Genkwa Flos

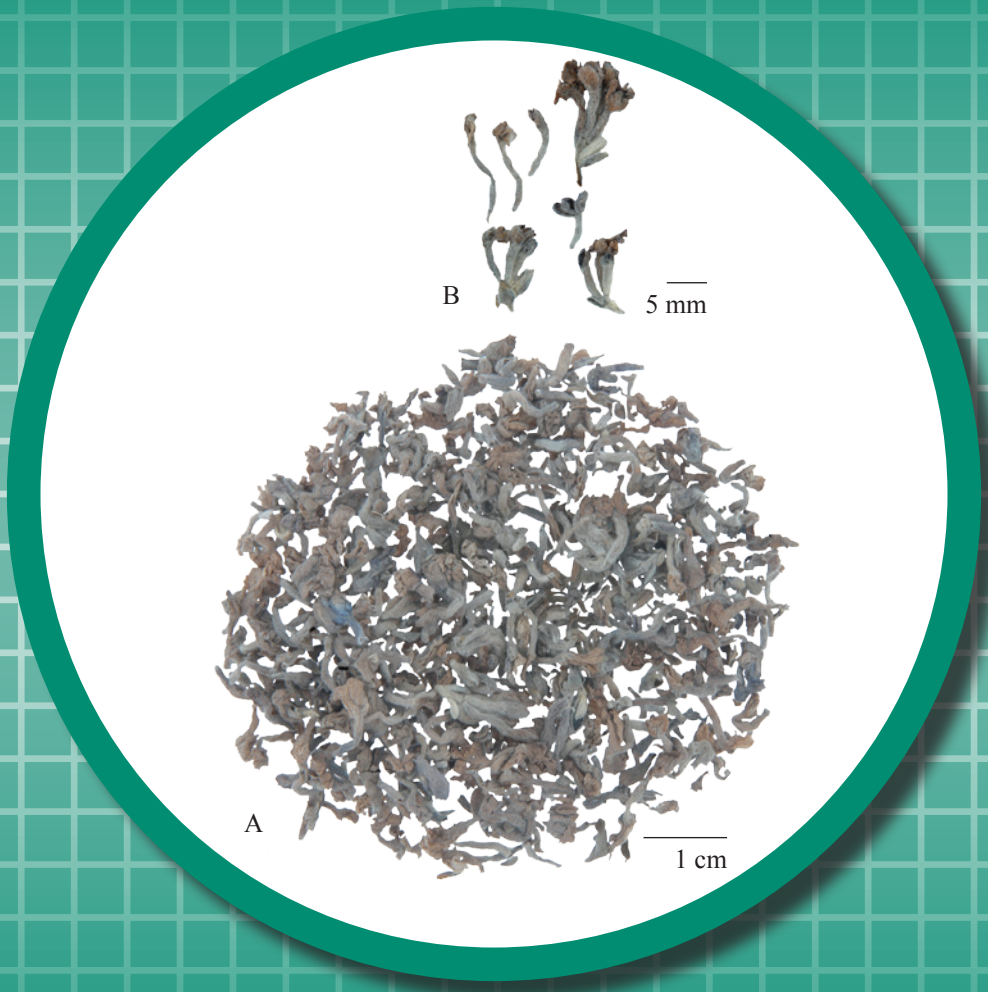

Figure 1 A photograph of Genkwa Flos

A. Genkwa Flos B. Magnified flower buds

1. NAMES

Official Name: Genkwa Flos

Chinese Name: 芫花

Chinese Phonetic Name: Yuanhua

2. SOURCE

Genkwa Flos is the dried flower bud of *Daphne genkwa* Sieb. et Zucc. (Thymelaeaceae). The flower bud is collected in spring before flowering, foreign matter removed, then dried under the sun, in a shaded area or baked at temperature about 50ºC or lower to dryness to obtain Genkwa Flos.

3. DESCRIPTION

Usually 3-7 flower buds, subtended by 1-2 bracts, clustered on short rachis, mostly fallen off into a single flower bud. Flower bud clavate, usually curved, 1-1.7 cm long, about 1.5 mm in diameter. Perianth tube pale purple to greyish-green, densely pubescent, 4-lobed, lobes pale purple to yellowish-brown. Texture soft. Odour slight; taste sweet and slightly pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour greyish-brown. Non-glandular hairs unicellular, usually curved, 50-720 μm long, 12-23 μm in diameter, walls relatively thick, some with warty protuberance. Cells of lower surface of perianth subpolygonal in surface view, with non-glandular hairs. Pollen grains pale yellow, subspherical, 23-39 μm in diameter, exine with distinct reticulate sculptures (Fig. 2).

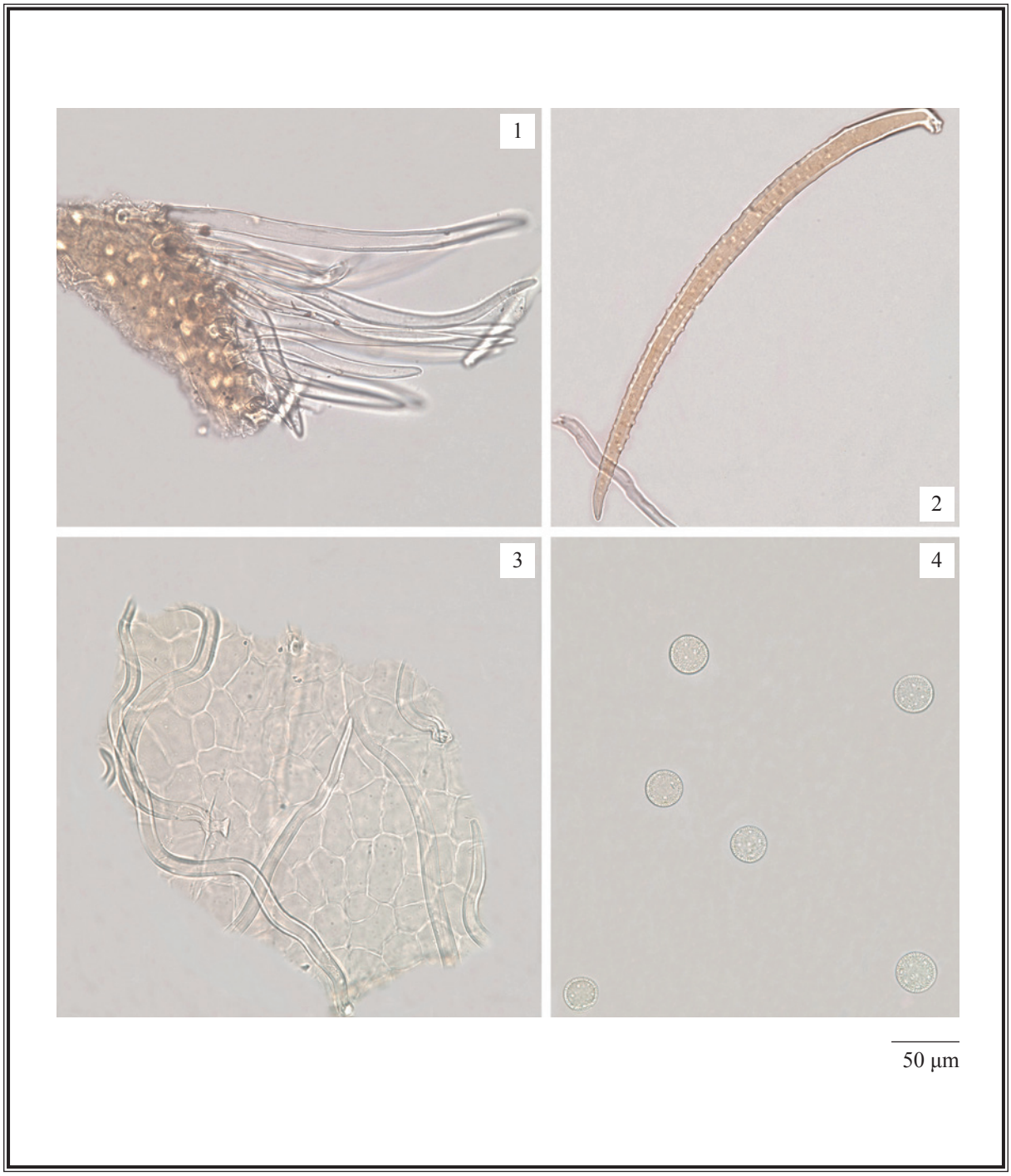

Figure 2 Microscopic features of powder of Genkwa Flos (under the light microscope)

1. Non-glandular hairs 2. Non-glandular hairs with warty protuberance
3. Cells of lower surface of perianth with non-glandular hairs (in surface view)
4. Pollen grains

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

Standard solution

Genkwanin standard solution

Weigh 1.0 mg of genkwanin CRS (Fig. 3) and dissolve in 0.5 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and formic acid (3:2:0.1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol. Sonicate (270 W) the mixture for 10 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

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 genkwanin standard solution (2 μL) and the test solution (3 μL) 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. Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

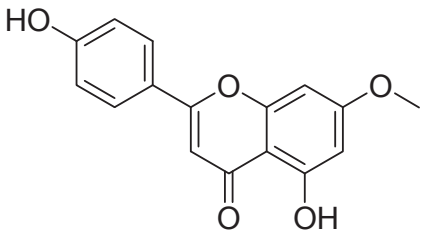

Figure 3 Chemical structure of genkwanin

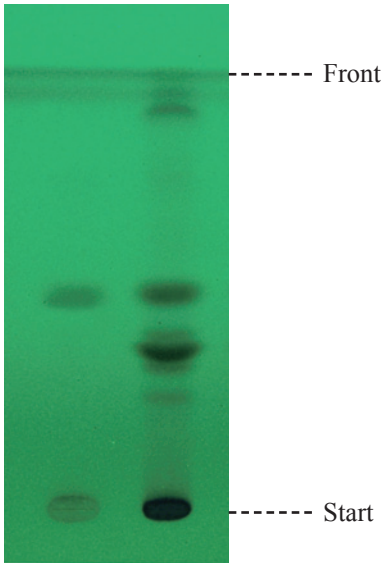

Figure 4 A reference HPTLC chromatogram of Genkwa Flos extract observed under UV light (254 nm)

1. Genkwanin 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 genkwanin (Fig. 4).

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

Standard solution

Genkwanin standard solution for fingerprinting, Std-FP (48 mg/L)

Weigh 1.2 mg of genkwanin CRS and dissolve in 25 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.5% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	95 → 15	5 → 85	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of genkwanin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of genkwanin should not be more than 5.0%; the RSD of the retention time of genkwanin peak should not be more than 2.0%; the column efficiency determined from genkwanin peak should not be less than 300000 theoretical plates.

The *R* value between peak 8 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the eight characteristic peaks of Genkwa Flos extract

Peak No.	RRT	Acceptable Range
1	0.50	± 0.03
2	0.51	± 0.03
3	0.53	± 0.03
4	0.54	± 0.03
5	0.57	± 0.03
6	0.77	± 0.03
7	0.89	± 0.03
8 (marker, genkwanin)	1.00	-

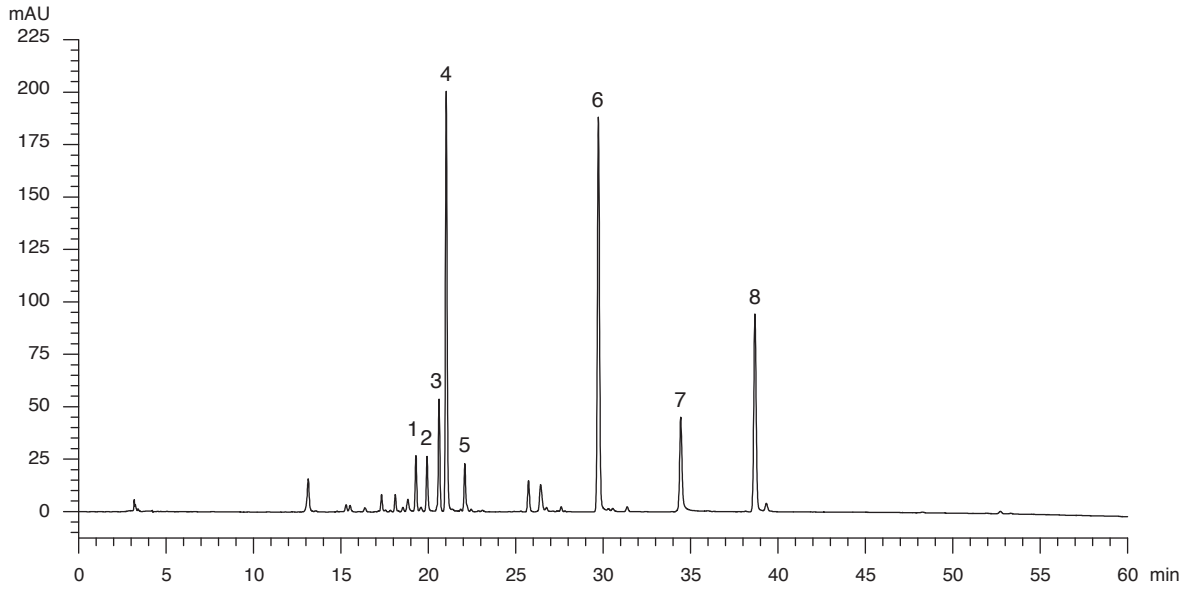

Figure 5 A reference fingerprint chromatogram of Genkwa Flos extract

For positive identification, the sample must give the above eight 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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 16.0%.
Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

7. ASSAY

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

Standard solution

Genkwanin standard stock solution, Std-Stock (240 mg/L)

Weigh accurately 2.4 mg of genkwanin CRS and dissolve in 10 mL of methanol.

Genkwanin standard solution for assay, Std-AS

Measure accurately the volume of the genkwanin Std-Stock, dilute with methanol to produce a series of solutions of 3, 12, 24, 48, 72 mg/L for genkwanin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.5% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	95 → 15	5 → 85	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of genkwanin Std-AS (24 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of genkwanin should not be more than 5.0%; the RSD of the retention time of genkwanin peak should not be more than 2.0%; the column efficiency determined from genkwanin peak should not be less than 300000 theoretical plates.

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

Limits

The sample contains not less than 0.087% of genkwanin ($C_{16}H_{12}O_5$), calculated with reference to the dried substance.