Cirsii Herba

Figure 1 A photograph of Cirsii Herba

A. Cirsii Herba B. Leaves C. Capitulum
D. Magnified capitulum with flower

1. NAMES

Official Name: Cirsii Herba

Chinese Name: 小薊

Chinese Phonetic Name: Xiaoji

2. SOURCE

Cirsii Herba is the dried aerial part of *Cirsium setosum* (Willd.) MB. (Asteraceae). The aerial part is collected at flowering in summer and autumn, foreign matter removed, then dried under the sun to obtain Cirsii Herba.

3. DESCRIPTION

Stems cylindrical, sometimes branched on the upper part, 5-30 cm long, 2-5 mm in diameter. Externally greyish-green to purple, with longitudinal ridges and white pubescence; texture fragile, easily broken; fracture hollow. Leaves simple, alternate, sessile or short-petioled; lamina crumpled or broken, when intact flattened out, elongated-elliptic to oblong-lanceolate, 3-12 cm long, 5-30 mm wide; margins entire or serrulate to pinnatipartite, the tips of serrations spinescent; the upper surface greenish-brown and the lower surface greyish-green, covered with white pubescences on both surfaces. Capitulum solitary or several disposed on a loose terminal; involucres campanulate, bracts 5-8 layers, yellowish-green; flowers purplish-red. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of cells. Hypodermal collenchyma present at angular regions. Cortex consists of several to more than 10 layers of cells. Pericyclic fibre in bundles, slightly lignified. Phloem consists of several layers of small square parenchymatous cells. Cambium indistinct. Xylem vessels located in middle and lower parts of xylem. Pith usually hollow [Fig. 2 (i)].

Leaf: Both upper and lower epidermis consist of 1 layer of cells, non-glandular hairs easily fallen off during transverse section processing. Mesophyll mainly consists of spongy cells. Xylem fibres in bundles, located in upper side of vessels. Vascular bundle collateral. Phloem consists of several layers of small parenchymatous cells. Phloem fibres in bundles. Crystals of calcium oxalate scattered in leaf [Fig. 2 (ii)].

Powder

Colour brownish-green to dark green. Epidermal cells polygonal in surface view, anticlinal walls straight, stomata and non-glandular hairs occur on both surfaces, stomata anomocytic or anisocytic. Non-glandular hairs consist of 3-10 cells or more, apical cells slender whip-shaped, shrunken and twisted. Crystals of calcium oxalate scattered in mesophyll cells, 1-25 μm in diameter; blue under the polarized microscope. Pollen grains subglobular, 30-50 μm in diameter, with spiny exine (Fig. 3).

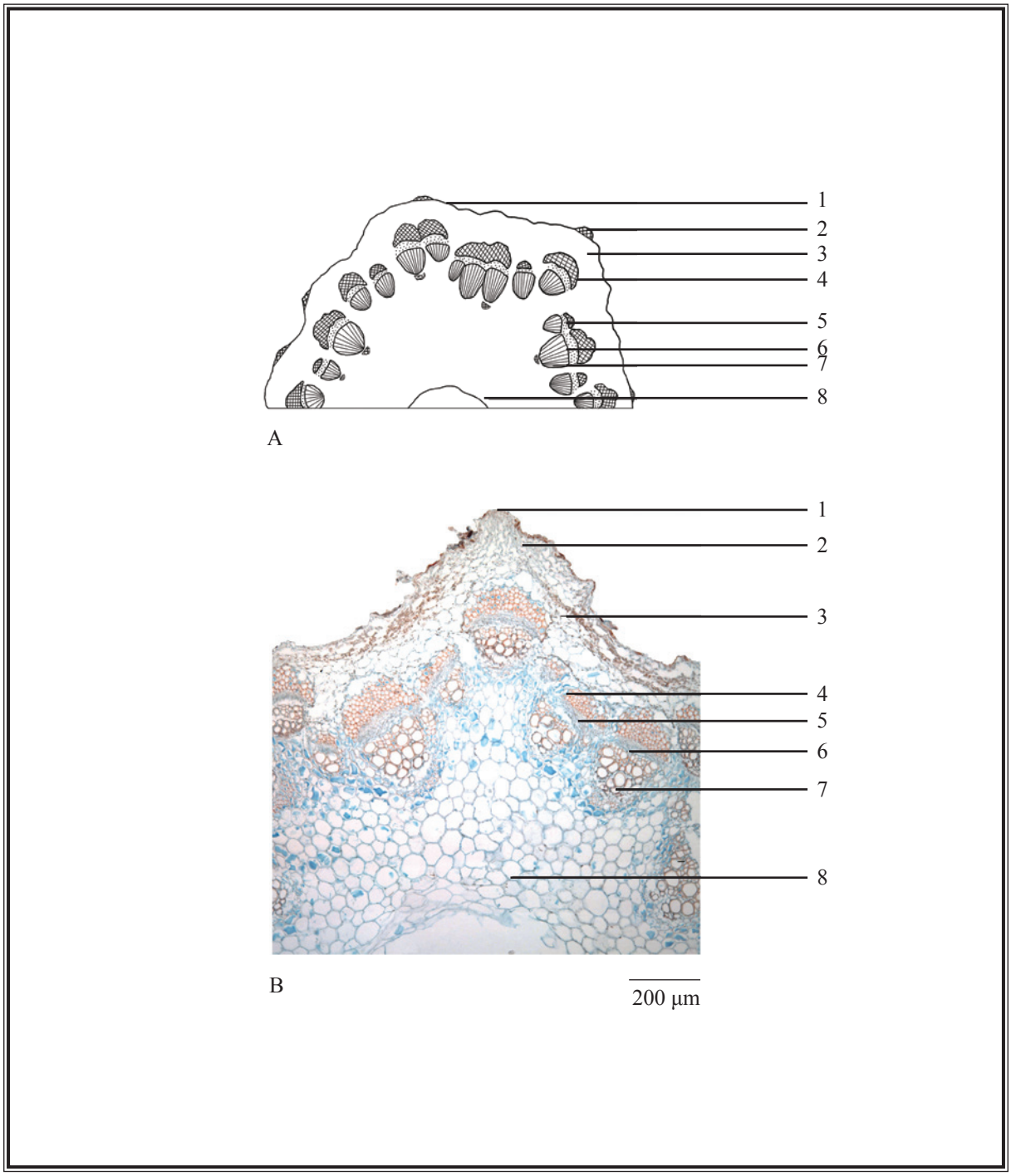

Figure 2 (i) Microscopic features of transverse section of stem of Cirsii Herba

A. Sketch B. Section illustration

1. Epidermis 2. Hypodermal collenchyma 3. Cortex 4. Pericyclic fibre
5. Phloem 6. Cambium 7. Xylem 8. Pith

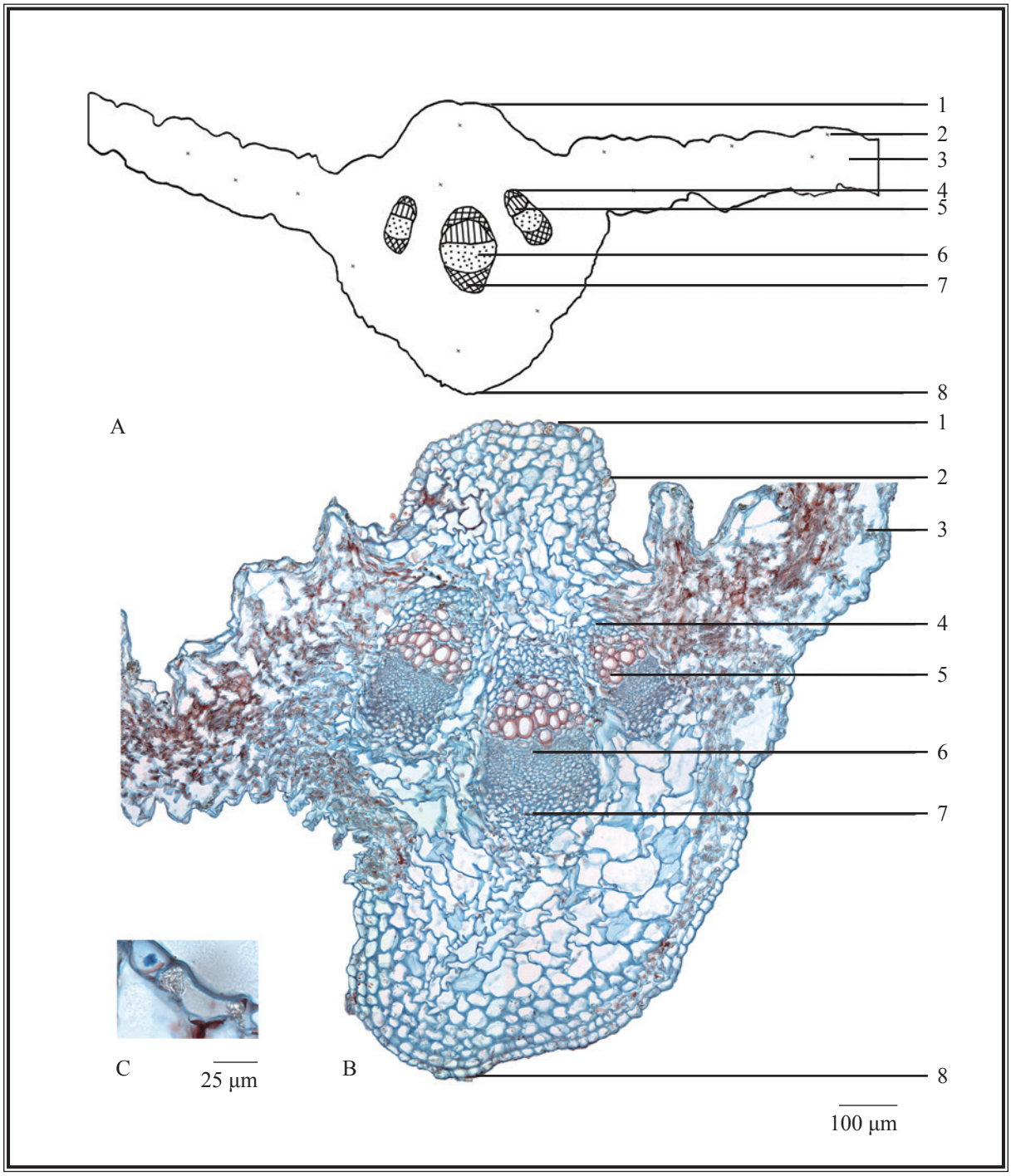

Figure 2 (ii) Microscopic features of transverse section of leaf of Cirsii Herba

A. Sketch B. Section illustration C. Crystals of calcium oxalate

1. Upper epidermis 2. Crystals of calcium oxalate 3. Mesophyll
4. Xylem fibres 5. Xylem 6. Phloem 7. Phloem fibres 8. Lower epidermis

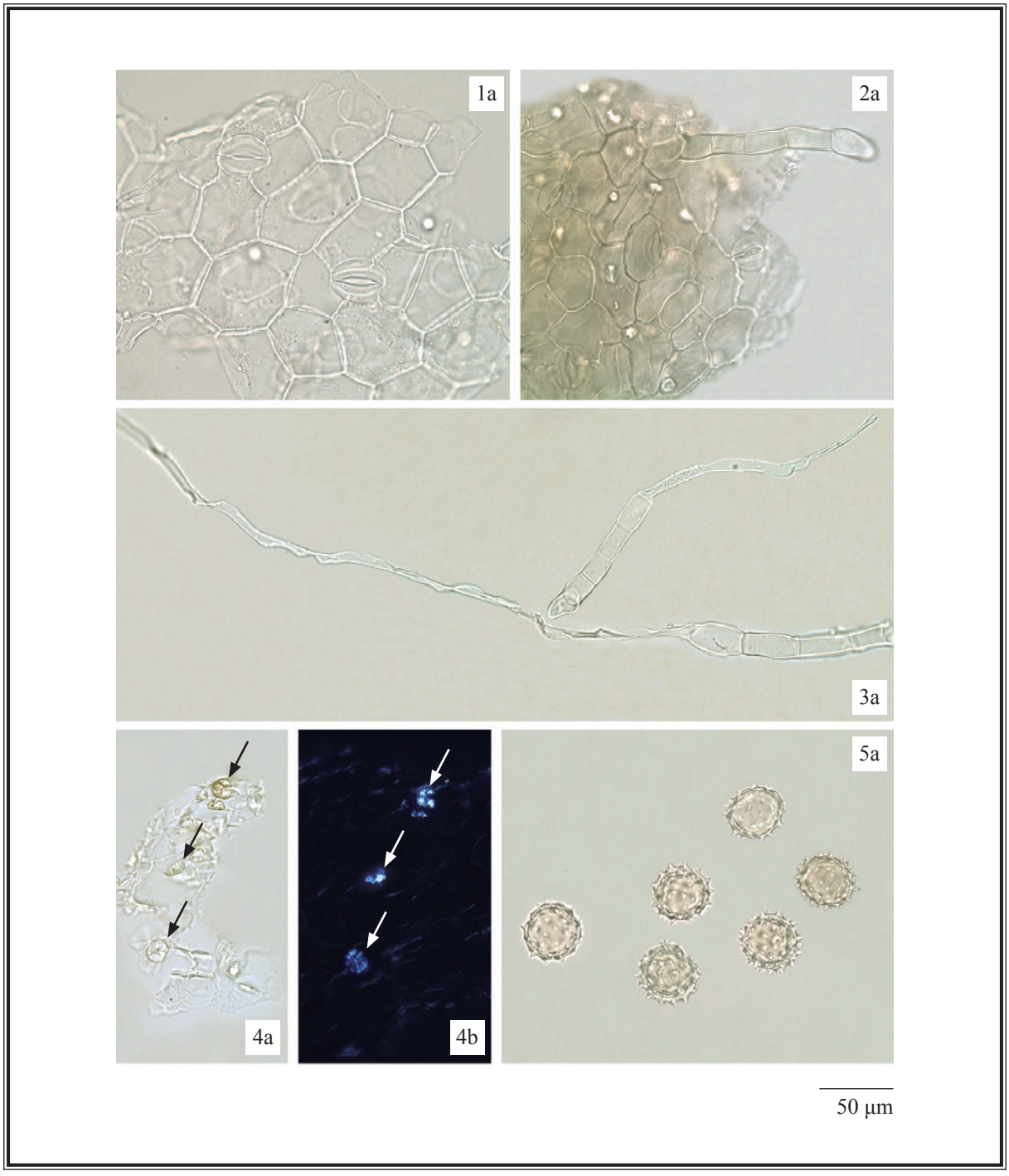

Figure 3 Microscopic features of powder of Cirsii Herba

1. Epidermal cells with stomata 2. Epidermal cells with non-glandular hair
3. Non-glandular hairs 4. Crystals of calcium oxalate 5. Pollen grains

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

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

Standard solution

Linarin (buddleoside) standard solution

Weigh 0.2 mg of linarin CRS (Fig. 4) and dissolve in 2 mL of ethanol. Place it in a water bath at about 90°C for 1 min.

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (8:1:1, v/v).

Spray reagent

Weigh 0.5 g of aluminium trichloride and dissolve in 50 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of ethanol. Sonicate (270 W) the mixture for 30 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 2 mL of ethanol and then filter.

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 linarin standard solution and the test solution (1 μL each) 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 4 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 UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV(A).

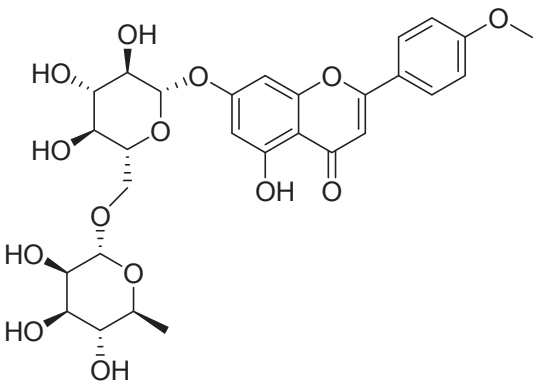

Figure 4 Chemical structure of linarin (buddleoside)

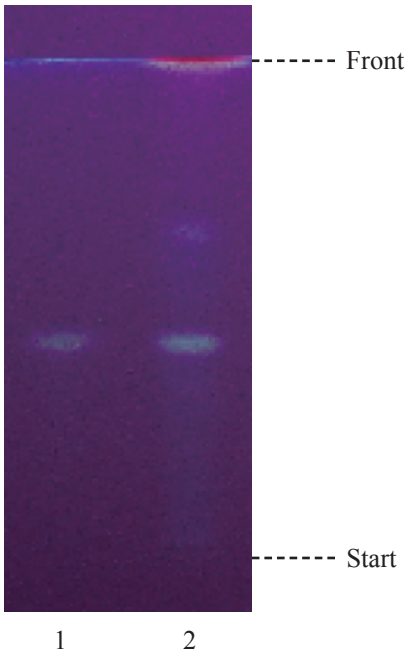

Figure 5 A reference HPTLC chromatogram of Cirsii Herba extract observed under UV light (366 nm) after staining

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. 5).

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

Standard solution

Linarin (buddleoside) standard solution for fingerprinting, Std-FP (160 mg/L)

Weigh 1.6 mg of linarin CRS and dissolve in 10 mL of ethanol (70%). Place it in a water bath at about 90°C for 1 min.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL round-bottomed flask, then add 20 mL of ethanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Transfer the solution to a 25-mL centrifuge tube. Centrifuge at about 2500 × *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 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)	Acetonitrile (%, v/v)	0.05% Trifluoroacetic acid (%, v/v)	Elution
0 – 10	13 → 24	87 → 76	linear gradient
10 – 38	24	76	isocratic
38 – 50	24 → 75	76 → 25	linear gradient
50 – 60	75	25	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 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 20000 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 linarin Std-FP and the test solution (5 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. 6) 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 Cirsii Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Cirsii Herba extract

Peak No.	RRT	Acceptable Range
1 (chlorogenic acid)	0.24	± 0.03
2	0.39	± 0.03
3 (marker, linarin)	1.00	-
4	1.45	± 0.03

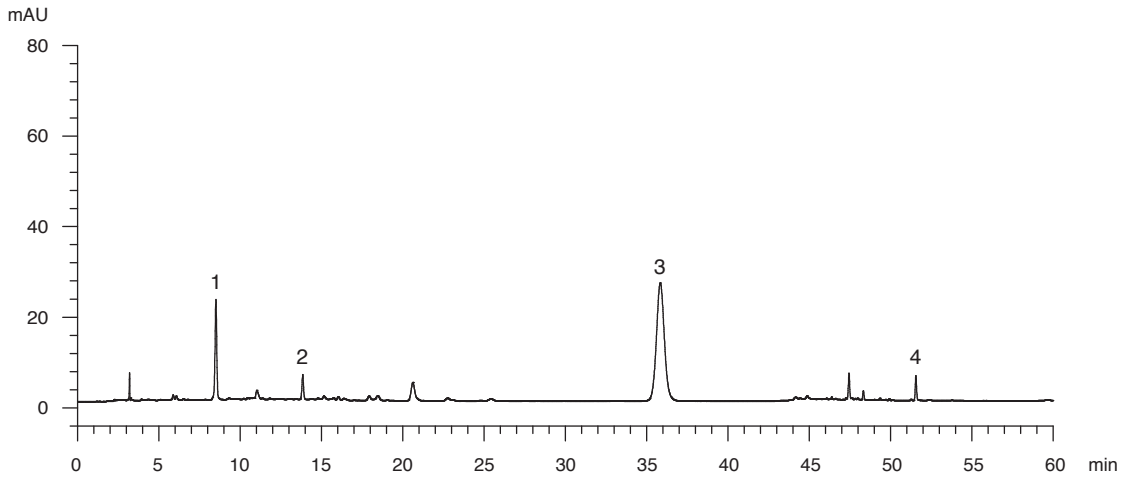

Figure 6 A reference fingerprint chromatogram of Cirsii Herba extract

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. 6).

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 19.5%.
Acid-insoluble ash: not more than 4.5%.

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 18.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Linarin (buddleoside) standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of linarin CRS and dissolve in 10 mL of methanol. Place it in a water bath at about 90°C for 1 min.

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 20, 60, 100, 140, 180 mg/L for linarin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants 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 (330 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 0.05% trifluoroacetic acid and acetonitrile (76:24, v/v). The elution time is about 35 min.

System suitability requirements

Perform at least five replicate injections, each using 5 μL of linarin Std-AS (100 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 10000 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 (5 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 5 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 0.82% of linarin ($C_{28}H_{32}O_{14}$), calculated with reference to the dried substance.