Farfarae Flos

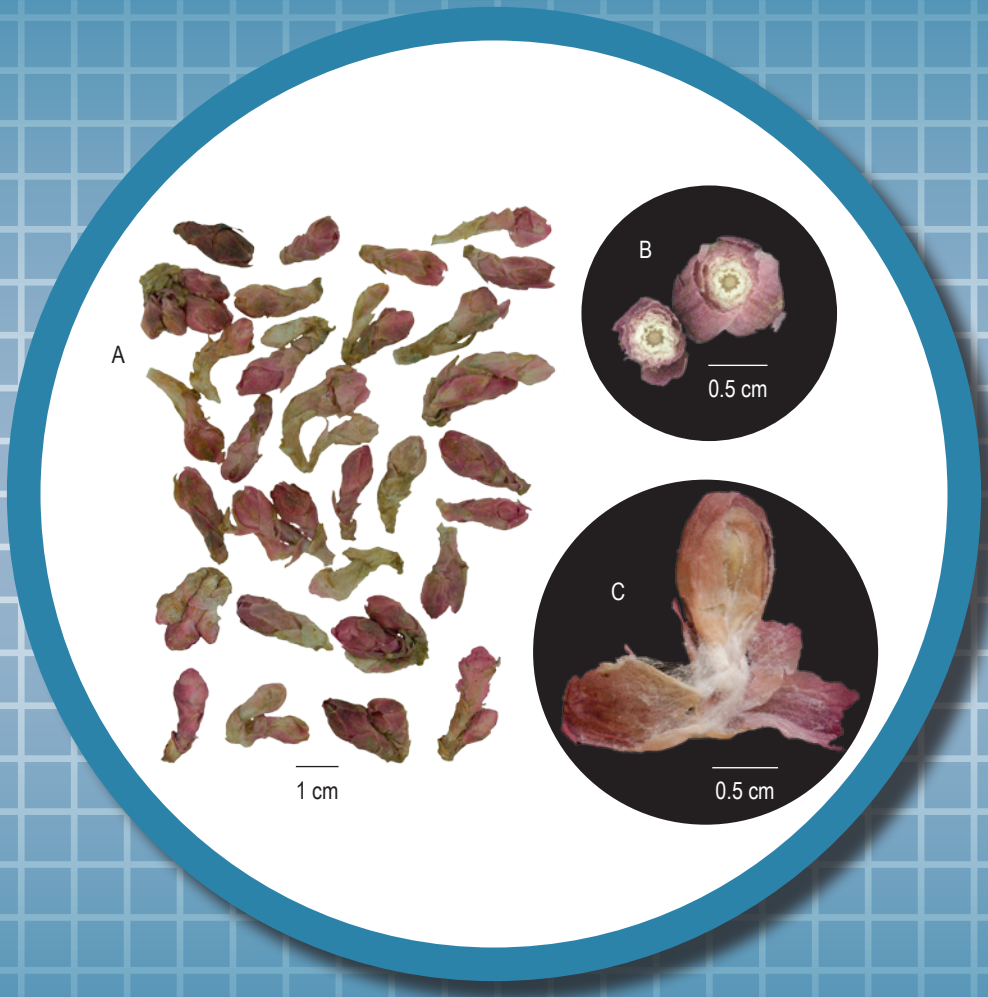

Figure 1 A photograph of Farfarae Flos

A. Whole pieces of Farfarae Flos B. Transverse section
C. After ripping of the bracts, the inner surface shows a dense cover of white flocky hairs

1. NAMES

Official Name: Farfarae Flos

Chinese Name: 款冬花

Chinese Phonetic Name: Kuandonghua

2. SOURCE

Farfarae Flos is the dried flower bud of *Tussilago farfara* L. (Asteraceae). The flower bud is collected in December or before the ground is frozen while the flower is still under the ground, pedicels and soil removed, then dried in the shade to obtain Farfarae Flos.

3. DESCRIPTION

Elongated-clavate. Solitary or 2–3 accreted at the base, 1–2.5 cm long, 5–10 mm in diameter. The upper part broader; the lower part gradually slender toward the base or with short pedicel, enclosed by numerous scaly bracts. Bracts externally purplish-red or pale red and the inner surface densely covered with white flocky hairs. Bud light in weight, showing white tomentose when ripped. Odour aromatic; taste slightly bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Rachis: Epidermis of rachis consists of 1 layer of subrounded or subsquare cells, covered with unevenly thickened cuticle. Cortex consists of 15–30 layers of subrounded parenchymatous cells. Endodermis sometimes visible. Vascular bundles arranged in a ring, frequently with secretory duct located closely to the top of each bundle, while sclerenchymatous cells usually scattered between the secretory duct and the phloem. Pith mainly consists of parenchymatous cells.

Bract: The upper epidermal cells of the bract subrounded, covered with cuticle; the lower ones subrectangular. A secretory duct located closely to the top of vascular bundle (Fig. 2).

Powder

Colour pale red. Pollen grains yellow, subspherical, 25-40 μm in diameter, some with fragments of anther. Inulin fairly abundant, with radial striations. Non-glandular hairs consist of 1-4 cells, abundant, stripe-shaped or twisted into masses, varying in length (occasionally up to 510 μm). Epidermal cells of bract subrectangular or polygonal in surface view, with intermittent fine stripes, stomata anomocytic, subsidiary cells 4-7. Glandular hairs pestle-like in shape, varying in length (occasionally up to 216 μm), 16-52 μm in diameter; head 4- to 6-celled, expanded, ellipsoidal; base consists of 2 columns side by side, several to more than 10 cells (Fig. 3).

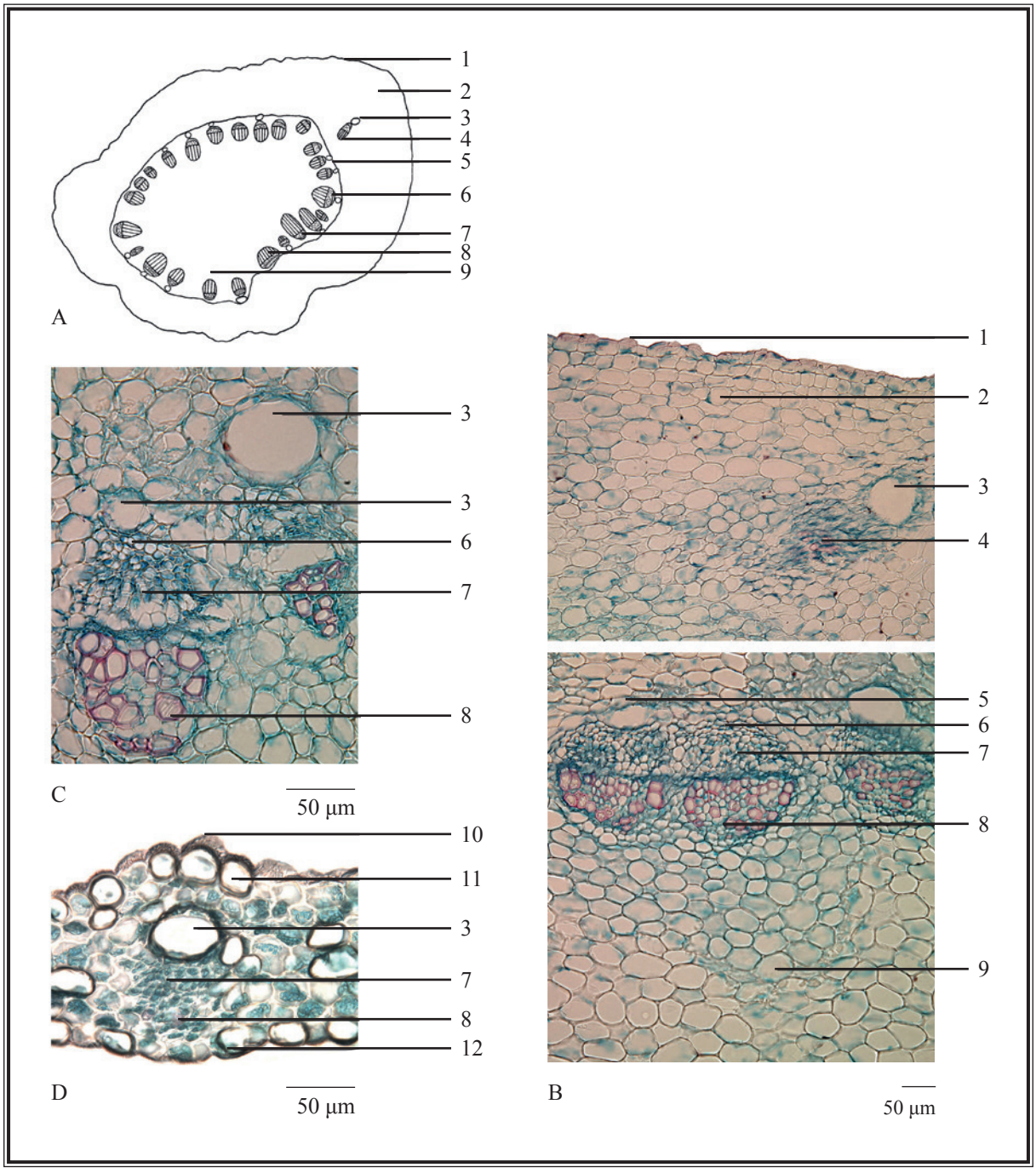

Figure 2 Microscopic features of transverse section of Farfarae Flos

A. Sketch of rachis B. Section illustration of rachis
C. Vascular bundles and secretory ducts D. Section illustration of bract

1. Epidermis 2. Cortex 3. Secretory duct 4. Bract trace vascular bundle
5. Endodermis 6. Sclerenchymatous cells 7. Phloem 8. Xylem
9. Pith 10. Cuticle 11. Upper epidermis of bract
12. Lower epidermis of bract

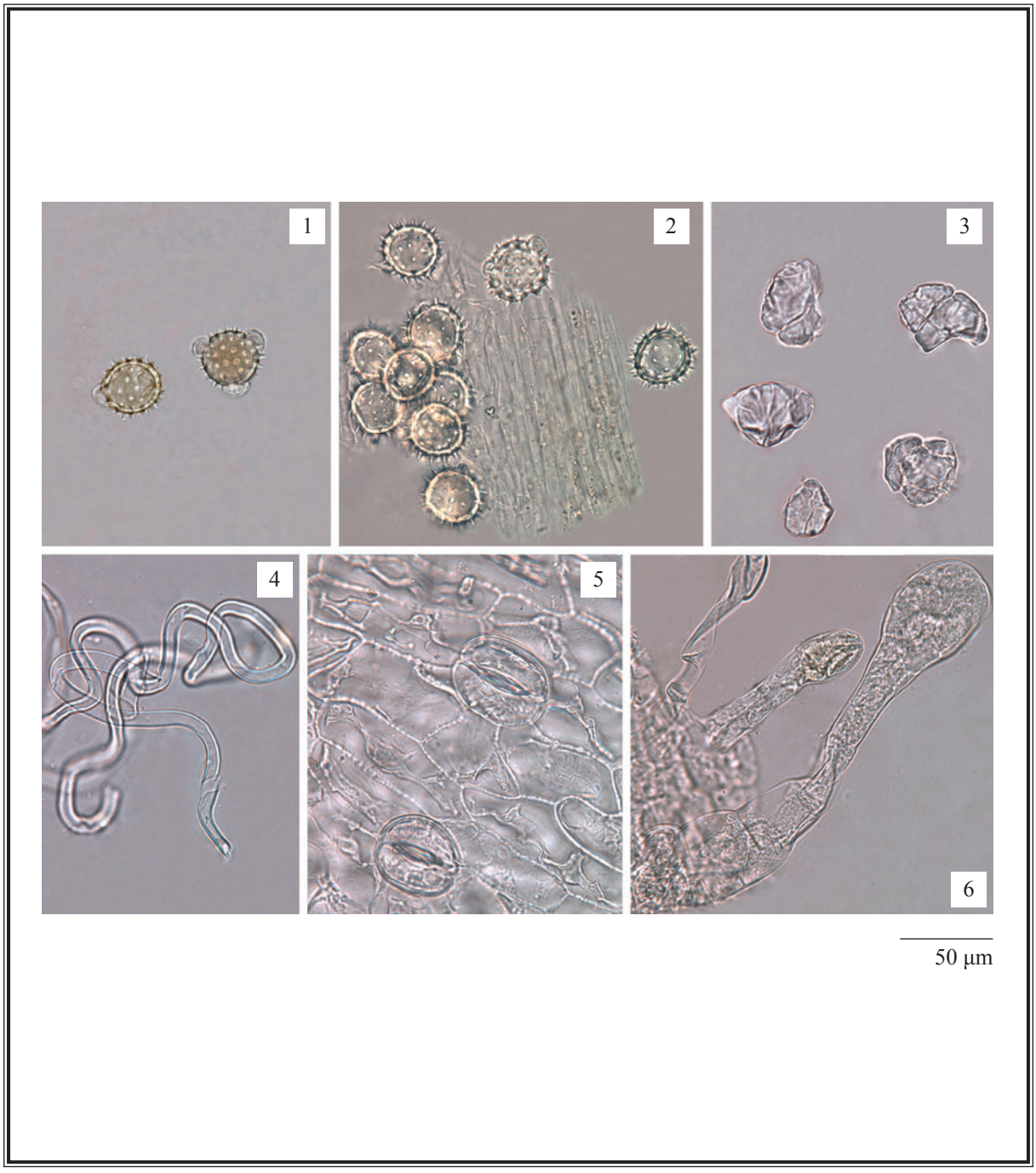

Figure 3 Microscopic features of powder of Farfarae Flos (under the light microscope)

1. Pollen grains 2. Pollen grains and fragments of anther 3. Inulins
4. Non-glandular hairs 5. Epidermal cells of bract with stomata
6. Glandular hairs

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

Standard solution

Tussilagone standard solution

Weigh 1.0 mg of tussilagone CRS (Fig. 4) and dissolve in 0.5 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80˚C) and ethyl acetate (4:1, 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 (160 W) the mixture for 1 h. 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 ethyl acetate.

Procedure

Carry out the method by using a TLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately tussilagone standard solution (2 µL) and the test solution (8 µL) to the plate. Develop over a path of about 4 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).

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 tussilagone.

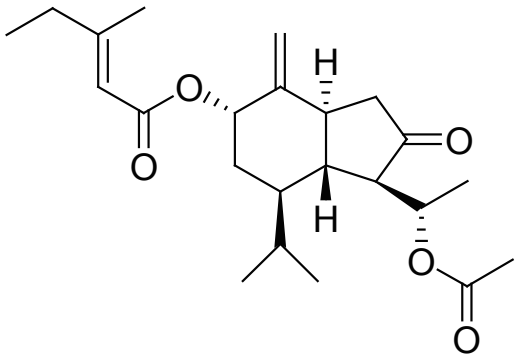

Figure 4 Chemical structure of tussilagone

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

Standard solution

Tussilagone standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.6 mg of tussilagone CRS and dissolve in 20 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (160 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 ethanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of the inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	90 → 64	10 → 36	linear gradient
20 – 30	64 → 10	36 → 90	linear gradient
30 – 60	10 → 5	90 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of tussilagone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of tussilagone should not be more than 5.0%; the RSD of the retention time of tussilagone peak should not be more than 2.0%; the column efficiency determined from tussilagone peak should not be less than 250000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Farfarae Flos extract

Peak No.	RRT	Acceptable Range
1	0.52	± 0.03
2	0.55	± 0.03
3	0.57	± 0.03
4 (marker, tussilagone)	1.00	-
5	1.08	± 0.03

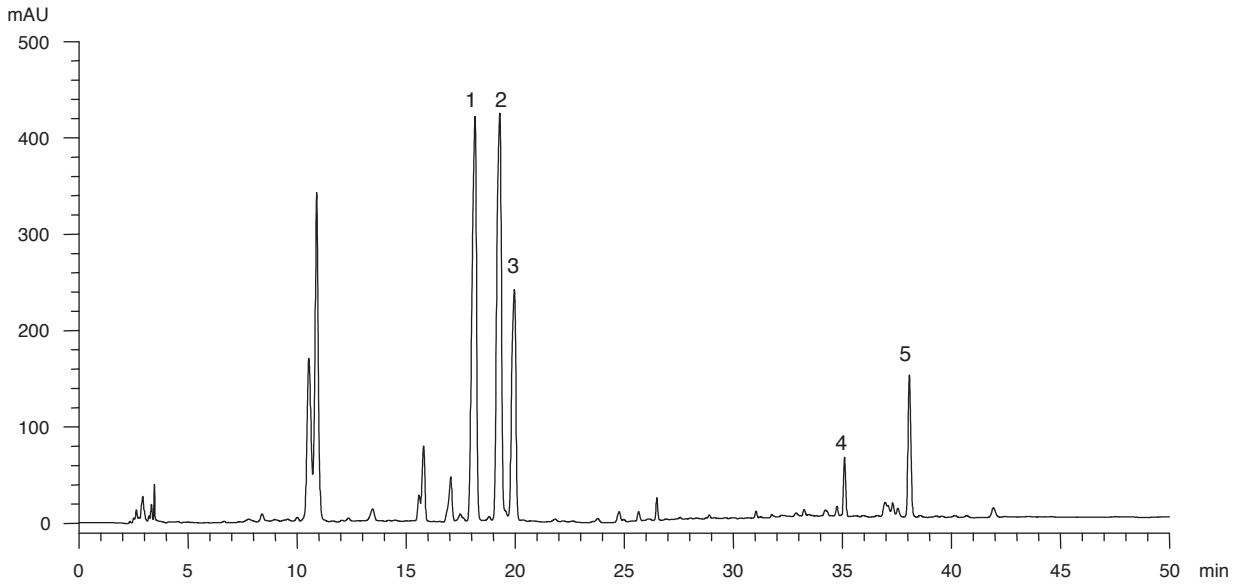

Figure 5 A reference fingerprint chromatogram of Farfarae Flos extract

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 11.0%.
Acid-insoluble ash: not more than 3.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 47.0%.
Ethanol-soluble extractives (cold extraction method): not less than 29.0%.

7. ASSAY

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

Standard solution

Tussilagone standard stock solution, Std-Stock (600 mg/L)

Weigh accurately 6.0 mg of tussilagone CRS and dissolve in 10 mL of ethanol.

Tussilagone standard solution for assay, Std-AS

Measure accurately the volume of the tussilagone Std-Stock, dilute with ethanol to produce a series of solutions of 0.6, 15, 30, 45, 60 mg/L for tussilagone.

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 ethanol. Sonicate (160 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 ethanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of the inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	90 → 64	10 → 36	linear gradient
20 – 30	64 → 10	36 → 90	linear gradient
30 – 60	10 → 5	90 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of tussilagone Std-AS (30 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of tussilagone should not be more than 5.0%; the RSD of the retention time of tussilagone peak should not be more than 2.0%; the column efficiency determined from tussilagone peak should not be less than 250000 theoretical plates.

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

Limits

The sample contains not less than 0.081% of tussilagone ($C_{23}H_{34}O_5$), calculated with reference to the dried substance.