Nelumbinis Receptaculum

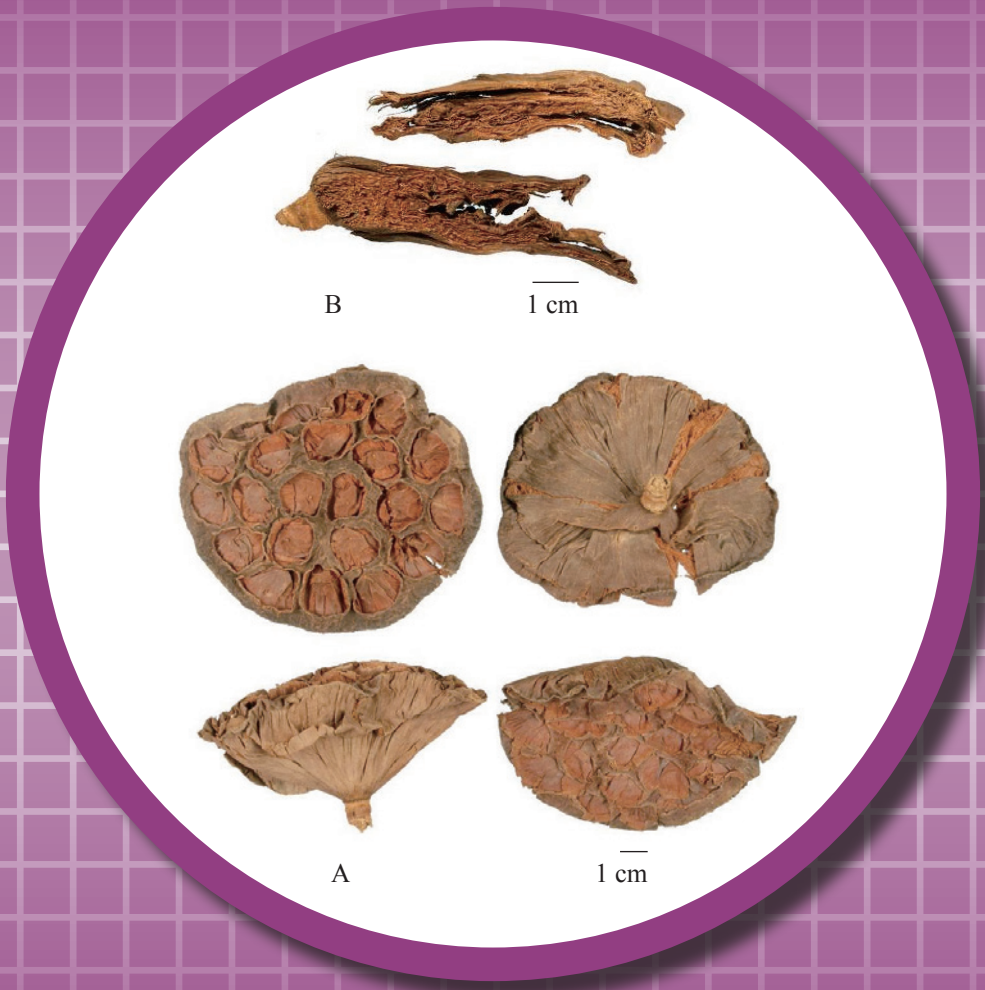

Figure 1 A photograph of Nelumbinis Receptaculum

A. Nelumbinis Receptaculum

B. Sliced pieces showing broken sponge-like fracture

1. NAMES

Official Name: Nelumbinis Receptaculum

Chinese Name: 蓮房

Chinese Phonetic Name: Lianfang

2. SOURCE

Nelumbinis Receptaculum is the dried receptacle of *Nelumbo nucifera* Gaertn. (Nymphaeaceae). The receptacle is collected in autumn when the fruit is ripe, fruits removed, then dried under the sun to obtain Nelumbinis Receptaculum.

3. DESCRIPTION

Obconical or funnel-shaped, frequently dilacerated, 53-140 mm in diameter, 3.8-7 cm high. Externally greyish-brown to purplish-brown, with fine longitudinal striations and wrinkles. The top with numerous rounded hollows and the base with the remnants of pedicel. Texture lax. Fracture sponge-like, brown. Odour slight; taste slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour purplish-brown to yellowish-brown. Epidermal cells polygonal in surface view, with double circle-shaped papillae. Brown cells subsquare to subrounded, walls slightly thickened, with reddish-brown contents in lumen. Clusters of calcium oxalate in rosette aggregates numerous, 8-52 μm in diameter; polychromatic under the polarized microscope. Fibres in bundles, pitted, 8-32 μm in diameter. Vessels mainly spiral, annular vessels occasionally found, 8-60 μm in diameter (Fig. 2).

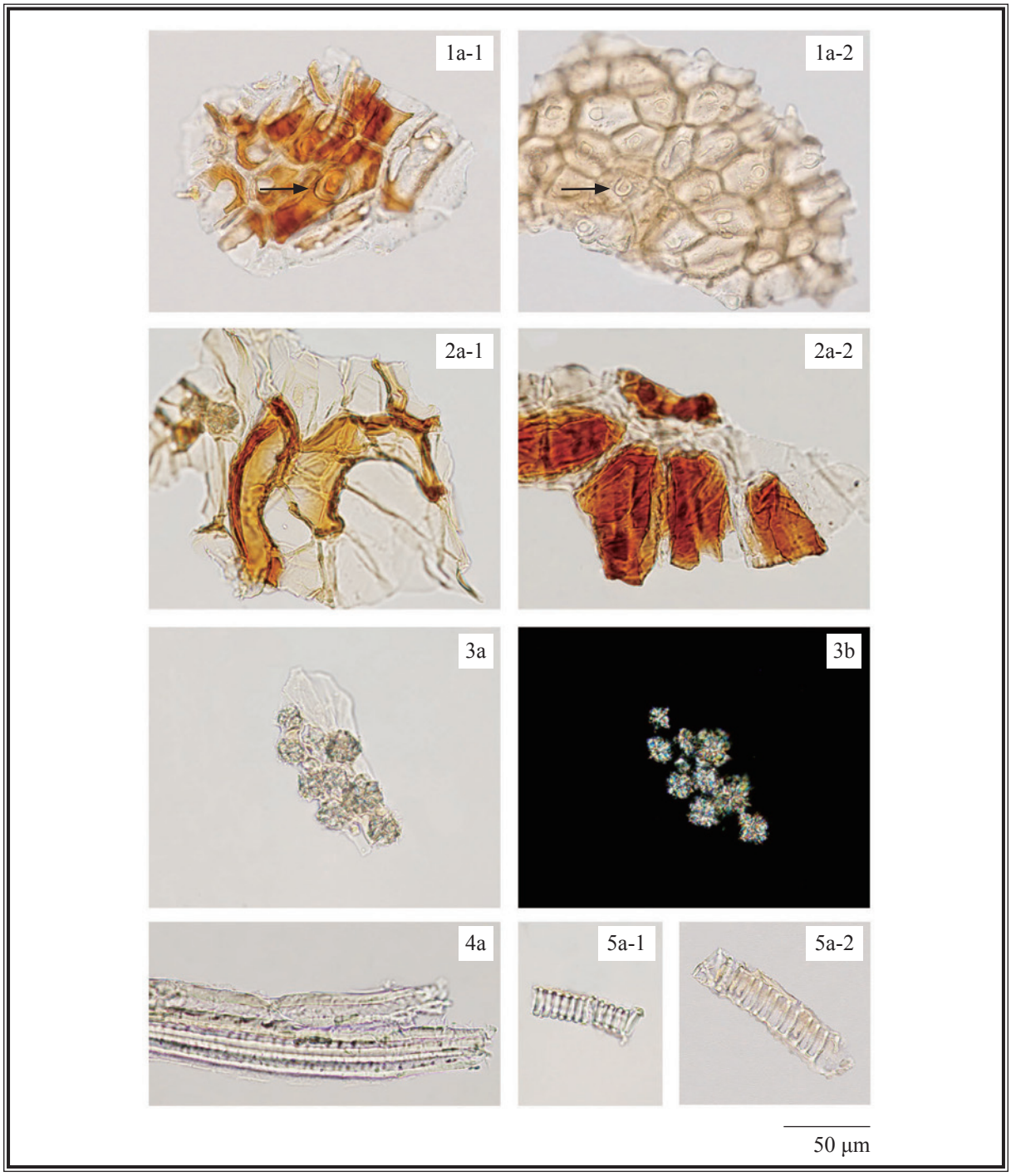

Figure 2 Microscopic features of powder of Nelumbinis Receptaculum

1. Epidermal cells (papillae ⟶) 2. Brown cells 3. Clusters of calcium oxalate 4. Fibres 5. Vessels (5-1 spiral vessel, 5-2 annular vessel)

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

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

Standard solution

Hyperoside standard solution

Weigh 1.0 mg of hyperoside CRS (Fig. 3) and dissolve in 1 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (8:1:1, v/v).

Spray reagent

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

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (95%). Heat the mixture in a water bath at about 60ºC for 1 h. Cool down to room temperature. 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 ethanol (95%). 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 hyperoside standard solution (0.5 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 8 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 (about 3 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

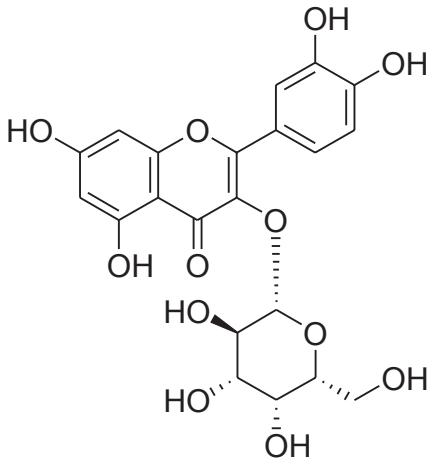

Figure 3 Chemical structure of hyperoside

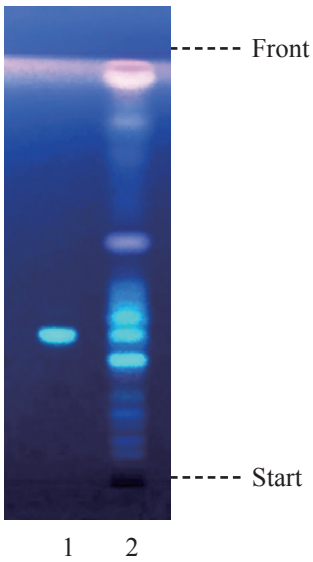

Figure 4 A reference HPTLC chromatogram of Nelumbinis Receptaculum extract observed under UV light (366 nm) after staining

1. Hyperoside 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 hyperoside (Fig. 4).

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

Standard solution

Hyperoside standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of hyperoside CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 7 mL of methanol (70%). Sonicate (200 W) the mixture for 30 min in a water bath at 50ºC. Centrifuge at about 5000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (360 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)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 10	15 → 17	85 → 83	linear gradient
10 – 35	17 → 18	83 → 82	linear gradient
35 – 42	18 → 28	82 → 72	linear gradient
42 – 50	28 → 60	72 → 40	linear gradient
50 – 55	60 → 90	40 → 10	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of hyperoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of hyperoside should not be more than 5.0%; the RSD of the retention time of hyperoside peak should not be more than 2.0%; the column efficiency determined from hyperoside 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. 5).

Procedure

Separately inject hyperoside Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of hyperoside peak in the chromatogram of hyperoside Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify hyperoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of hyperoside Std-FP. The retention times of hyperoside 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 Nelumbinis Receptaculum extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Nelumbinis Receptaculum extract

Peak No.	RRT	Acceptable Range
1	0.66	± 0.03
2 (marker, hyperoside)	1.00	-
3	1.05	± 0.03
4 (quercetin-3-*O*-β-D-glucuronide)	1.13	± 0.03
5	1.73	± 0.04

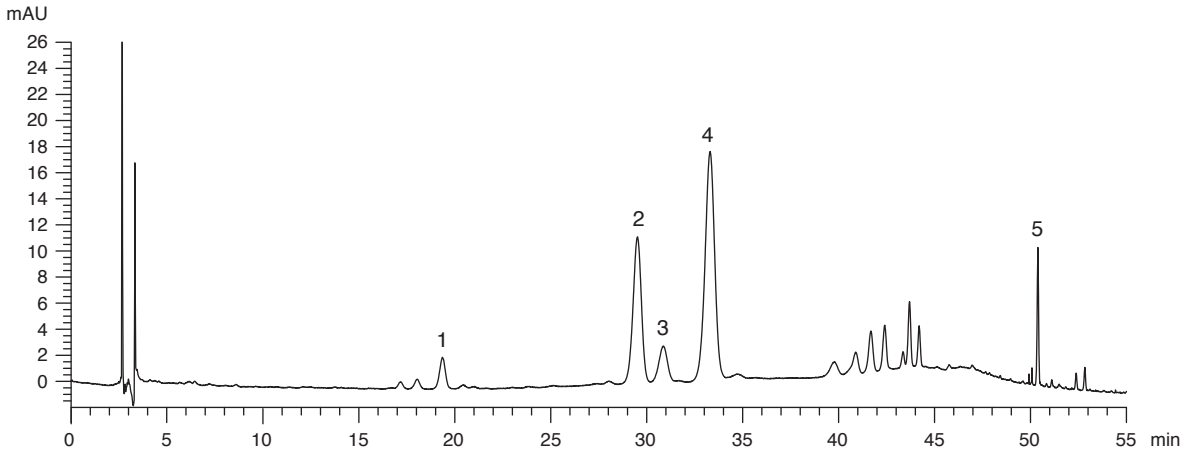

Figure 5 A reference fingerprint chromatogram of Nelumbinis Receptaculum extract

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 fingerprint chromatogram (Fig. 5).

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 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 7.0%.
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

Hyperoside standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of hyperoside CRS and dissolve in 10 mL of methanol (70%).

Hyperoside standard solution for assay, Std-AS

Measure accurately the volume of the hyperoside Std-Stock, dilute with methanol (70%) to produce a series of solutions of 1, 2, 5, 10, 20 mg/L for hyperoside.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 7 mL of methanol (70%). Sonicate (200 W) the mixture for 30 min in a water bath at 50ºC. Centrifuge at about 5000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (360 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)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 10	15 → 17	85 → 83	linear gradient
10 – 35	17 → 18	83 → 82	linear gradient
35 – 42	18 → 28	82 → 72	linear gradient
42 – 50	28 → 60	72 → 40	linear gradient
50 – 55	60 → 90	40 → 10	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of hyperoside Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of hyperoside should not be more than 5.0%; the RSD of the retention time of hyperoside peak should not be more than 2.0%; the column efficiency determined from hyperoside peak should not be less than 20000 theoretical plates.

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

Limits

The sample contains not less than 0.024% of hyperoside ($C_{21}H_{20}O_{12}$), calculated with reference to the dried substance.