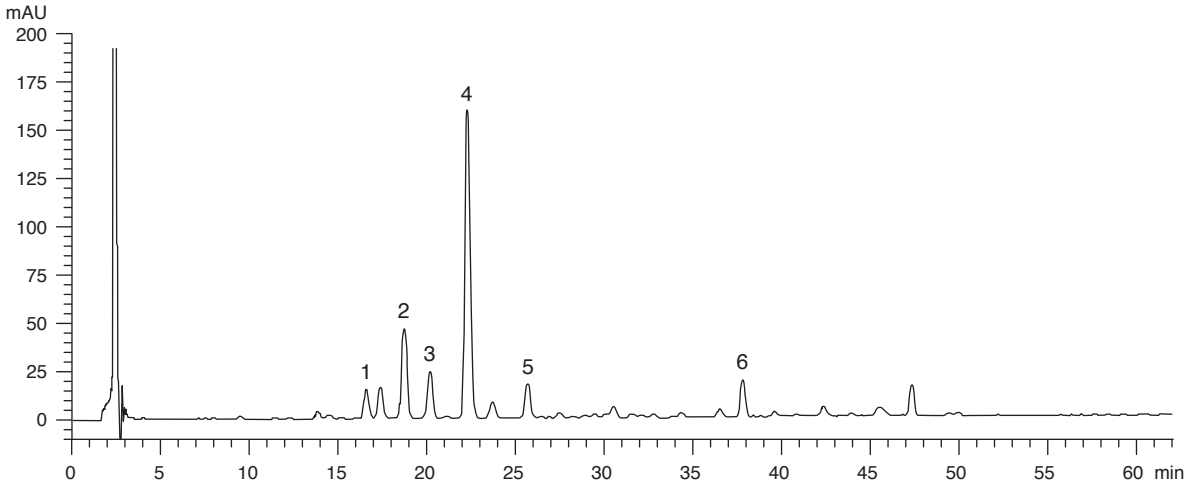

Figure 6 A reference fingerprint chromatogram of Dryopteridis Crassirhizomatis Rhizoma extract

For positive identification, the sample must give the above six 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 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.5 Ash *(Appendix IX)*

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

5.6 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 22.0%.
Ethanol-soluble extractives (hot extraction method): not less than 28.0%.

7. ASSAY

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

Reagents

0.1 M Citric acid solution

Weigh 9.6 g of citric acid and dissolve in 500 mL of water.

0.1 M Disodium hydrogen phosphate solution

Weigh 17.8 g of disodium hydrogen phosphate and dissolve in 500 mL of water.

Disodium hydrogen phosphate-citric acid buffer solution (pH 5.0)

Pipette 50 mL of 0.1 M disodium hydrogen phosphate solution, 50 mL of 0.1 M citric acid solution and 900 mL of water to a 1500-mL beaker. Adjust the pH value to 5.0 with 0.1 M citric acid solution.

Extraction solution

Weigh 2.5 g of L-ascorbic acid and dissolve it in a mixture of 100 mL of dimethyl sulphoxide and 400 mL of methanol. Freshly prepare the solution.

Standard solution

Dryocrassin standard stock solution, Std-Stock (400 mg/L)

Weigh accurately 2.0 mg of dryocrassin CRS and place it in a 5-mL volumetric flask, then dissolve in 1 mL of dimethyl sulphoxide. Make up to the mark with extraction solution. Keep at about -10ºC.

Dryocrassin standard solution for assay, Std-AS

Measure accurately the volume of the dryocrassin Std-Stock, dilute with extraction solution to produce a series of solutions of 20, 40, 100, 200, 400 mg/L for dryocrassin. Keep at about -10ºC.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of extraction solution. Sonicate (270 W) the mixture for 20 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with extraction solution. Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and make up to the mark with extraction solution. Filter through a 0.45-μm PTFE filter. Freshly prepare the test solution.

Chromatographic system

The liquid chromatograph is equipped with a DAD (302 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 30ºC 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)	Methanol (%, v/v)	Disodium hydrogen phosphate-citric acid buffer solution (pH 5.0) (%, v/v)	Elution
0 – 60	70 → 100	30 → 0	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of dryocrassin Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of dryocrassin should not be more than 5.0%; the RSD of the retention time of dryocrassin peak should not be more than 2.0%; the column efficiency determined from dryocrassin peak should not be less than 15000 theoretical plates.

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

Limits

The sample contains not less than 2.0% of dryocrassin ($C_{43}H_{48}O_{16}$), calculated with reference to the dried substance.