Flos Magnoliae

Figure 1 A photograph of Flos Magnoliae

1. NAMES

Official Name: Flos Magnoliae

Chinese Name: 辛夷

Chinese Phonetic Name: Xinyi

2. SOURCE

Flos Magnoliae is the dried flower bud of *Magnolia biondii* Pamp. (Magnoliaceae). The flower bud is collected in late winter to early spring, before the flower opens by removing it from the branchlet, then dried in a shaded area to obtain Flos Magnoliae.

3. DESCRIPTION

The flower bud is silky-haired, elongated-ovoid, resembling the tip of a writing brush, 1.2-3.0 cm long, 8-16 mm in diameter. Usually the base with short pedicel, which exhibits whitish dotted lenticels. Bracts 2-3 layers, each layer consists of 2 segments, subtending small scaly buds between the 2 layers of bracts: outer surface of bracts densely covered with greyish-white, greyish-yellow or greyish-green pubescence, inner surface brownish, glabrous. Perianth consists of 9 brownish tepals; 3 tepals in the outermost whorl, linear, sepaloid, about 1/4 of the length of the inner 2 whorls; each of the 2 inner whorls consist of 3 tepals. Stamens and pistils numerous, spirally arranged. Texture light and fragile. Odour aromatic; taste pungent, cool and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Pedicel transverse section

One layer of the epidermal cells, which resemble stone cells, are differentiated to form non-glandular hairs. The non-glandular hairs consist of 1-3 cells. A few groups of oil cells and stone cells are found in the cortex. Stone cells subround, fusiform or irregular, 34-206 μm long, 16-99 μm in diameter, mostly with striations. Vascular bundles arranged in a ring. A few groups of oil cells and stone cells are also found in the pith (Fig. 2).

Powder

Colour greyish-green or pale yellowish-green. Non-glandular hairs numerous, scattered and frequently broken, consisting of 1-3 cells when whole, unicellular hairs are also found, cell wall 3-12 μm thick, the basal cells short, thick and inflated, with extremely thickened wall, like that of stone cells; bright yellowish-white under the polarized microscope. Stone cells in groups, elliptical, irregular or branched, with a brownish-yellow secretion inside. Epidermal cells of the tepals flat-squared, anticlinal wall beaded; stoma present. Oil cells numerous, subround, oil droplets easily observed (Fig. 3).

4.2 Physicochemical Identification

Reagent

Potassium iodobismuthate solution R_1

Dissolve 0.85 g of bismuth subnitrate in 10 mL of glacial acetic acid and 40 mL of water, then add 20 mL of aqueous potassium iodide solution (40%, w/v).

Procedure

Weigh 2.0 g of the powdered sample and put into a test tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. Transfer the solution to a test tube. Add 2 drops of potassium iodobismuthate solution R_1. An orange or orangish-brown precipitate is observed.

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

Standard solutions

Fargesin standard solution

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

Magnolin standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane and diethyl ether (5:1, v/v).

Spray reagent

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

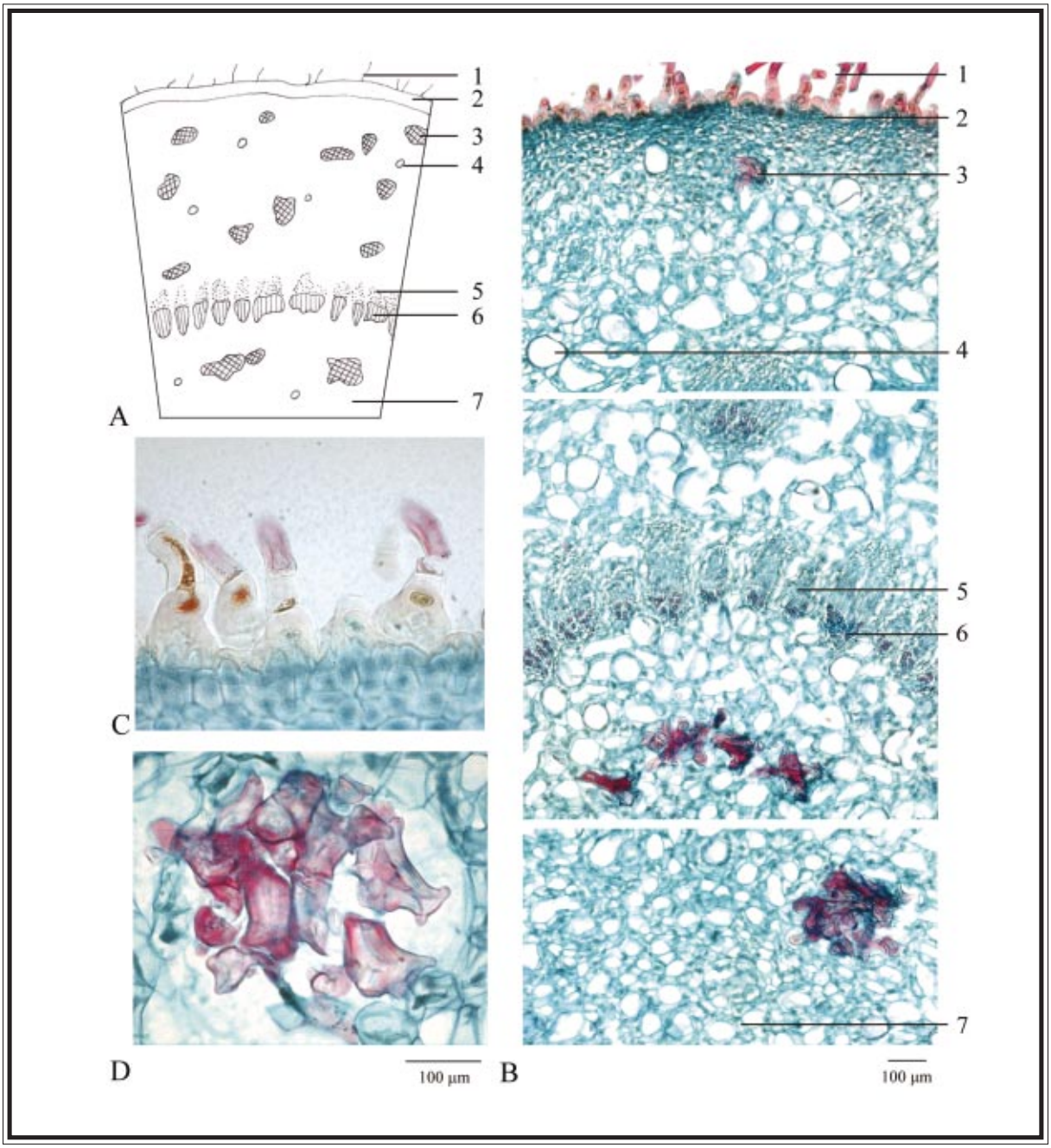


Figure 2 Microscopic features of transverse section of pedicel of Flos Magnoliae

A. Sketch B. Section illustration C. Trichome (Non-glandular hairs) D. A group of stone cells

1. Trichome (Non-glandular hairs) 2. Epidermis 3. Stone cells 4. Oil cells 5. Phloem 6. Xylem 7. Pith

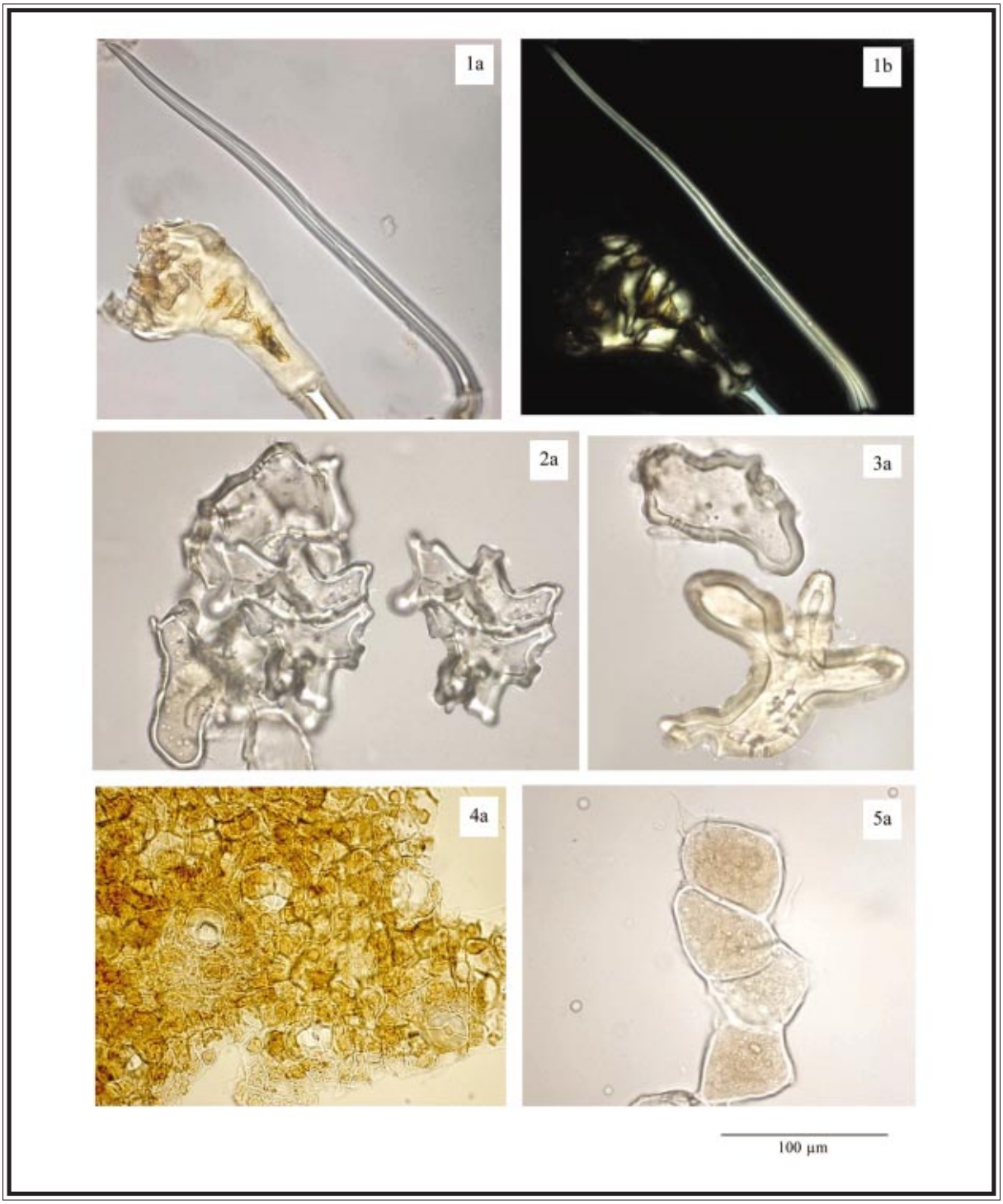


Figure 3 Microscopic features of powder of Flos Magnoliae

1. Non-glandular hairs showing the base cells 2. Several group of stone cells 3. Stone cells
4. Epidermal cells of tepals with stomata 5. Oil cells

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

Test solution

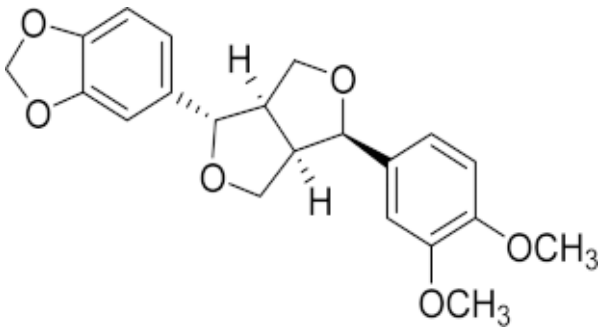
Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800× *g* for 10 min and then filter.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately fargesin standard solution, magnolin standard solution and the test solution (1 µL each) to the plate. Develop over a path of about 5 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 70°C until the spots or bands become visible (about 10 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of fargesin and magnolin.

(i)



(ii)

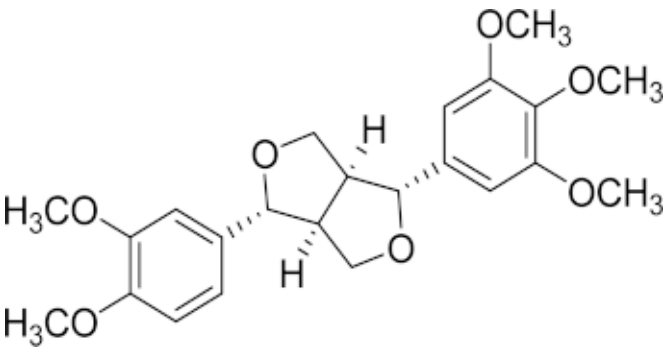


Figure 4 Chemical structures of (i) fargesin and (ii) magnolin

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

Standard solution

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

Weigh 1.0 mg of magnolin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.25 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 5 min. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 40	60 → 45	40 → 55	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 μL of magnolin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of magnolin should not be more than 3.0%; the RSD of the retention time of magnolin peak should not be more than 2.0%; the column efficiency determined from magnolin peak should not be less than 15000 theoretical plates.

The *R* value between peak 1 and peak 2 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject magnolin Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of magnolin peak in the chromatogram of the magnolin Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify magnolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of magnolin Std-FP. The retention times of magnolin 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 Flos Magnoliae extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the six characteristic peaks of Flos Magnoliae extract

Peak No.	RRT	Acceptable Range
1	0.94	±0.03
2 (marker, magnolin)	1.00	-
3	1.07	±0.03
4	1.16	±0.03
5	1.30	±0.04
6 (fargesin)	1.50	±0.05

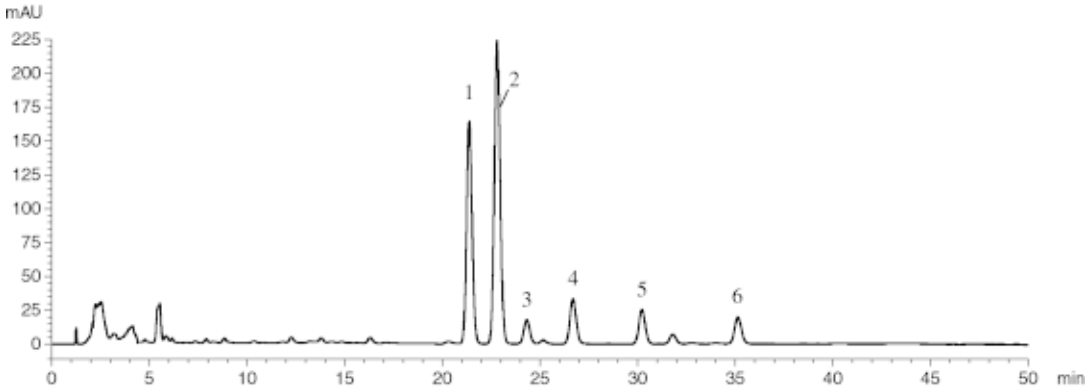


Figure 5 A reference fingerprint chromatogram of Flos Magnoliae extract

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. 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 1.0%.

5.6 **Ash** *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 **Water Content** *(Appendix X)*: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 10.0 mg of magnolin CRS and dissolve in 10 mL of methanol.

Magnolin standard solution for assay, Std-AS

Measure accurately the volume of the magnolin Std-Stock, dilute with methanol to produce a series of solutions of 20, 40, 60, 100, 200 mg/L for magnolin.

Test solution

Weigh accurately 0.25 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of methanol. Sonicate (490 W) the mixture for 90 min. Centrifuge at about 1800× *g* for 5 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction thrice. Combine the extracts and make up to the mark with methanol. Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (280 nm) and a column (4.6 × 250 mm) packed

with ODS bonded silica gel (5 µm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 40	60 → 45	40 → 55	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of magnolin Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of magnolin should not be more than 3.0%; the RSD of the retention time of magnolin peak should not be more than 2.0%; the column efficiency determined from magnolin peak should not be less than 15000 theoretical plates.

The *R* value between magnolin 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 magnolin Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of magnolin against the corresponding concentrations of magnolin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify magnolin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of magnolin Std-AS. The retention times of magnolin peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of magnolin in the test solution, and calculate the percentage content of magnolin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 3.0% of magnolin ($C_{23}H_{28}O_7$), calculated with reference to the dried substance.