Centipedae Herba

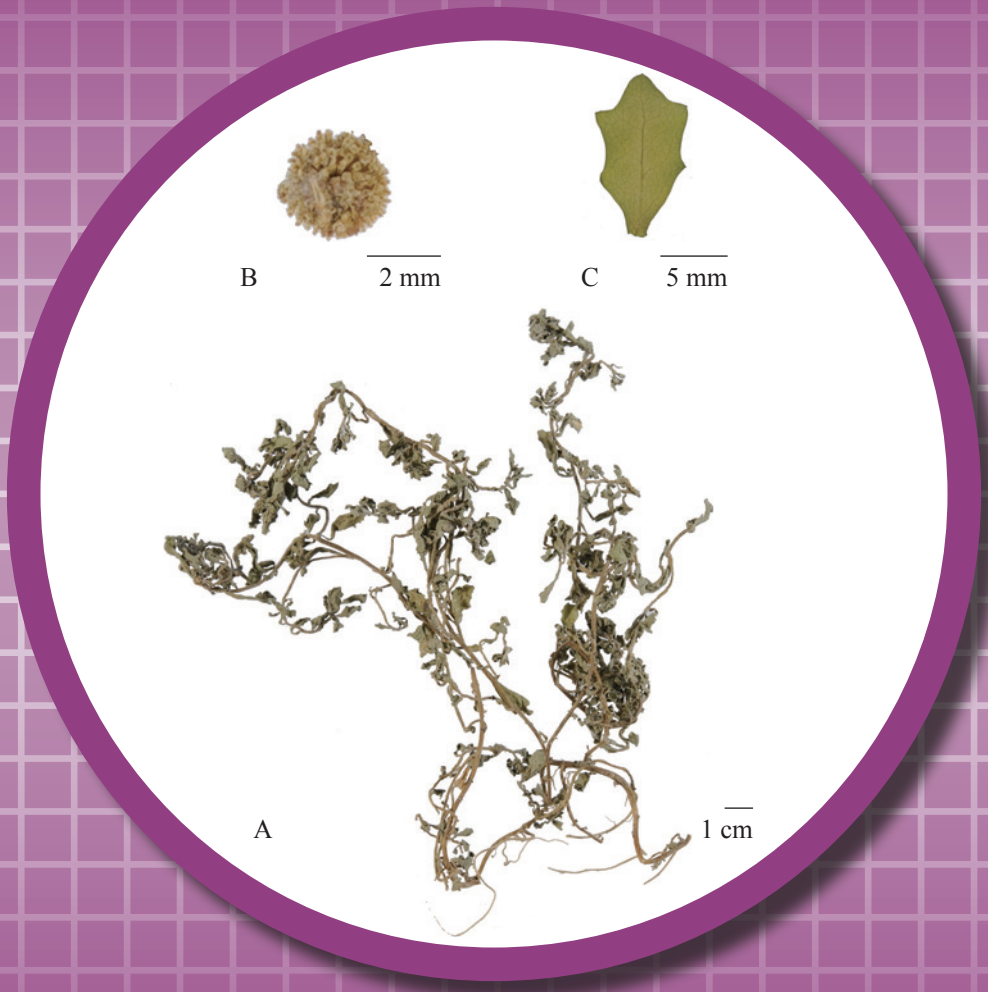

Figure 1 A photograph of Centipedae Herba

A. Centipedae Herba
B. Magnified image of capitulum with numerous flowers
C. Magnified image of leaf

1. NAMES

Official Name: Centipedae Herba

Chinese Name: 鵝不食草

Chinese Phonetic Name: Ebushicao

2. SOURCE

Centipedae Herba is the dried whole plant of *Centipeda minima* (L.) A. Br. et Aschers. (Asteraceae). The whole plant containing rootlets is collected in summer and autumn at flowering, soil removed, then dried under the sun to obtain Centipedae Herba.

3. DESCRIPTION

Tangled masses of stems and leaves. Rootlets fine, pale yellow. Stems slender, frequently branched; texture fragile, easily broken, fracture yellowish-white. Leaves small, nearly sessile; lamina mostly crimpled and broken, spatulate-shaped when intact flattened out, externally greyish-green or brown, margin 3-5-dentate. Capitulum yellow to yellowish-brown. Odour slightly fragrant, irritating with prolonged smelling; taste bitter and slightly pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Epidermal cells subrounded, elongated tangentially, the walls finely thickened. Cortex consists of 5-8 layers of cells, the cells subrounded, relative large, with numerous clefts. Endodermis distinct. Phloem narrow, cells elongated tangentially. Xylem broad, vessels arranged radially [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of cells, the cells subrounded or elongated tangentially. Cortex consists of 5-8 layers of cells, clefts large. Fibres 4-15 in bundles, located on outer side of the phloem. Phloem narrow, the cells elongated tangentially. Xylem broad, vessels arranged radially. Pith distinct [Fig. 2 (ii)].

Powder

Colour greyish-green to greyish-brown. Glandular hairs paramecium-like in surface view, cells arranged in pairs, containing yellow contents. Non-glandular hairs biseriate, one unicellular, slightly short, the other bicellular, basal cell relatively short, apical cell hooked or rolled, with fine cuticular striations on the surface of 2/3 of upper part. Pollen grains pale yellow, subglobular, 11-23 μm in diameter, with 3 germinal furrows, exine spiny. Epidermal cells of corolla yellow, rectangular to subpolygonal in surface view, with cuticular striations, cells extended outwards to form tomentose protuberance. Epidermal cells of stem rectangular or subpolygonal, walls slightly thickened, with indistinct cuticular striations, stomata visible. Epidermal cells of leaf subpolygonal, anticlinal walls thin and sinuous in surface view, stomata anomocytic, subsidiary cells 4-6 (Fig. 3).

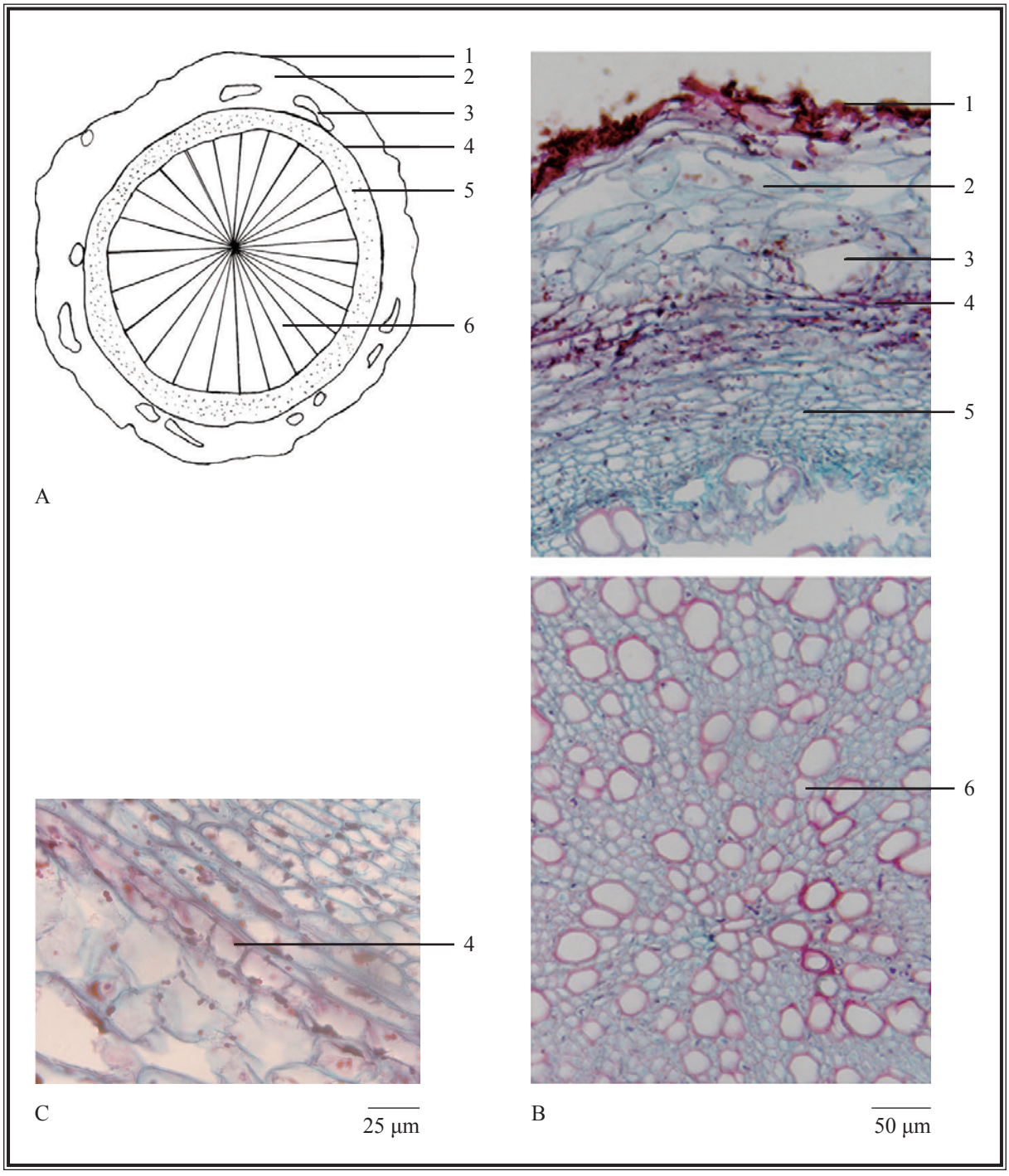

Figure 2 (i) Microscopic features of transverse section of root of Centipedae Herba

A. Sketch B. Section illustration C. Endodermis

1. Epidermis 2. Cortex 3. Cleft 4. Endodermis 5. Phloem 6. Xylem

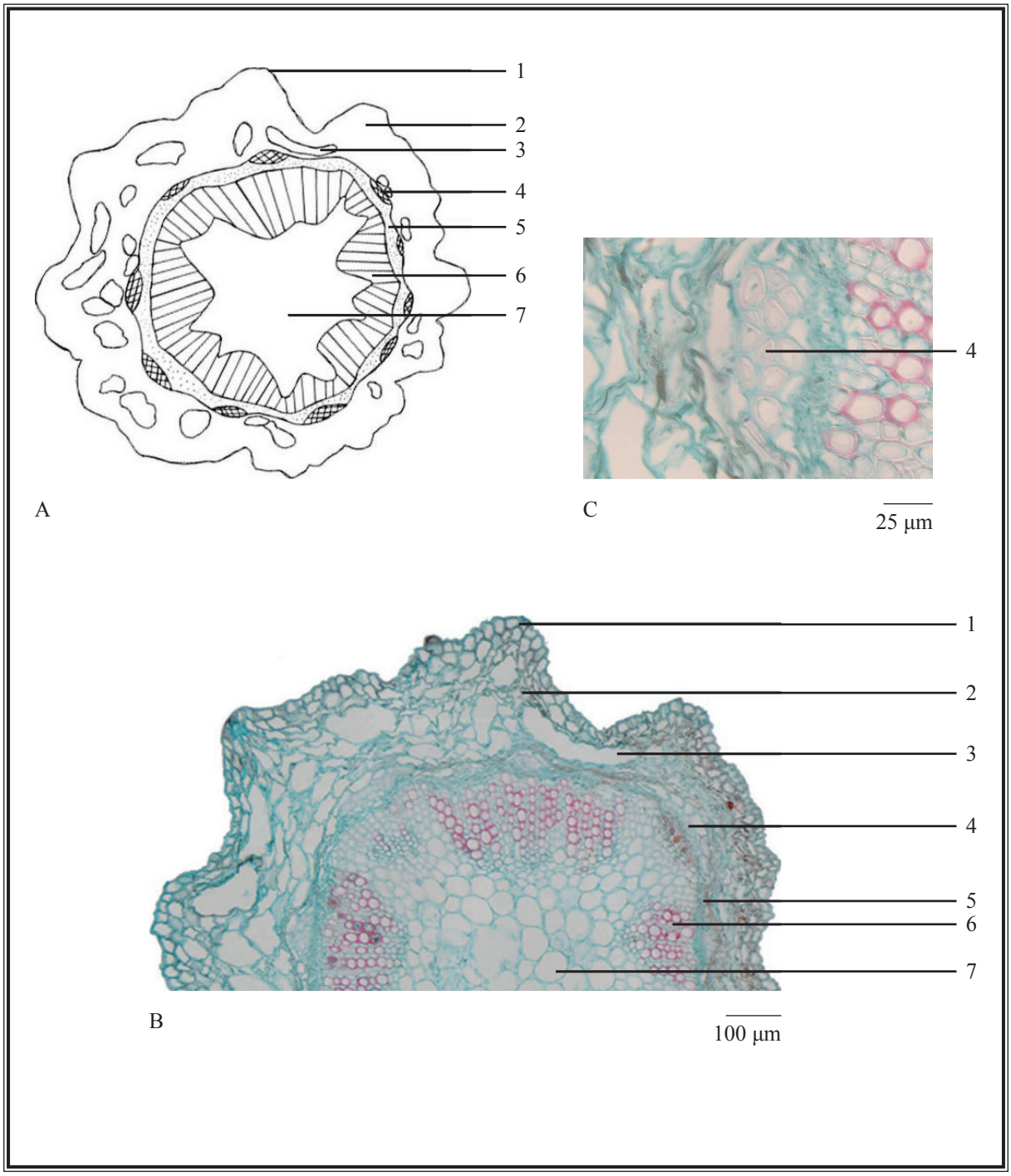

Figure 2 (ii) Microscopic features of transverse section of stem of Centipedae Herba

A. Sketch B. Section illustration C. Fibres

1. Epidermis 2. Cortex 3. Cleft 4. Fibres 5. Phloem 6. Xylem 7. Pith

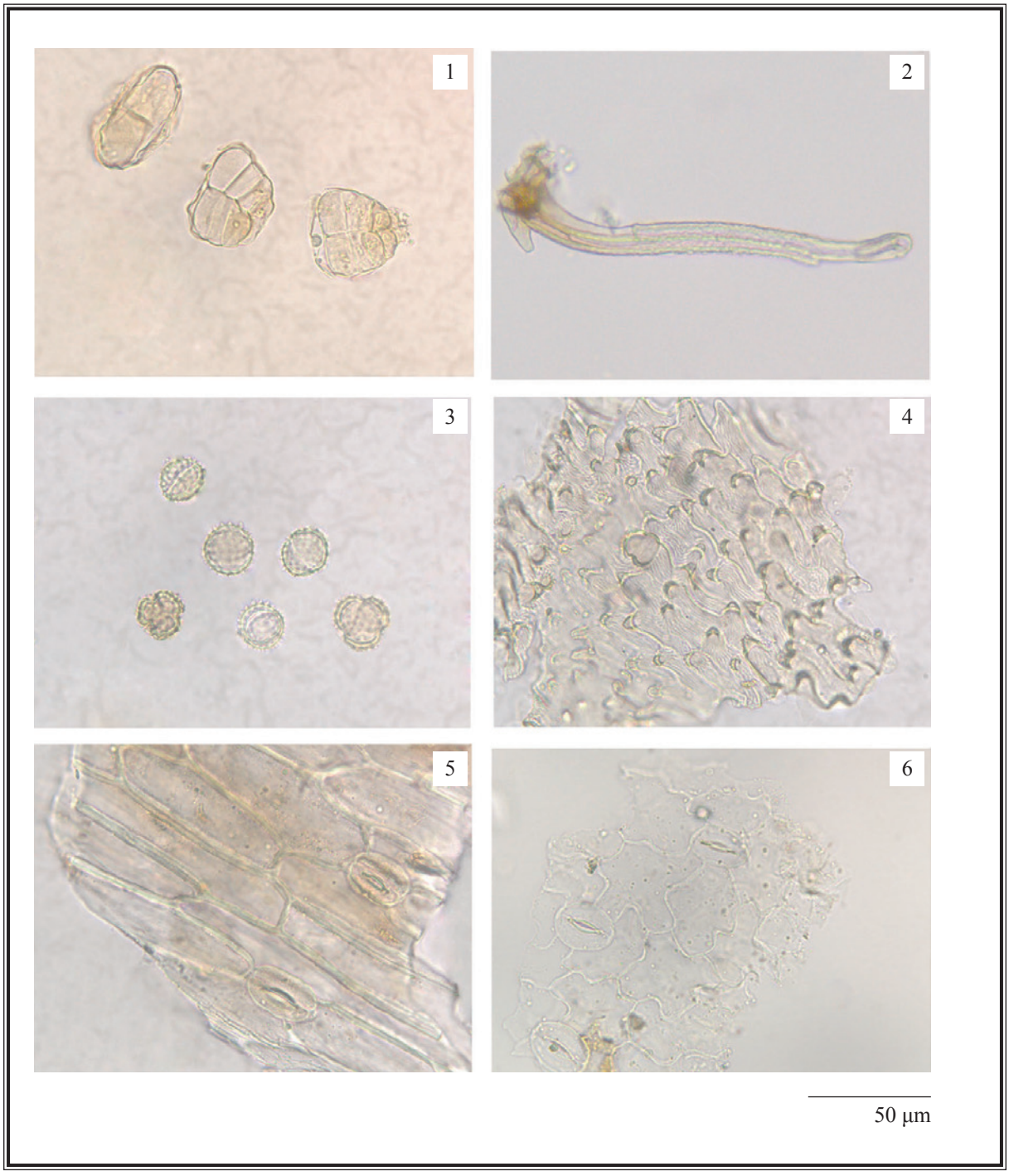

Figure 3 Microscopic features of powder of Centipedae Herba (under the light microscope)

1. Glandular hairs 2. Non-glandular hair 3. Pollen grains 4. Epidermal cells of corolla
5. Epidermal cells of stem with stomata 6. Epidermal cells of leaf with stomata

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

Standard solution

3,5-Dicaffeoylquinic acid standard solution

Weigh 1.0 mg of 3,5-dicaffeoylquinic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate, formic acid and water (5:5:2:0.1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (150 W) the mixture for 1 h. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately 3,5-dicaffeoylquinic acid standard solution (1 μL) and the test solution (10 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. 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. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

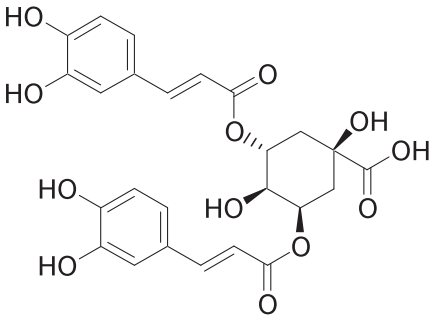

Figure 4 Chemical structure of 3,5-dicaffeoylquinic acid

Figure 5 A reference HPTLC chromatogram of Centipedae Herba extract observed under UV light (366 nm)

1. 3,5-Dicaffeoylquinic acid 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 3,5-dicaffeoylquinic acid (Fig. 5).

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

Standard solution

3,5-Dicaffeoylquinic acid standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of 3,5-dicaffeoylquinic acid CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethanol (50%). Sonicate (150 W) the mixture for 30 min. 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, 190 Å pore size and 12% carbon loading). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.08% Trifluoroacetic acid (%, v/v)	Elution
0 – 30	12 → 22	88 → 78	linear gradient
30 – 60	22 → 35	78 → 65	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of 3,5-dicaffeoylquinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of 3,5-dicaffeoylquinic acid should not be more than 5.0%; the RSD of the retention time of 3,5-dicaffeoylquinic acid peak should not be more than 2.0%; the column efficiency determined from 3,5-dicaffeoylquinic acid peak should not be less than 55000 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 3,5-dicaffeoylquinic acid Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention time of 3,5-dicaffeoylquinic acid peak in the chromatogram of 3,5-dicaffeoylquinic acid Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify 3,5-dicaffeoylquinic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 3,5-dicaffeoylquinic acid Std-FP. The retention times of 3,5-dicaffeoylquinic acid 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 six characteristic peaks of Centipedae Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Centipedae Herba extract

Peak No.	RRT	Acceptable Range
1	0.28	± 0.03
2	0.41	± 0.03
3 (marker, 3,5-dicaffeoylquinic acid)	1.00	-
4	1.17	± 0.03
5	1.34	± 0.03
6	1.73	± 0.04

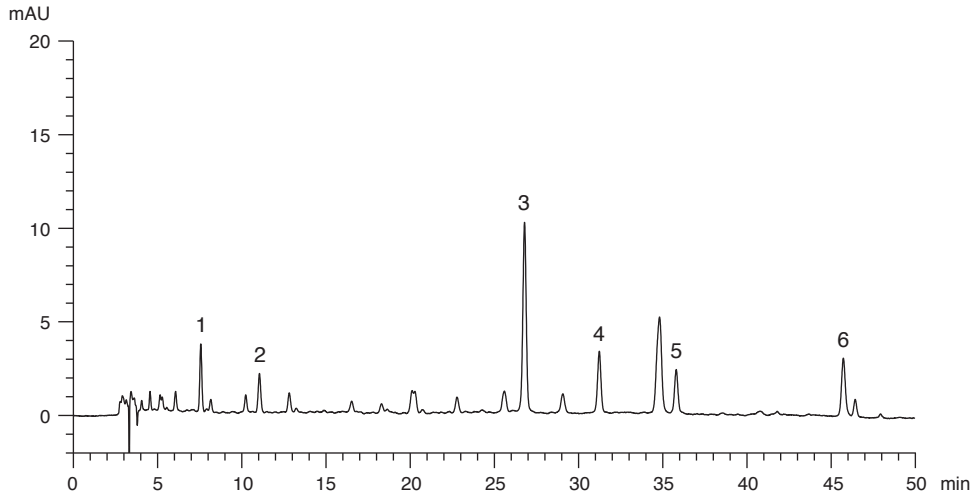

Figure 6 A reference fingerprint chromatogram of Centipedae Herba extract

For positive identification, the sample must give the above six 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 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 22.5%.
Acid-insoluble ash: not more than 11.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution

3,5-Dicaffeoylquinic acid standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of 3,5-dicaffeoylquinic acid CRS and dissolve in 10 mL of ethanol (50%).

3,5-Dicaffeoylquinic acid standard solution for assay, Std-AS

Measure accurately the volume of the 3,5-dicaffeoylquinic acid Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 0.5, 2.5, 10, 60, 120 mg/L for 3,5-dicaffeoylquinic acid.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 7 mL of ethanol (50%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). 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, 190 Å pore size and 12% carbon loading). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.08% trifluoroacetic acid and acetonitrile (82:18, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 5 μL of 3,5-dicaffeoylquinic acid Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 3,5-dicaffeoylquinic acid should not be more than 5.0%; the RSD of the retention time of 3,5-dicaffeoylquinic acid peak should not be more than 2.0%; the column efficiency determined from 3,5-dicaffeoylquinic acid peak should not be less than 8000 theoretical plates.

The *R* value between 3,5-dicaffeoylquinic acid 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 3,5-dicaffeoylquinic acid Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of 3,5-dicaffeoylquinic acid against the corresponding concentrations of 3,5-dicaffeoylquinic acid Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 μL of the test solution into the HPLC system and record the chromatogram. Identify 3,5-dicaffeoylquinic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 3,5-dicaffeoylquinic acid Std-AS. The retention times of 3,5-dicaffeoylquinic acid 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 3,5-dicaffeoylquinic acid in the test solution, and calculate the percentage content of 3,5-dicaffeoylquinic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.046% of 3,5-dicaffeoylquinic acid ($C_{25}H_{24}O_{12}$), calculated with reference to the dried substance.