Buddlejae Flos

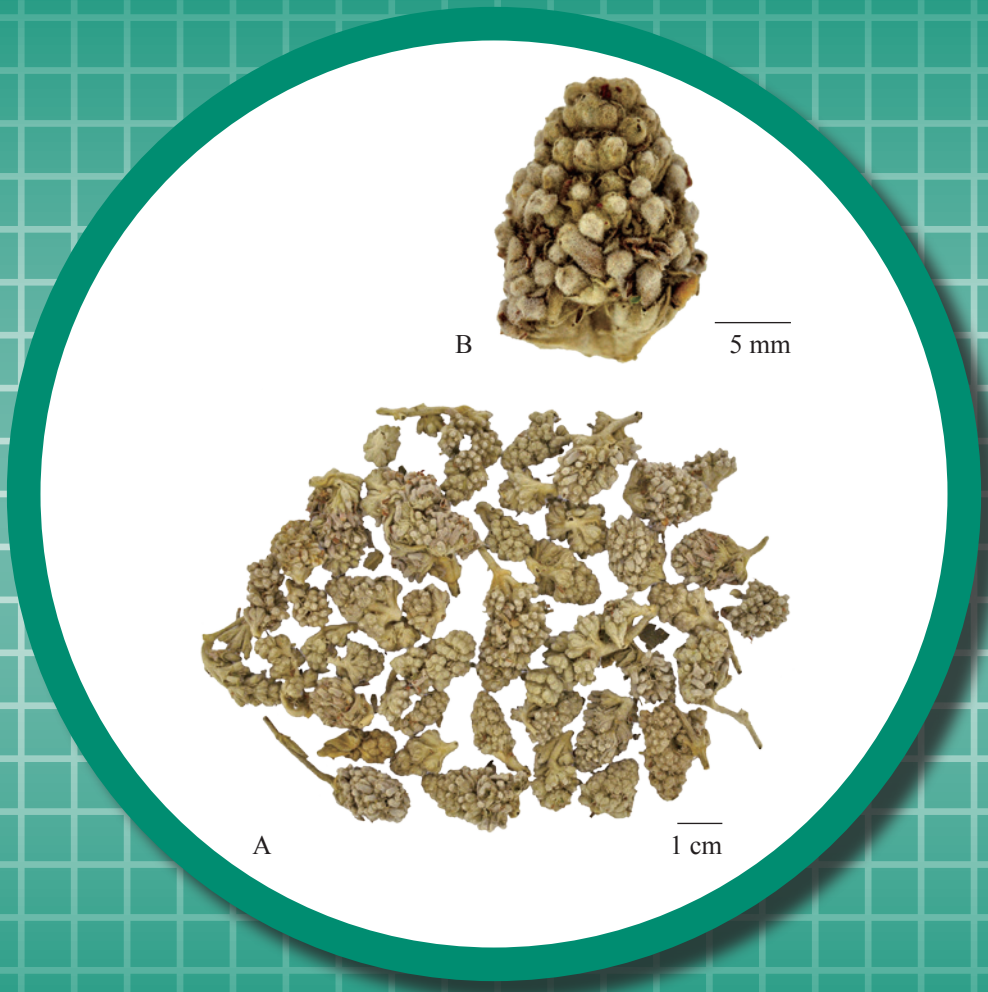

Figure 1 A photograph of Buddlejae Flos

A. Buddlejae Flos B. Magnified flower bud and inflorescence

1. NAMES

Official Name: Buddlejae Flos

Chinese Name: 密蒙花

Chinese Phonetic Name: Mimenghua

2. SOURCE

Buddlejae Flos is the dried flower bud and inflorescence of *Buddleja officinalis* Maxim. (Loganiaceae). The flower bud and inflorescence are collected in spring before flowering, foreign matter removed, then dried under the sun to obtain Buddlejae Flos.

3. DESCRIPTION

Small, terminal branches of the irregularly conical inflorescence, bearing densely clustered flower buds, 1.0-4.5 cm long. Externally greyish-yellow to brownish-yellow, densely pubescent. Flower buds subglobular or short clavate-shaped, those on the upper part slightly larger, 0.5-1.2 cm long, 1.5-2.5 mm in diameter; calyx campanulate, apex 4-dentate; corolla tubular, of the same length as calyx or slightly longer, apex 4-lobed, lobes ovate; stamens 4, attached at the middle of corolla tube. Texture soft. Odour slightly aromatic; taste slightly bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour brown. Non-glandular hairs numerous, usually 4-celled, 2 cells uniseriate arranged at the base, another 2 cells biseriate arranged at the apex; each cell bifurcated, each furcate 34-568 μm long, 7-28 μm in diameter, walls extremely thick, lumens linear; another type of non-glandular hairs few, unicellular, walls with numerous spiny protuberance. Pollen grains globose, 11-21 μm in diameter, exine smooth, with 3 germinal pores. Glandular hairs consist of 1- to 2-celled head and 1- to 2-celled stalk, 2-celled head biseriate arranged in dumbbell-shaped or butterfly-shaped in surface view. Anther cells annular thickened. Epidermal cells of corolla tip villiform. Epidermal cells of corolla lobe subrounded to polygonal (Fig. 2).

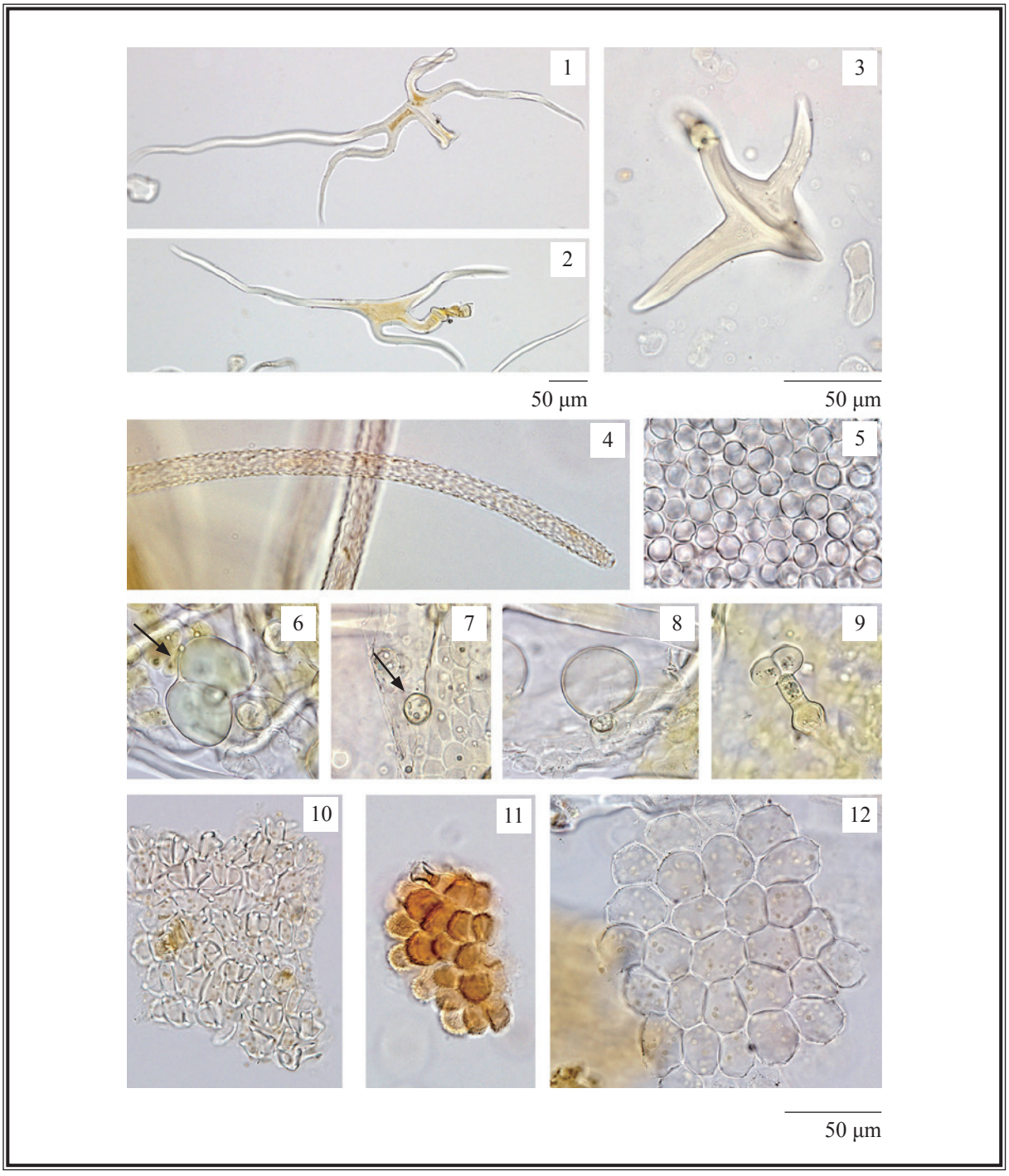

Figure 2 Microscopic features of powder of Buddlejae Flos (under the light microscope)

1-3. Non-glandular hairs 4. Unicellular non-glandular hairs 5. Pollen grains
6-7. Glandular hair (in surface view) 8-9. Glandular hair (in lateral view)
10. Anther cells 11. Epidermal cells of corolla tip 12. Epidermal cells of corolla lobe

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

Standard solution

Linarin (buddleoside) standard solution

Weigh 1.0 mg of linarin CRS (Fig. 3) and dissolve in 4 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, water and formic acid (7:3:1.2:1, v/v).

Spray reagent

Weigh 2.5 g of ferric trichloride and dissolve in 50 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 methanol. Sonicate (220 W) the mixture for 30 min. Filter the mixture.

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 linarin standard solution (12 µL) and the test solution (4 µL) to the plate. 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 dry in air. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

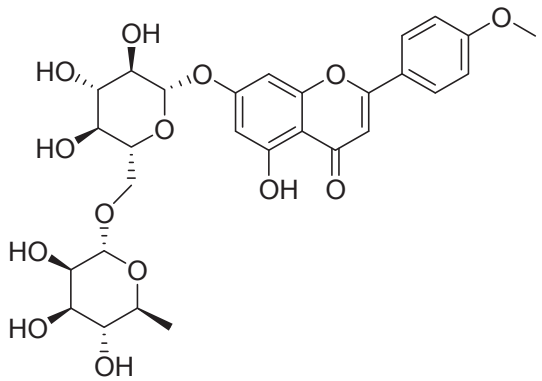

Figure 3 Chemical structure of linarin (buddleoside)

Figure 4 A reference HPTLC chromatogram of Buddlejae Flos extract observed under visible light after staining

1. Linarin 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 linarin (Fig. 4).

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

Standard solution

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

Weigh 0.1 mg of linarin CRS and dissolve in 5 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (326 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)	1% Acetic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 60	65 → 35	35 → 65	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of linarin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of linarin should not be more than 5.0%; the RSD of the retention time of linarin peak should not be more than 2.0%; the column efficiency determined from linarin peak should not be less than 25000 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 linarin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of linarin peak in the chromatogram of linarin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify linarin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of linarin Std-FP. The retention times of linarin 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 four characteristic peaks of Buddlejae Flos extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Buddlejae Flos extract

Peak No.	RRT	Acceptable Range
1	0.94	± 0.03
2 (marker, linarin)	1.00	-
3	1.13	± 0.03
4 (apigenin)	1.27	± 0.03

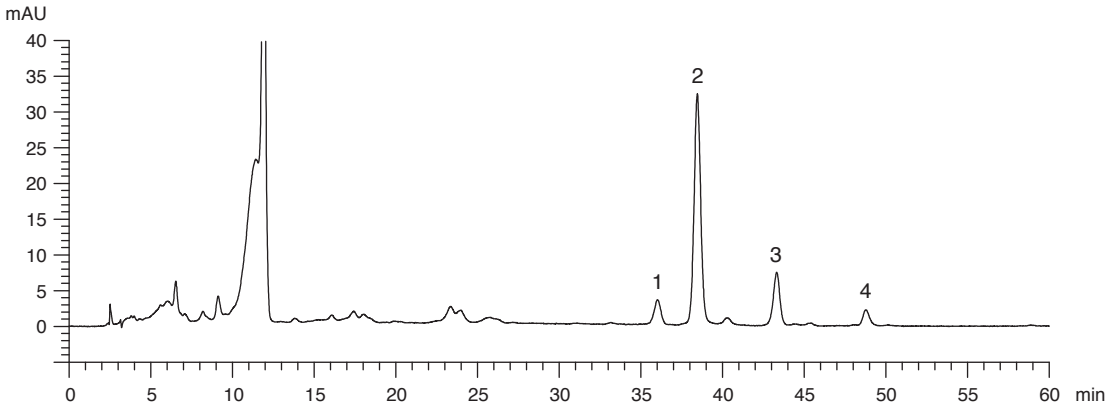

Figure 5 A reference fingerprint chromatogram of Buddlejae Flos extract

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 XVII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.5%.
Acid-insoluble ash: not more than 4.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

Linarin (buddleoside) standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 0.5 mg of linarin CRS and dissolve in 5 mL of methanol.

Linarin standard solution for assay, Std-AS

Measure accurately the volume of the linarin Std-Stock, dilute with methanol to produce a series of solutions of 1, 10, 20, 30, 40 mg/L for linarin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 15 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (326 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. The mobile phase is a mixture of 1% acetic acid and methanol (50:50, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of linarin Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of linarin should not be more than 5.0%; the RSD of the retention time of linarin peak should not be more than 2.0%; the column efficiency determined from linarin peak should not be less than 4500 theoretical plates.

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

Limits

The sample contains not less than 0.68% of linarin ($C_{28}H_{32}O_{14}$), calculated with reference to the dried substance.