Geranii Caroliniani Herba

Figure 1 A photograph of Geranii Caroliniani Herba

A. Geranii Caroliniani Herba B. Magnified image of upper surface of leaf
C. Magnified image of lower surface of leaf D. Magnified image of branch
E. Magnified image of fruit and fruit persistent style
F. Magnified image of fruit and its segment G. Magnified image of stem

1. NAMES

Official name: Geranii Caroliniani Herba

Chinese name: 野老鸛草

Chinese phonetic name: Yelaoguancao

2. SOURCE

Geranii Caroliniani Herba is the dried aerial part of *Geranium carolinianum* L. (Geraniaceae). The aerial part is collected in summer and autumn when the fruit is beginning to ripe, foreign matter removed, then dried under the sun to obtain Geranii Caroliniani Herba.

3. DESCRIPTION

Stems varying in length and up to 50 cm long, 1-7 mm in diameter, frequently branched, nodes swollen, externally greyish-yellow to purplish, longitudinally furrowed and sparsely pubescent, texture fragile, fracture yellowish-white, sometimes hollow. Leaves opposite, slenderly petioled, lamina palmatisect, 5-7 parted, lobes stripe-shaped and pinnatipartite about 3-5 at upper part. Persistent calyx visible. Capsule spherical, 0.3-0.5 cm long, covered with scabrous pubescence, persistent style 1-1.5 cm long, beak-shaped, sometimes 5-lobed, curled upward. Odour slight, taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Non-glandular hairs found on the surface of epidermis, abundant, mostly unicellular. Epidermis consists of 1 layer of cells, covered with a thin layer of cuticle. Cortex parenchymatous cells irregular arranged. Pericycle fibres arranged in a ring, slightly lignified. Vascular bundles collateral, arranged in an interrupted ring beneath pericycle fibres. Phloem broad. Xylem composed of vessels, few fibres and parenchymatous cells. Pith relatively large, mainly consisting of parenchymatous cells [Fig. 2 (i)].

Leaf: Petiole base consists of 5-7 collateral vascular bundles. Upper and lower epidermis consists of 1 layer of epidermal cell. Palisade tissue consists of 2 layers of cylindrical cells. Vascular bundles surrounded by sclerenchyma. 1-7 layers of sclerenchymatous cells present on the inner side of upper and lower epidermis of vein. The spongy tissue consists of irregular shaped parenchymatous cells. Vascular bundles collateral, xylem consists of lignified vessels, phloem crescent-shaped. Glandular and non-glandular hairs visible on the upper and lower epidermis, mostly fallen off after staining process; the head of glandular hairs elliptical, consisting of 1-2 cells, stalk mostly 1-celled, occasionally 2 cells visible; non-glandular hairs mostly unicellular. Clusters of calcium oxalate occasionally visible [Fig. 2(ii)].

Powder

Colour greyish-green. Upper epidermal cells subpolygonal, with straight or slightly sinuous anticlinal walls. Lower epidermal cells irregular in shape, with sinuous anticlinal wall. Stomata anomocytic, subsidiary cells 4-5. Head of glandular hairs elliptical, consisting of 1-2 cells, stalk mostly 1-celled, occasionally 2 cells visible. Non-glandular hairs mostly unicellular, bent or straight, surface with fine warty protrusions. Clusters of calcium oxalate in rosette aggregate, 3-13 μm in diameter, occasionally visible in leaf, but densely distributed in persistent calyx fragment; bright white under the polarized microscope (Fig. 3).

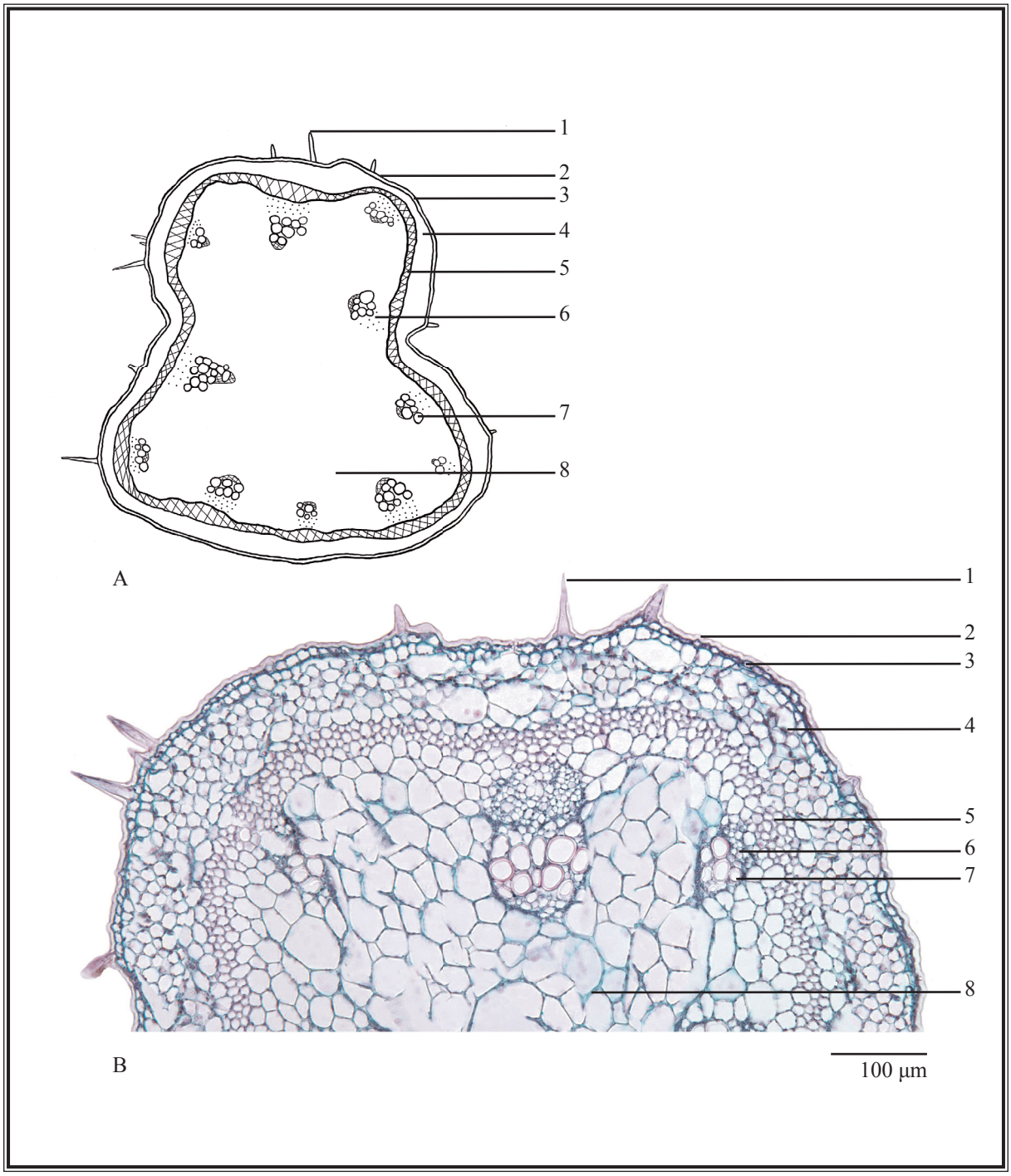

Figure 2 (i) Microscopic features of transverse section of stem of Geranii Caroliniani Herba

A. Sketch B. Section illustration

1. Non-glandular hair 2. Cuticle 3. Epidermis 4. Cortex 5. Pericycle fibre
6. Phloem 7. Xylem 8. Pith

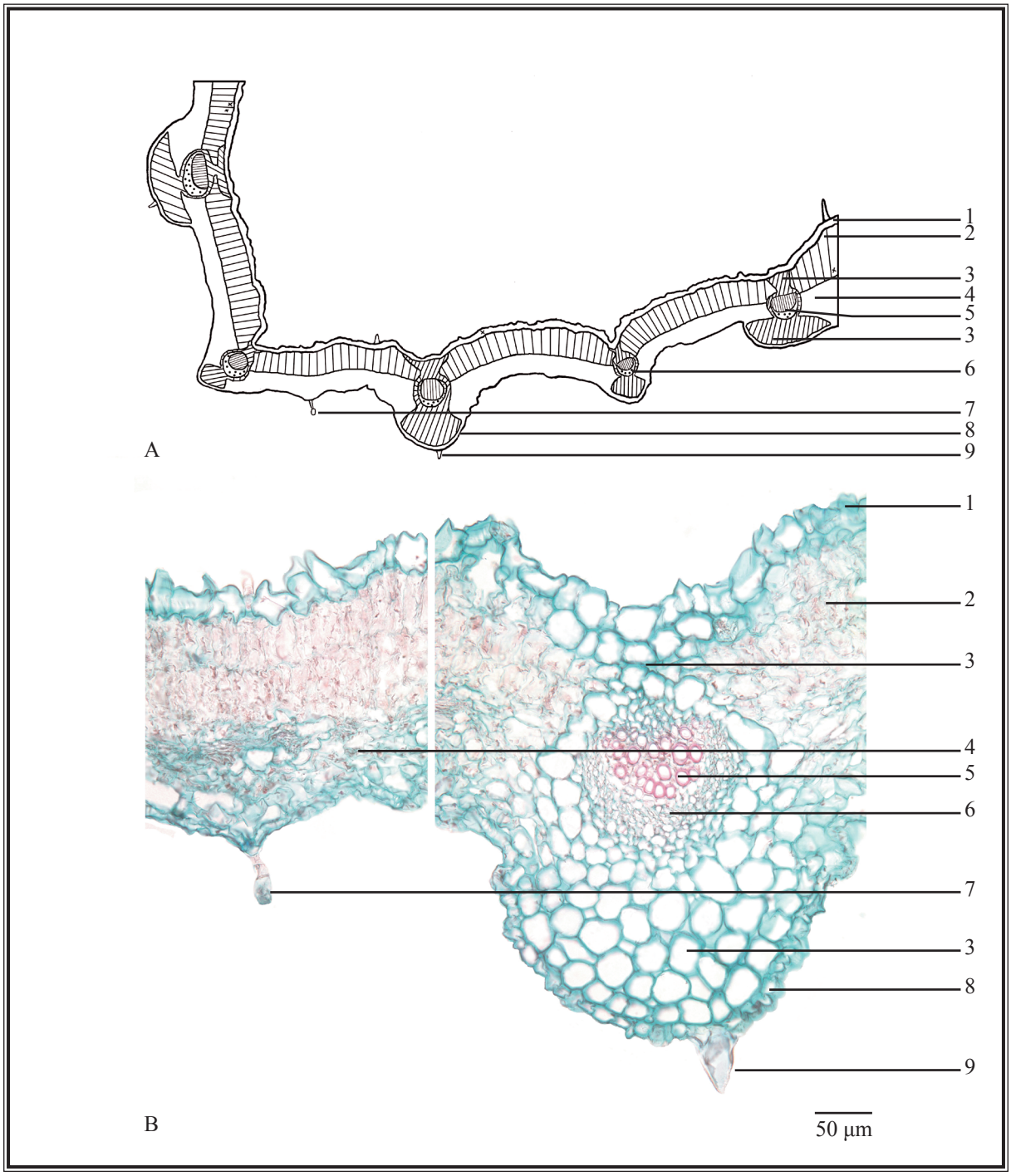

Figure 2 (ii) Microscopic features of transverse section of leaf of Geranii Caroliniani Herba

A. Sketch B. Section illustration (petiole base)

1. Upper epidermis 2. Palisade tissue 3. Sclerenchyma 4. Spongy tissue 5. Xylem
6. Phloem 7. Glandular hair 8. Lower epidermis 9. Non-glandular hair

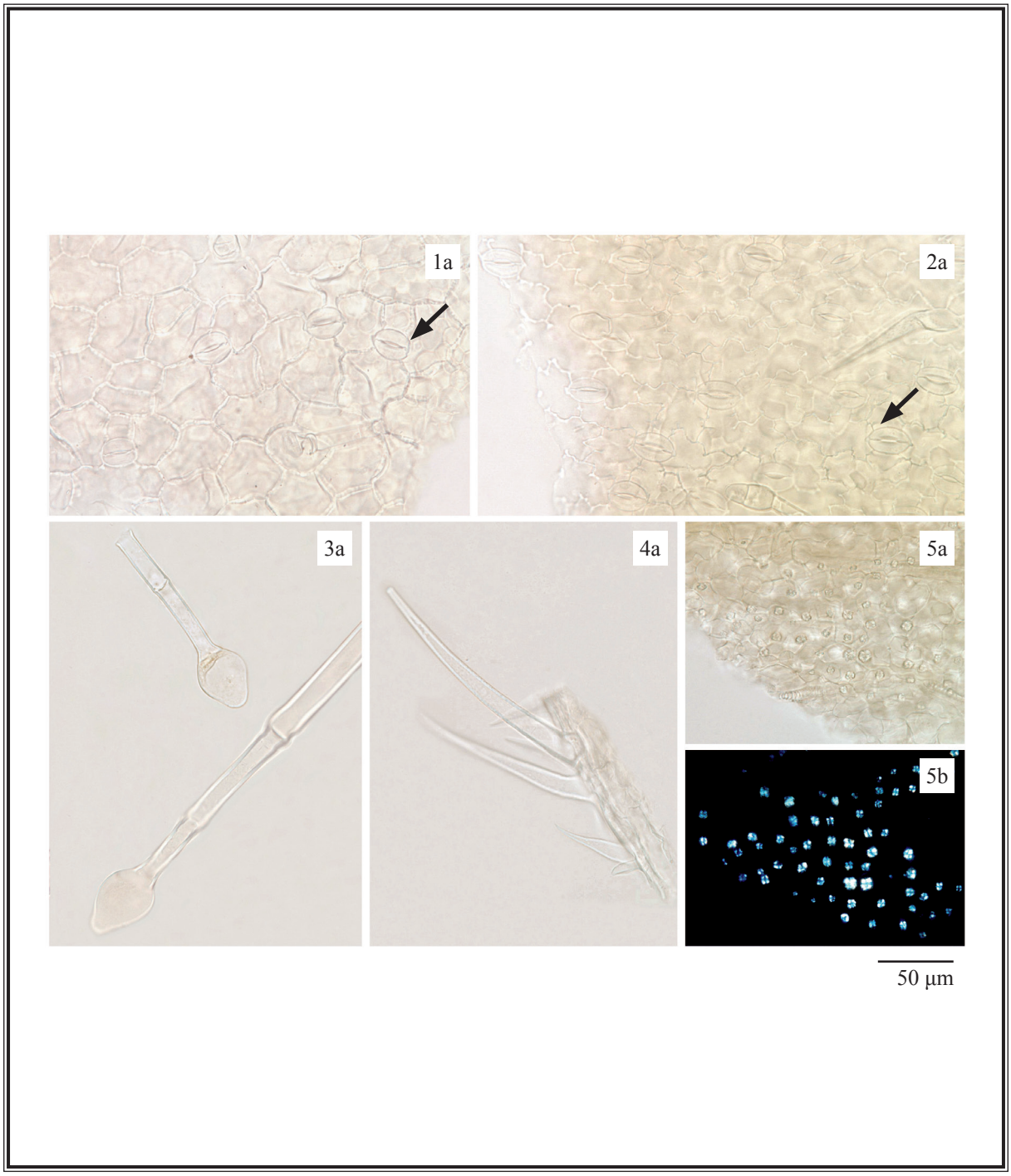

Figure 3 Microscopic features of powder of Geranii Caroliniani Herba

1. Upper epidermal cells with anomocytic stomata (→)
2. Lower epidermal cells with anomocytic stomata (→) 3. Glandular hairs
4. Non-glandular hairs 5. Clusters of calcium oxalate in calyx cells

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

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

Standard solution

Corilagin standard solution

Weigh 1.0 mg of corilagin CRS (Fig. 4) and dissolve in 1 mL of water.

Developing solvent system

Prepare a mixture of dichloromethane, formic acid and methanol (6: 1.5: 1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL round-bottomed flask, then add 10 mL of water. Reflux the mixture for 30 min. Cool down to room temperature. Filter through a 0.45-μm nylon 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 corilagin standard solution (3 μL) and the test solution (1 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).

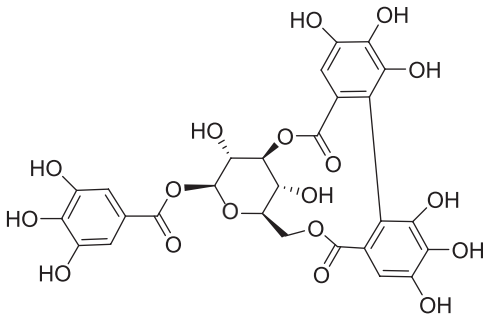

Figure 4 Chemical structure of corilagin

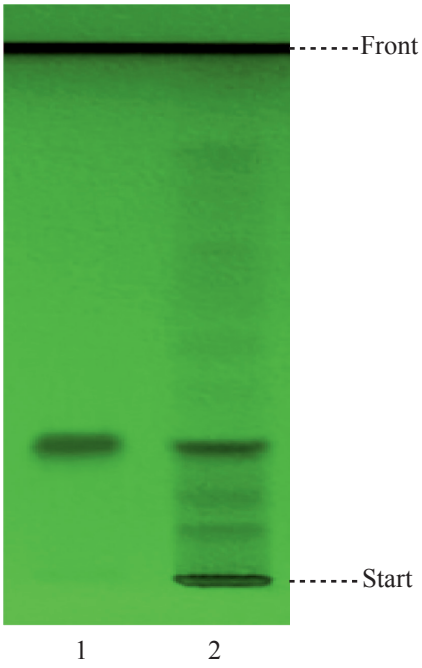

Figure 5 A reference HPTLC chromatogram of Geranii Caroliniani Herba extract observed under UV light (254 nm)

1. Corilagin 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 corilagin (Fig. 5).

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

Standard solution

Corilagin standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of corilagin CRS and dissolve in 10 mL of water.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of water. Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with 25 mL of water. Combine the extract. Evaporate the combined extract to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in water. Transfer the extract to a 50-mL volumetric flask and make up to the mark with water. Filter through a 0.2-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (266 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (1.7 μm particle size, 130 Å pore size and 185 m^2/g surface area). The flow rate is about 0.2 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 – 3	93	7	isocratic
3 – 4	93 → 85	7 → 15	linear gradient
4 – 16	85 → 65	15 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 1 μL of corilagin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of corilagin should not be more than 5.0%; the RSD of the retention time of corilagin peak should not be more than 2.0%; the column efficiency determined from corilagin peak should not be less than 100000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject corilagin Std-FP and the test solution (1 μL each) into the UHPLC system and record the chromatograms. Measure the retention time of corilagin peak in the chromatogram of corilagin Std-FP and the retention times of the seven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify corilagin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of corilagin Std-FP. The retention times of corilagin 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 seven characteristic peaks of Geranii Caroliniani Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the seven characteristic peaks of Geranii Caroliniani Herba extract

Peak No.	RRT	Acceptable Range
1	0.26	± 0.03
2	0.94	± 0.03
3 (marker, corilagin)	1.00	-
4	1.02	± 0.03
5	1.23	± 0.03
6	1.26	± 0.03
7	1.56	± 0.04

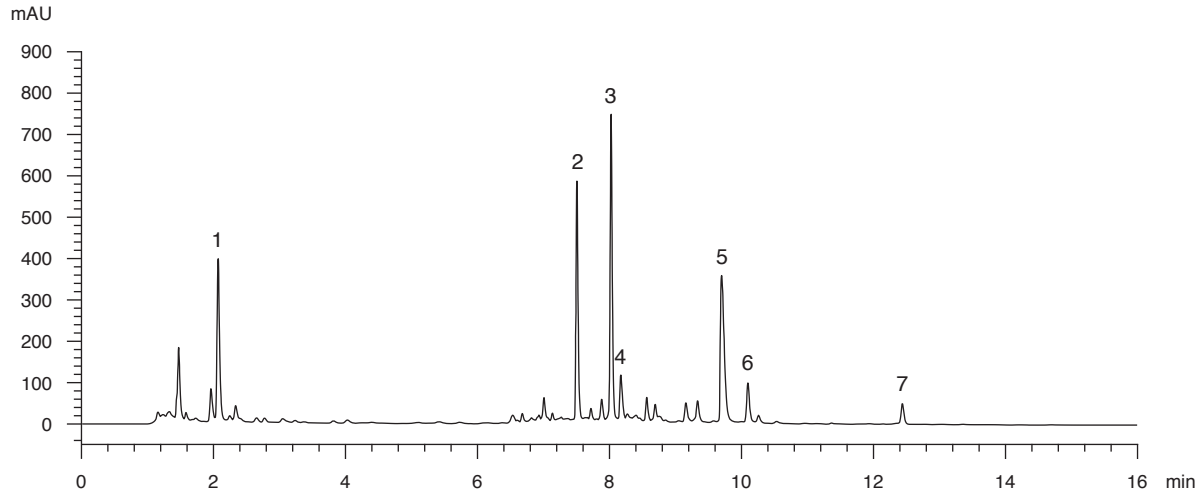

Figure 6 A reference fingerprint chromatogram of Geranii Caroliniani Herba extract

For positive identification, the sample must give the above seven characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 6).

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 10.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 24.0%.
Ethanol-soluble extractives (cold extraction method): not less than 18.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 5.0 mg of corilagin CRS and dissolve in 5 mL of water.

Corilagin standard solution for assay, Std-AS

Measure accurately the volume of the corilagin Std-Stock, dilute with water to produce a series of solutions of 5, 25, 100, 200, 300 mg/L for corilagin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of water. Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue with 25 mL of water. Combine the extract. Evaporate the combined extract to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in water. Transfer the extract to a 50-mL volumetric flask and make up to the mark with water. Filter through a 0.2-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (266 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (1.7 μm particle size, 130 Å pore size and 185 m^2/g surface area). The flow rate is about 0.2 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 – 3	93	7	isocratic
3 – 4	93 → 85	7 → 15	linear gradient
4 – 16	85 → 65	15 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 1 μL of corilagin Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of corilagin should not be more than 5.0%; the RSD of the retention time of corilagin peak should not be more than 2.0%; the column efficiency determined from corilagin peak should not be less than 100000 theoretical plates.

The *R* value between corilagin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of corilagin Std-AS (1 μL each) into the UHPLC system and record the chromatograms. Plot the peak areas of corilagin against the corresponding concentrations of corilagin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 1 µL of the test solution into the UHPLC system and record the chromatogram. Identify corilagin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of corilagin Std-AS. The retention times of corilagin 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 corilagin in the test solution, and calculate the percentage content of corilagin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.0% of corilagin ($C_{27}H_{22}O_{18}$), calculated with reference to the dried substance.

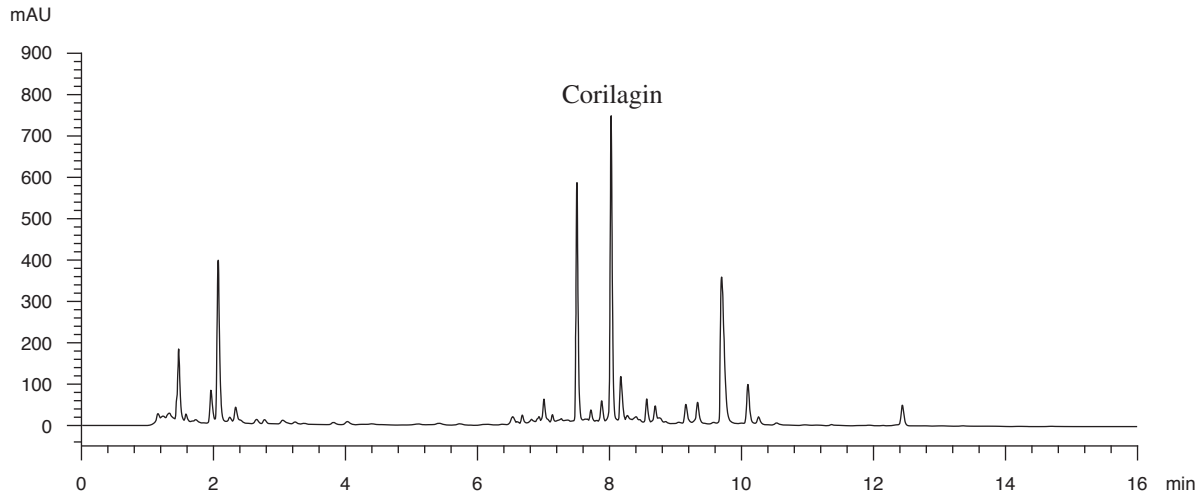

Figure 7 A reference assay chromatogram of Geranii Caroliniani Herba extract