

# Rhododendri Mollis Flos



**Figure 1** A photograph of Rhododendri Mollis Flos

A. Rhododendri Mollis Flos

B. Magnified image of flower (spread view after soaking)

## 1. NAMES

Official Name: *Rhododendri Mollis Flos*

Chinese Name: 鬧羊花

Chinese Phonetic Name: Naoyanghua

## 2. SOURCE

*Rhododendri Mollis Flos* is the dried flower of *Rhododendron molle* G. Don (Ericaceae). The flower is collected at the beginning of flowering period in April and May, dried in a shaded area or under the sun to obtain *Rhododendri Mollis Flos*.

## 3. DESCRIPTION

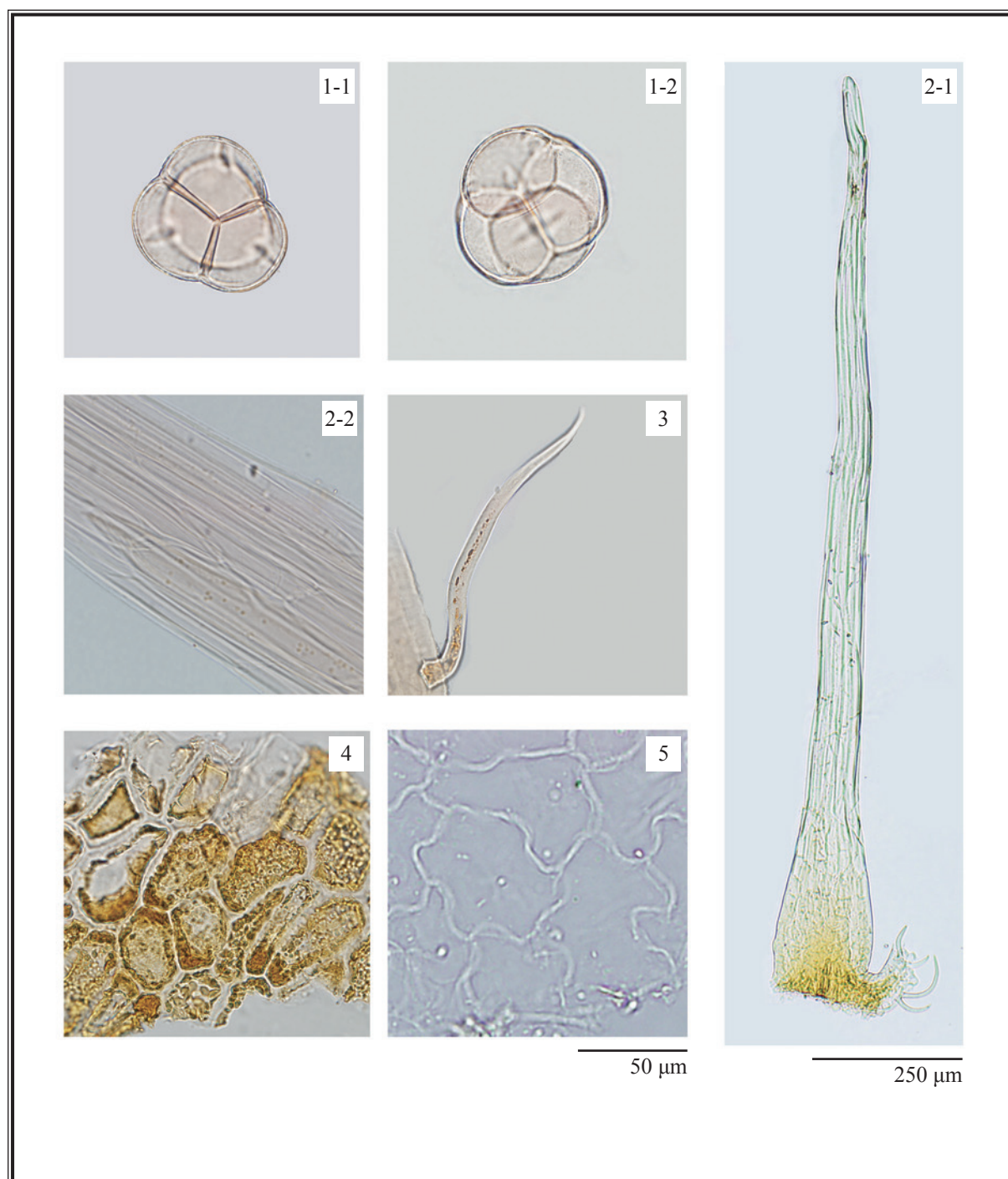
Mostly one or two flowers remaining on peduncle, greyish-yellow to yellowish-brown, shrunken. Sepals 5-lobed, semirounded to triangular, margins with slender hairs. Corolla campanulate, tube relatively long, 1-2.5 cm long, with contorted 5-lobed apex. Petals broadly ovate, with obtuse or retuse apex. Stamens 5, filaments curved, as long as or slightly longer than corolla, pubescent below the middle part, anthers reddish-brown, poricidal. Pistil 1, stigma cephaloid. Pedicel 1-2.8 cm long, brown, pubescent. Odour slight (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification (Appendix III)

#### Powder

Colour yellowish-brown. Pollen grains tetrahedral, 58-97 µm in diameter, with 3 germinal pores. Non-glandular hairs of calyx multicellular, arranged in several rows, 29-68 µm in diameter. Non-glandular hairs of corolla unicellular, 10-20 µm in diameter, up to 400 µm long or longer, wall thin, some with visible warty protuberance. Epidermal cells of pollen sac subpolygonal to subrounded, 13-31 µm in diameter, arranged orderly and tightly, wall slightly thickened, some with distinct pits, cells contain yellowish-brown contents. Epidermal cells of corolla rectangular, subsquare or irregular in shape, 26-78 µm in diameter, with thin and sinuous walls (Fig. 2).



**Figure 2** Microscopic features of powder of *Rhododendri Mollis Flos* (under the light microscope)

1. Pollen grains (1-1 with germinal pores, 1-2 tetrahedron)
2. Non-glandular hair of calyx (2-1 in full view, 2-2 in partial view)
3. Non-glandular hair of corolla
4. Epidermal cells of pollen sac
5. Epidermal cells of corolla

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

### Standard solutions

#### *Rhodojaponin II standard solution*

Weigh 1.0 mg of rhodojaponin II CRS (Fig. 3) and dissolve in 5 mL of ethanol (70%).

#### *Rhodojaponin III standard solution*

Weigh 1.0 mg of rhodojaponin III CRS (Fig. 3) and dissolve in 5 mL of ethanol (70%).

### Developing solvent system

Prepare a mixture of ethyl acetate, methanol, acetone and formic acid (10:1:1:0.2, v/v).

### Spray reagent

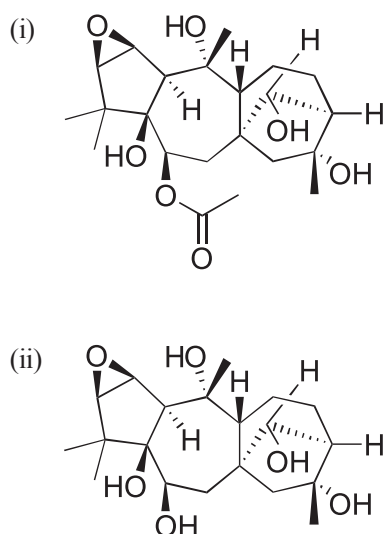
Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

### Test solution

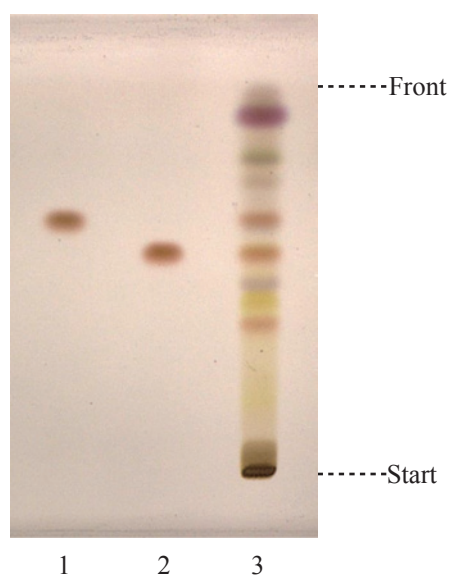
Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (70%). Sonicate (400 W) the mixture for 15 min. Filter the mixture.

### Procedure

Carry out the method by using a HPTLC silica gel G60 plate and a freshly prepared developing solvent system as described above. Apply separately rhodojaponin II standard solution, rhodojaponin III standard solution and the test solution (10 µL each) to the plate. Develop over a path of about 7 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 105°C until the spots or bands become visible (about 20 min). Examine the plate under visible light. Calculate the  $R_f$  values by using the equation as indicated in Appendix IV (A).



**Figure 3** Chemical structures of (i) rhodojaponin II and (ii) rhodojaponin III



**Figure 4** A reference HPTLC chromatogram of *Rhododendri Mollis Flos* extract observed under visible light after staining

1. Rhodojaponin II standard solution    2. Rhodojaponin III standard solution    3. Test solution

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 rhodojaponin II and rhodojaponin III (Fig. 4).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solutions

*Rhodojaponin II standard solution for fingerprinting, Std-FP (200 mg/L)*

Weigh 2.0 mg of rhodojaponin II CRS and dissolve in 10 mL of ethanol (70%).

*Rhodojaponin III standard solution for fingerprinting, Std-FP (200 mg/L)*

Weigh 2.0 mg of rhodojaponin III CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 4.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 40 mL of ethanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with ethanol (70%). Combine the solutions and make up to the mark with ethanol (70%). Pipette 25 mL of the solution to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (70%). Transfer the solution to a 5-mL volumetric flask and make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a ELSD [drift tube temperature: 85°C; nebulizer gas (air) flow: 3.0 L/min] and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35°C 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)	0.5% Formic acid (% v/v)	Elution
0 – 16	38 → 58	62 → 42	linear gradient
16 – 22	58 → 80	42 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of rhodojaponin II Std-FP and rhodojaponin III Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of rhodojaponin II and rhodojaponin III should not be more than 5.0%; the RSD of the retention times of rhodojaponin II and rhodojaponin III peaks should not be more than 2.0%; the column efficiencies determined from rhodojaponin II and rhodojaponin III peaks should not be less than 25000 and 6000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

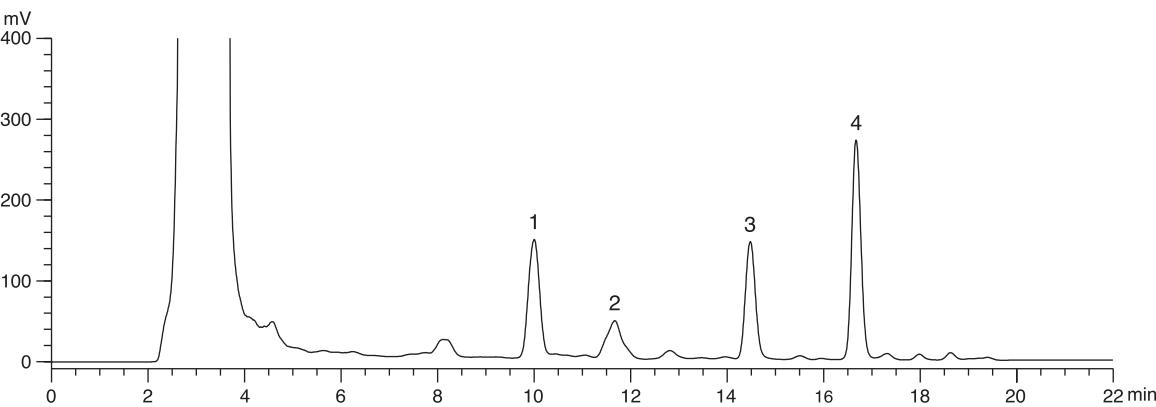
Procedure

Separately inject rhodojaponin II Std-FP, rhodojaponin III Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention times of rhodojaponin II and rhodojaponin III peaks in the chromatograms of rhodojaponin II Std-FP, rhodojaponin III Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify rhodojaponin II and rhodojaponin III peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of rhodojaponin II Std-FP and rhodojaponin III Std-FP. The retention times of rhodojaponin II and rhodojaponin III peaks in the chromatograms of the test solution and the corresponding Std-FP 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 *Rhododendri Mollis Flos* extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the four characteristic peaks of *Rhododendri Mollis Flos* extract

Peak No.	RRT	Acceptable Range
1 (rhodojaponin III)	0.60	± 0.03
2 (hyperoside)	0.70	± 0.03
3 (quercitrin)	0.87	± 0.03
4 (marker, rhodojaponin II)	1.00	-



**Figure 5** A reference fingerprint chromatogram of *Rhododendri Mollis Flos* 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. 5).

## 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 XVI*): meet the requirements.

**5.5 Foreign Matter** (*Appendix VIII*): not more than 2.0%.

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

Total ash: not more than 10.0%.

Acid-insoluble ash: not more than 4.0%.

**5.7 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 18.0%.

Ethanol-soluble extractives (hot extraction method): not less than 14.0%.

## 7. ASSAY

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

### Standard solution

Mixed rhodojaponin II and rhodojaponin III standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 5.0 mg of rhodojaponin II CRS and 5.0 mg of rhodojaponin III CRS, and dissolve in 5 mL of ethanol (70%).



Mixed rhodojaponin II and rhodojaponin III standard solution for assay, Std-AS

Measure accurately the volume of the mixed rhodojaponin II and rhodojaponin III Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 20, 40, 100, 200, 400 mg/L for both rhodojaponin II and rhodojaponin III.

Test solution

Weigh accurately 4.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 40 mL of ethanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with ethanol (70%). Combine the solutions and make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a ELSD [drift tube temperature: 85°C; nebulizer gas (air) flow: 3.0 L/min] and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35°C 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)	0.5% Formic acid (% v/v)	Elution
0 – 16	38 → 58	62 → 42	linear gradient
16 – 22	58 → 80	42 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed rhodojaponin II and rhodojaponin III Std-AS (100 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of rhodojaponin II and rhodojaponin III should not be more than 5.0%; the RSD of the retention times of rhodojaponin II and rhodojaponin III peaks should not be more than 2.0%; the column efficiencies determined from rhodojaponin II and rhodojaponin III peaks should not be less than 25000 and 6000 theoretical plates respectively.

The *R* value between rhodojaponin II peak and the closest peak; and the *R* value between rhodojaponin III peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

### Calibration curves

Inject a series of the mixed rhodojaponin II and rhodojaponin III Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of rhodojaponin II and rhodojaponin III against the natural logarithm of the corresponding concentrations of the mixed rhodojaponin II and rhodojaponin III Std-AS. Obtain the slopes, y-intercepts and the  $r^2$  values from the corresponding 5-point calibration curves.

### Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify rhodojaponin II and rhodojaponin III peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed rhodojaponin II and rhodojaponin III Std-AS. The retention times of rhodojaponin II and rhodojaponin III peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of rhodojaponin II and rhodojaponin III in the test solution, and calculate the percentage contents of rhodojaponin II and rhodojaponin III in the sample by using the equations as indicated in Appendix IV(B).

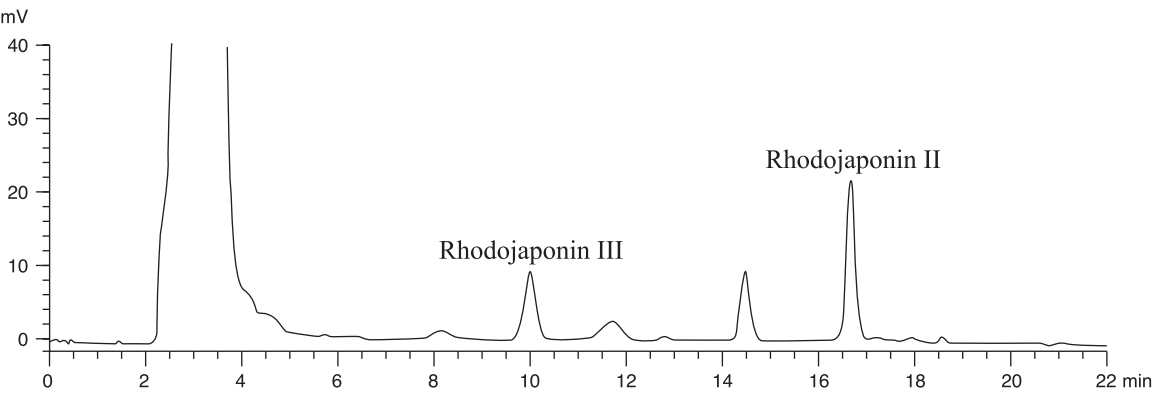
### Limits

The sample contains not less than 0.12% of the total content of rhodojaponin II ( $C_{22}H_{34}O_7$ ) and rhodojaponin III ( $C_{20}H_{32}O_6$ ), calculated with reference to the dried substance.

## 8. CAUTION

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.

**Rhododendri Mollis Flos ( 鬧羊花 )**



**Figure 1** A reference assay chromatogram of Rhododendri Mollis Flos extract