Cirsii Japonici Herba

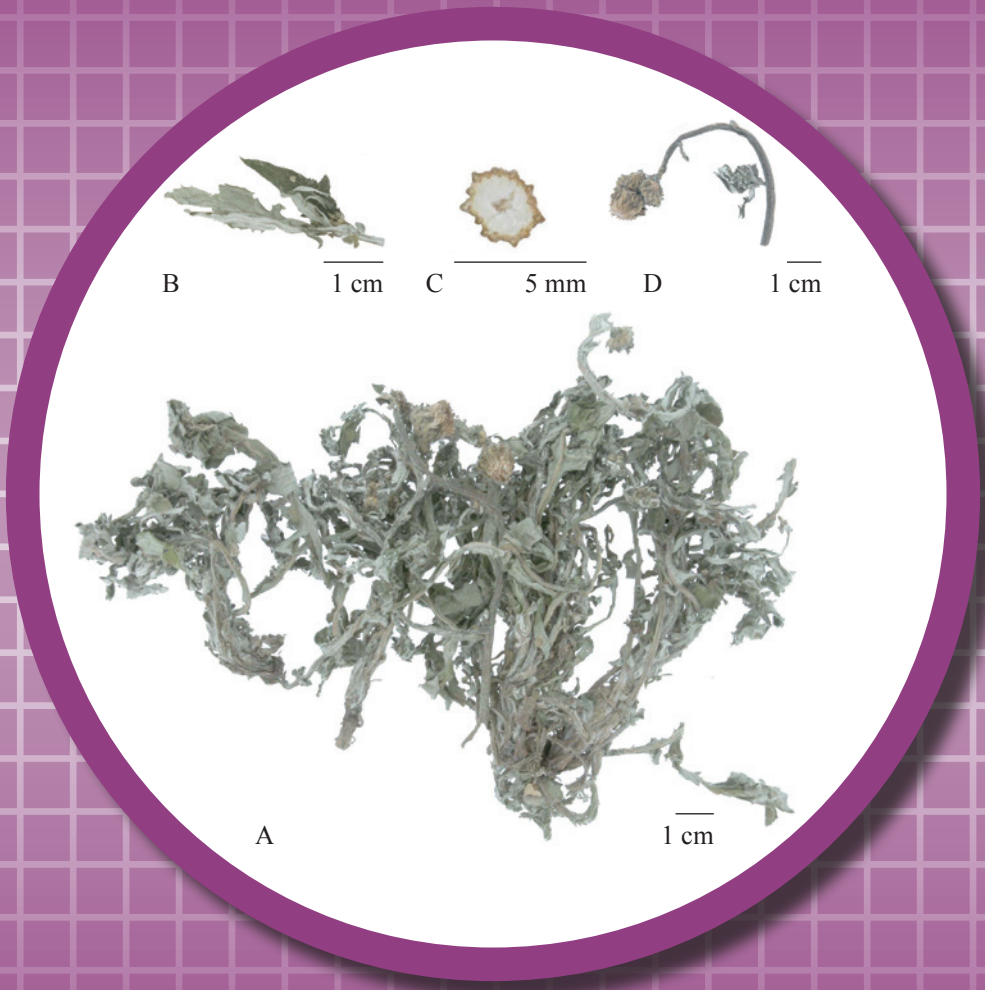

Figure 1 A photograph of Cirsii Japonici Herba

A. Cirsii Japonici Herba B. Magnified image of leaves
C. Magnified image of transverse section of stem D. Capitulum

1. NAMES

Official Name: Cirsii Japonici Herba

Chinese Name: 大薊

Chinese Phonetic Name: Daji

2. SOURCE

Cirsii Japonici Herba is the dried aerial part of *Cirsium japonicum* Fisch. ex DC. (Asteraceae). The aerial part is collected in summer and autumn at flowering, foreign matter removed, then dried under the sun to obtain Cirsii Japonici Herba.

3. DESCRIPTION

Stems cylindrical, base up to 12 mm in diameter; externally greyish-green to greenish-brown, with several longitudinal ridges and filamentous hairs; fracture greyish-white, pith lax or hollowed. Leaves crumpled, mostly broken, when intact flattened out, oblanceolate or obovate-elliptical, pinnatipartite, margin with unequal spines; the upper surface greyish-green or yellowish-brown and lower surface lighter in colour, with greyish-white filamentous hairs on both surfaces. Capitulum disposed on terminal, globose or elliptical, involucre campaniform, bract imbricate; feathery pappus greyish-white. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of cells, usually shrunken. Non-glandular hairs easily fallen off during transverse section processing. Cortical parenchymatous cells prolonged tangentially. Hypodermal collenchyma consists of 3-11 layers of cells, relatively distinct at prominent regions. Cambium indistinct. Vascular bundle collateral. Phloem fibres, vessels and xylem fibers slightly lignified. Pith usually hollow in the centre [Fig. 2 (i)].

Leaf: Blade cells usually shrunken. Both upper and lower epidermis consist of 1 layer of cells, non-glandular hairs easily fallen off during transverse section processing. Lower part of leave

deep wave-shaped, especially protrude near midrib. Hypodermal collenchyma consists of 2-9 layers of cells, relatively distinct at prominent regions. Vascular bundle collateral. Phloem fibres and xylem fibres in bundles, slightly lignified. Crystals of calcium oxalate aggregated in fan-shaped, rounded or irregular, scattered in leaf [Fig. 2 (ii)].

Powder

Colour brownish-green to dark green. Non-glandular hairs consist of 4-18 cells, mostly broken, with a slender whip-shaped apical cell, bent or twisted, 3-7 μm in diameter. Lower epidermal cells subrectangular in surface view, with sinuous anticlinal walls. Stomata anomocytic or anisocytic, subsidiary cells 3-5. Crystals of calcium oxalate aggregated in fan-shaped, rounded or irregular in shape; bluish-white under the polarized microscope (Fig. 3).

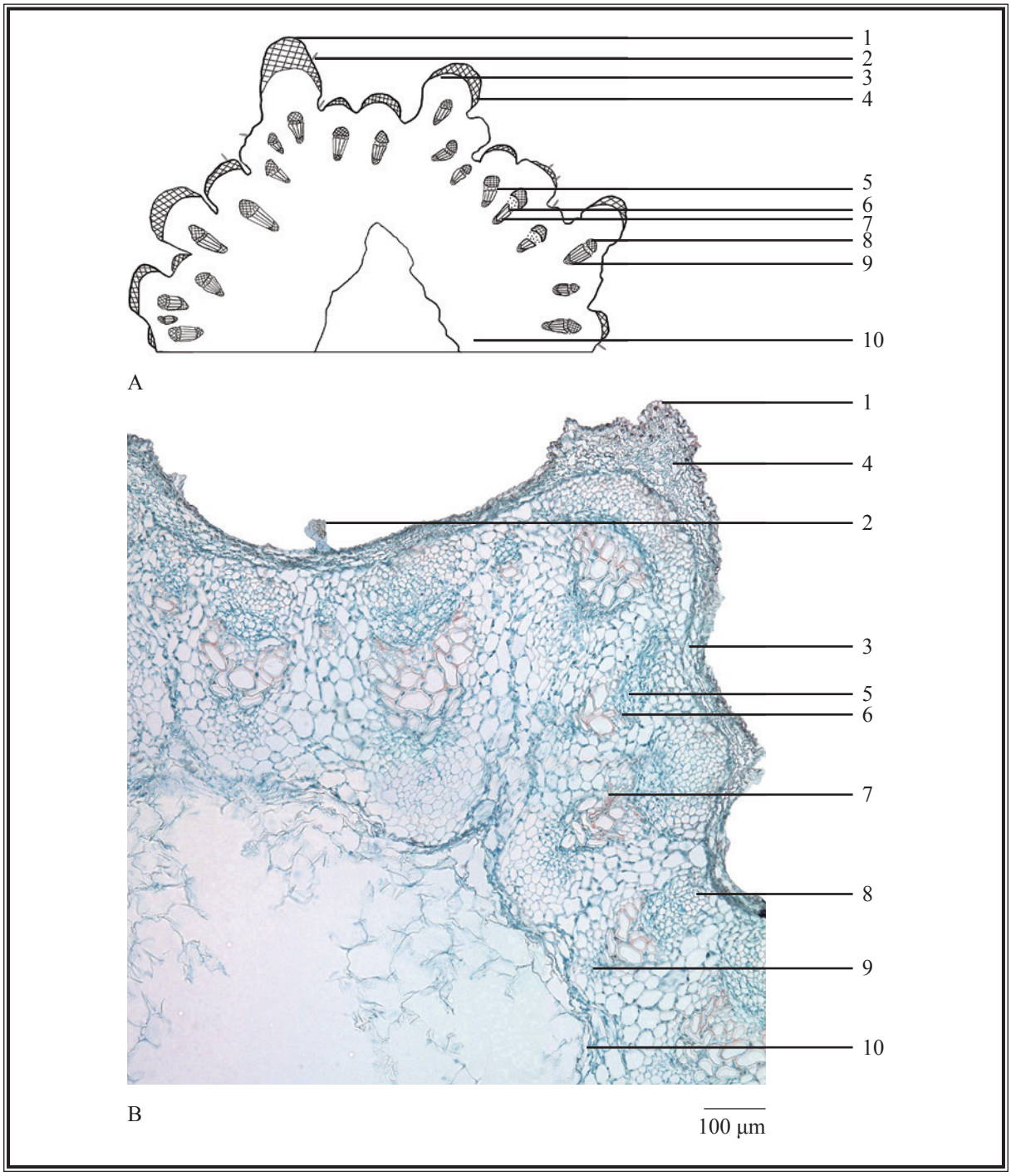

Figure 2 (i) Microscopic features of transverse section of stem of Cirsii Japonici Herba

A. Sketch B. Section illustration

1. Epidermis 2. Non-glandular hair 3. Cortex 4. Hypodermal collenchyma 5. Phloem 6. Cambium 7. Xylem 8. Phloem fibre 9. Xylem fibre 10. Pith

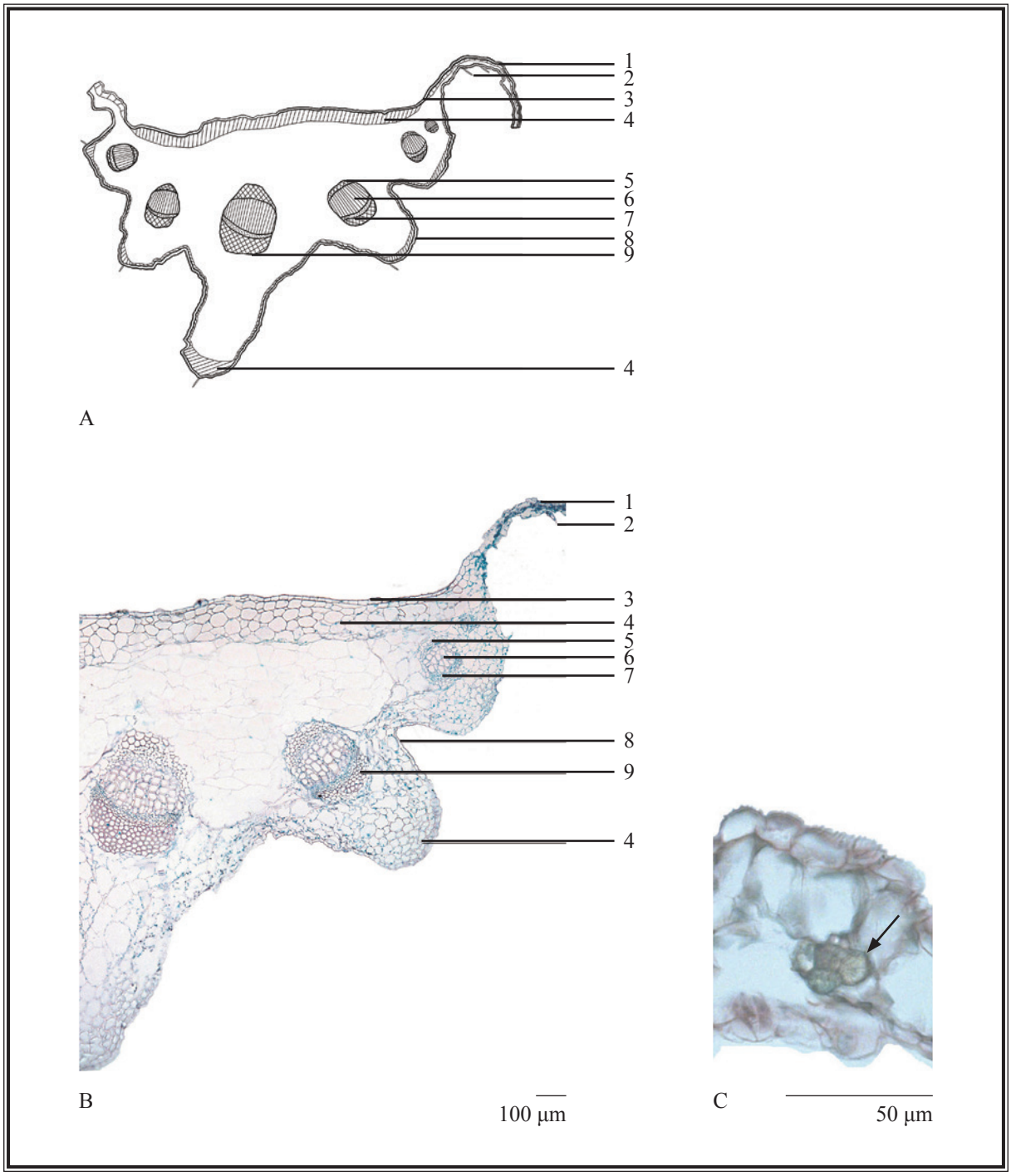

Figure 2 (ii) Microscopic features of transverse section of leaf of Cirsii Japonici Herba

A. Sketch B. Section illustration of leaf (midrib with blade) C. Crystals of calcium oxalate

1. Blade 2. Non-glandular hair 3. Upper epidermis 4. Hypodermal collenchyma 5. Xylem fibres 6. Xylem 7. Phloem 8. Lower epidermis 9. Phloem fibres

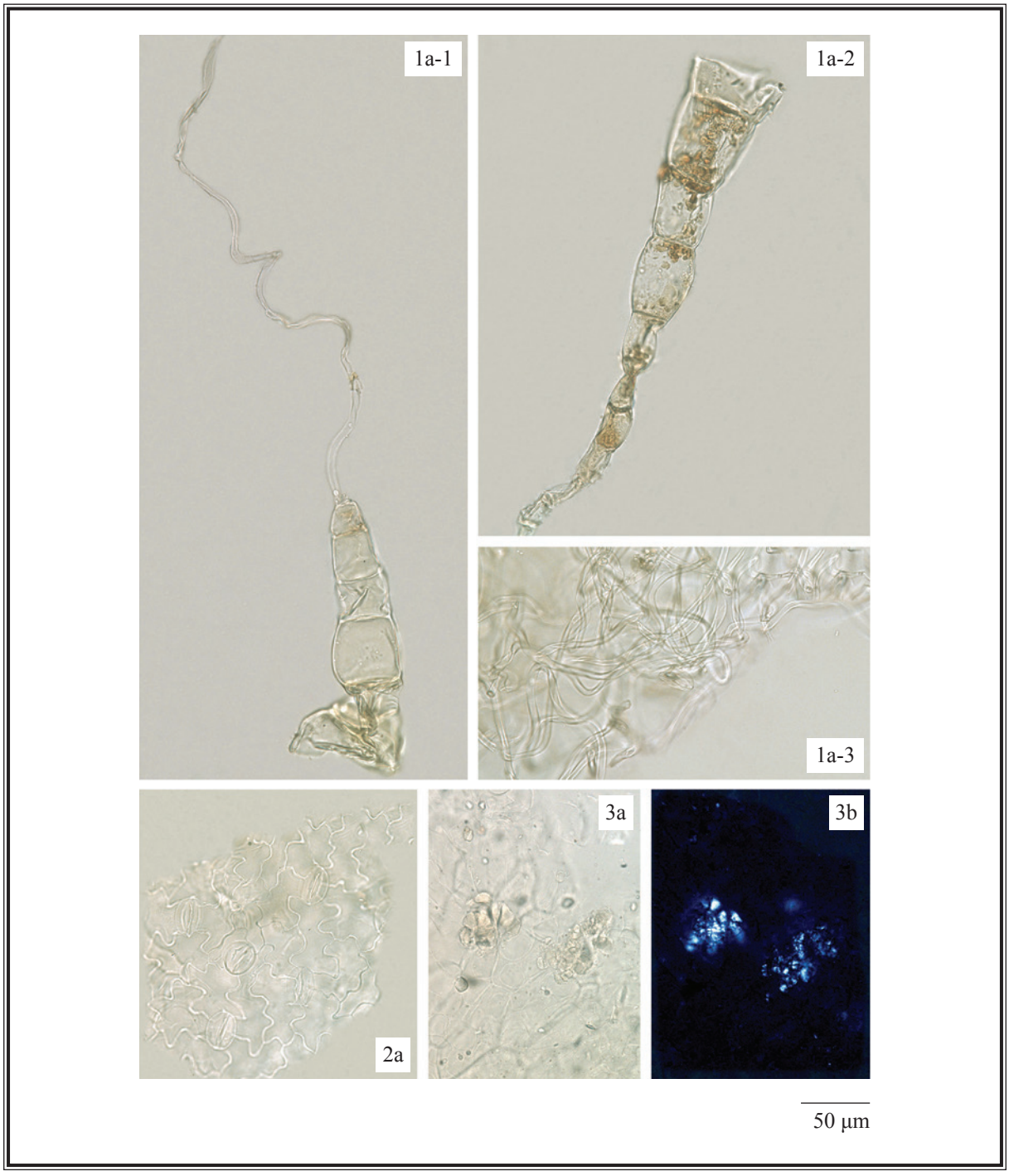

Figure 3 Microscopic features of powder of Cirsii Japonici Herba

1. Non-glandular hair (1-1 non-glandular hair, 1-2 base, 1-3 apex)
2. Lower epidermal cells with stomata 3. Crystals of calcium oxalate

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 90ºC 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 0.5 mL of ethanol.

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).

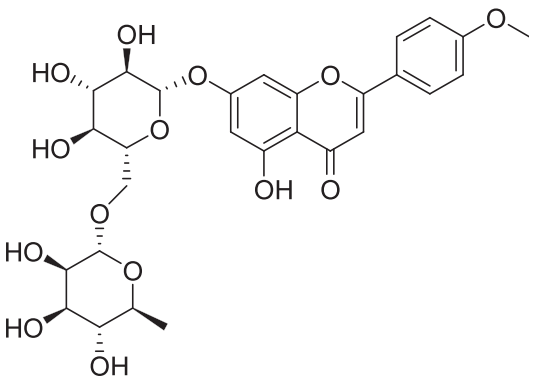

Figure 4 Chemical structure of linarin (buddleoside)

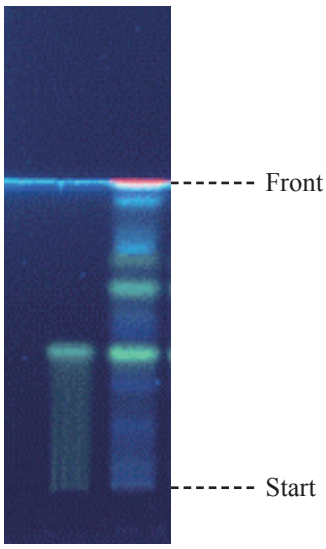

Figure 5 A reference HPTLC chromatogram of Cirsii Japonici Herba extract observed under UV light (366 nm) after staining

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 90ºC 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 18000 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. 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 three 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 three characteristic peaks of Cirsii Japonici Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (chlorogenic acid)	0.24	± 0.03
2 (marker, linarin)	1.00	-
3	1.48	± 0.03

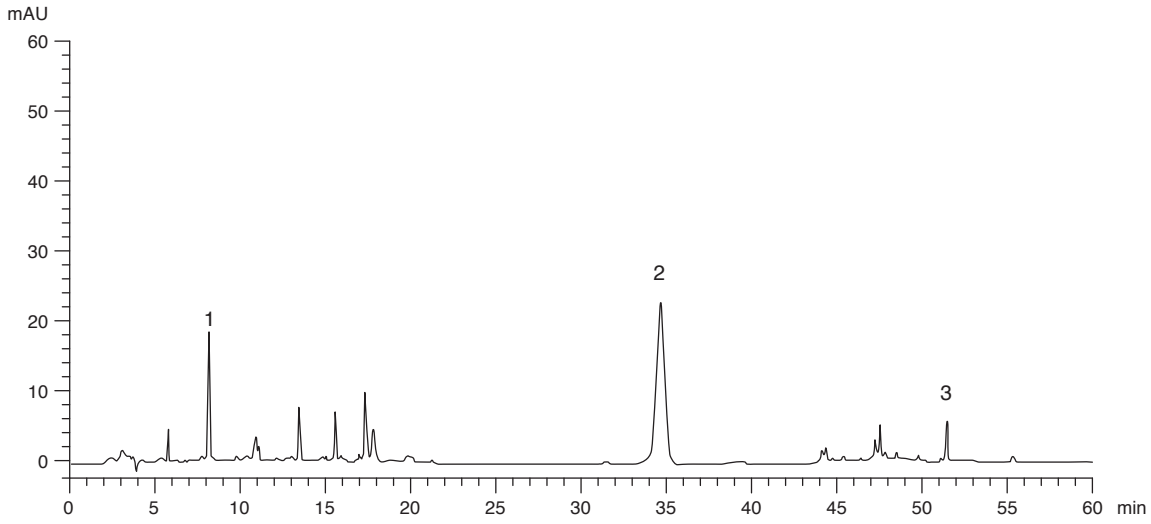

Figure 6 A reference fingerprint chromatogram of Cirsii Japonici Herba extract

For positive identification, the sample must give the above three 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 17.0%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 19.0%.
Ethanol-soluble extractives (hot extraction method): not less than 19.0%.

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 90ºC 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 4, 12, 20, 60, 100 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 (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 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 0.31% of linarin ($C_{28}H_{32}O_{14}$), calculated with reference to the dried substance.