Lobeliae Chinensis Herba

Figure 1 A photograph of Lobeliae Chinensis Herba

A. Lobeliae Chinensis Herba

B. Leaves and flower on an intact stem

1. NAMES

Official Name: Lobeliae Chinensis Herba

Chinese Name: 半邊蓮

Chinese Phonetic Name: Banbianlian

2. SOURCE

Lobeliae Chinensis Herba is the dried herb of *Lobelia chinensis* Lour. (Campanulaceae). The herb is collected in the summer, washed clean, then dried under the sun to obtain Lobeliae Chinensis Herba.

3. DESCRIPTION

Usually wound up into masses. Rhizomes short, cylindrical, 1-2 mm in diameter, externally pale brown or pale brownish-yellow, longitudinally fine. Roots slender, yellow, bearing lateral rootlets. Stem slender, branched, greyish-green, with obvious nodes, some bearing fibrous roots. Leaves alternate, greenish-brown, sessile, lamina mostly crumpled, when whole, narrowly lanceolate to long-ovate, 0.8-3.2 cm long, 0.2-0.5 cm wide. Pedicels slender, flower small, solitary and axillary, corolla tubular at the base, 5-lobed on the upper part, oblique on one side. Odour slight, taste slightly sweet and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification

Transverse section

Root: Epidermis consists of 1 layer of cells, covered with cuticle. Cortex broad, parenchymatous cells irregular. Endodermis distinct, cells rectangular or square, casparian strips visible. Vascular bundle small. Pericycle consists of 1 layer of parenchymatous cells. Laticiferous tubes scattered in the phloem. Phloem bundles arranged alternatively with xylem bundles. Xylem bundles 3-5 [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of cells, covered with cuticle, cells square or subsquare walls thickened. Cortex broad, parenchymatous cells polygonal and sparsely arranged. Crystals of calcium oxalate occasionally found in the cortex. Endodermis distinct, cells rectangular or square, casparian strips visible. Vascular bundle small. Phloem arranged in a ring, scattered with laticiferous tubes. Xylem radially arranged. Pith located in the central [Fig. 2 (ii)].

Leaf: Upper and lower epidermis each consists of 1 layer of subsquare cells, tangentially elongated, covered with cuticle. Palisade tissue consists of 1 layer of cells, across the upper part of the midrib. Spongy tissue cells subrounded. Vascular bundle small, collateral, irregular in shape. Unicellular non-glandular hairs occasionally arise from the lower epidermis [Fig. 2 (iii)].

Powder

Colour greenish-yellow to brownish-yellow. Epidermal cells of leaf with sinuous anticlinal walls. Epidermal cells of stem irregularly rectangular. Stomata found in the surface of leaf and stem, anomocytic, subsidiary cells 3-7. Laticiferous tubes occur alongside vessels, containing granular and oily contents. Parenchymatous cells contain inulin, inulin fan-shaped or irregular in shape; green under the polarized microscope. Crystals of calcium oxalate frequently occur beside vessels, sometimes arranged in a row; polychromatic under the polarized microscope. Spiral vessels and reticulate vessels visible, 6-67 μm in diameter. Non-glandular hairs occasionally visible, occurring in two types: first type slender, consisting of 1-3 cells, walls slightly thickened; second type short conical, unicellular, with warty walls on the surface (Fig. 3).

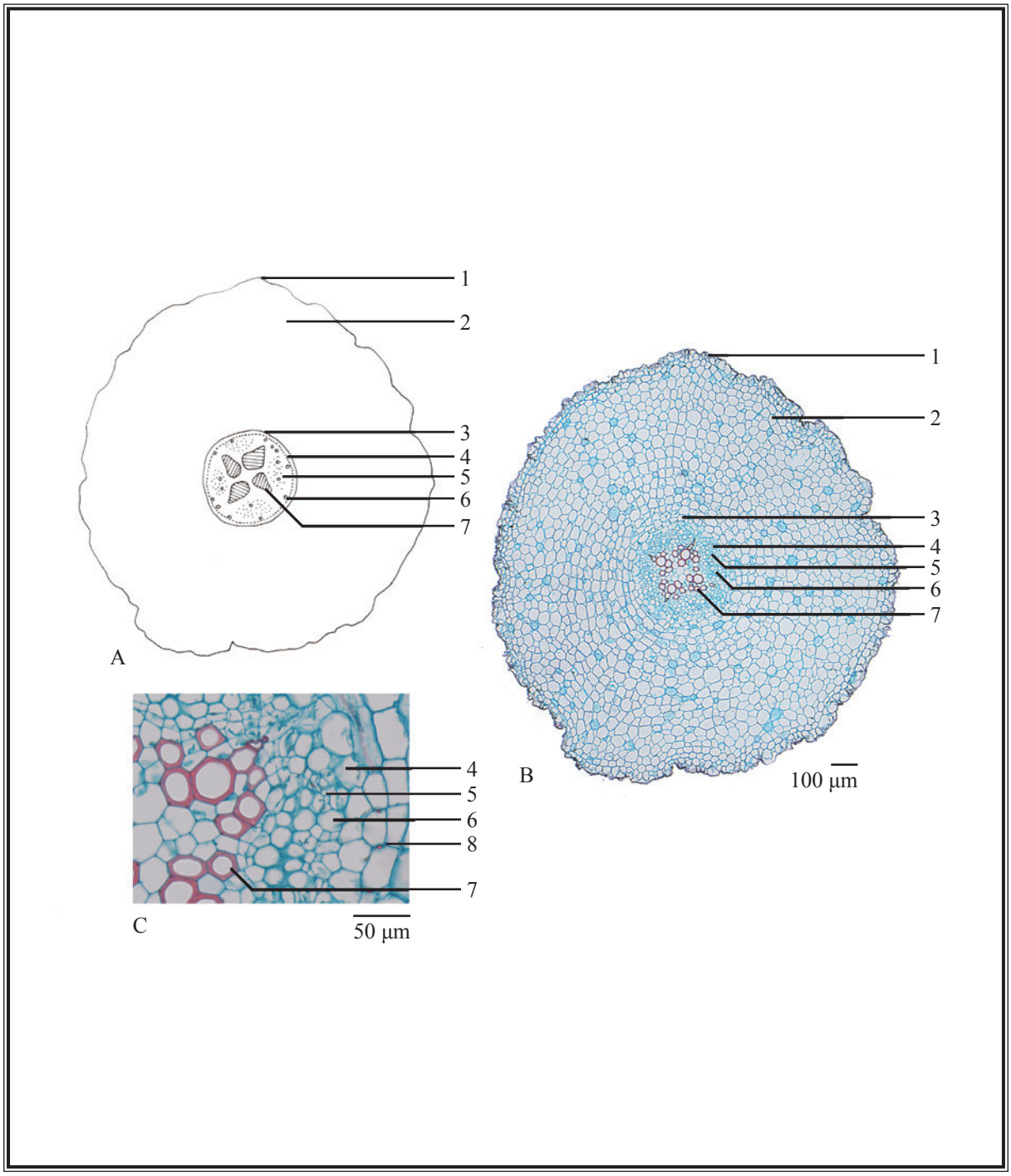

Figure 2 (i) Microscopic features of the transverse section of root of Lobeliae Chinensis Herba

A. Sketch B. Section illustration C. Section magnified

1. Epidermis 2. Cortex 3. Endodermis 4. Pericycle 5. Phloem
6. Laticiferous tube 7. Xylem 8. Casparian strip

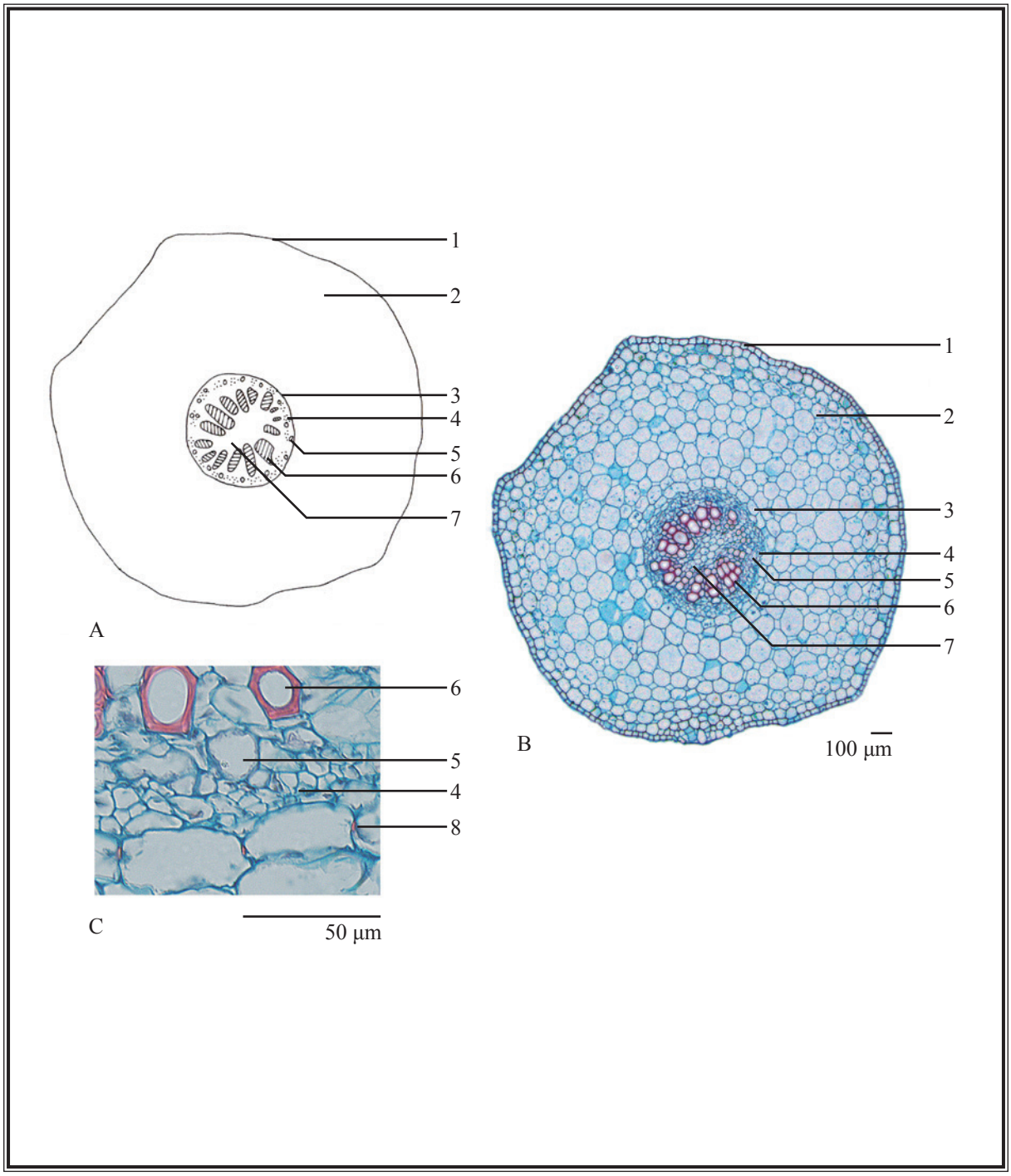

Figure 2 (ii) Microscopic features of the transverse section of stem of Lobeliae Chinensis Herba

A. Sketch B. Section illustration C. Section magnified

1. Epidermis 2. Cortex 3. Endodermis 4. Phloem 5. Laticiferous tube
6. Xylem 7. Pith 8. Casparian strip

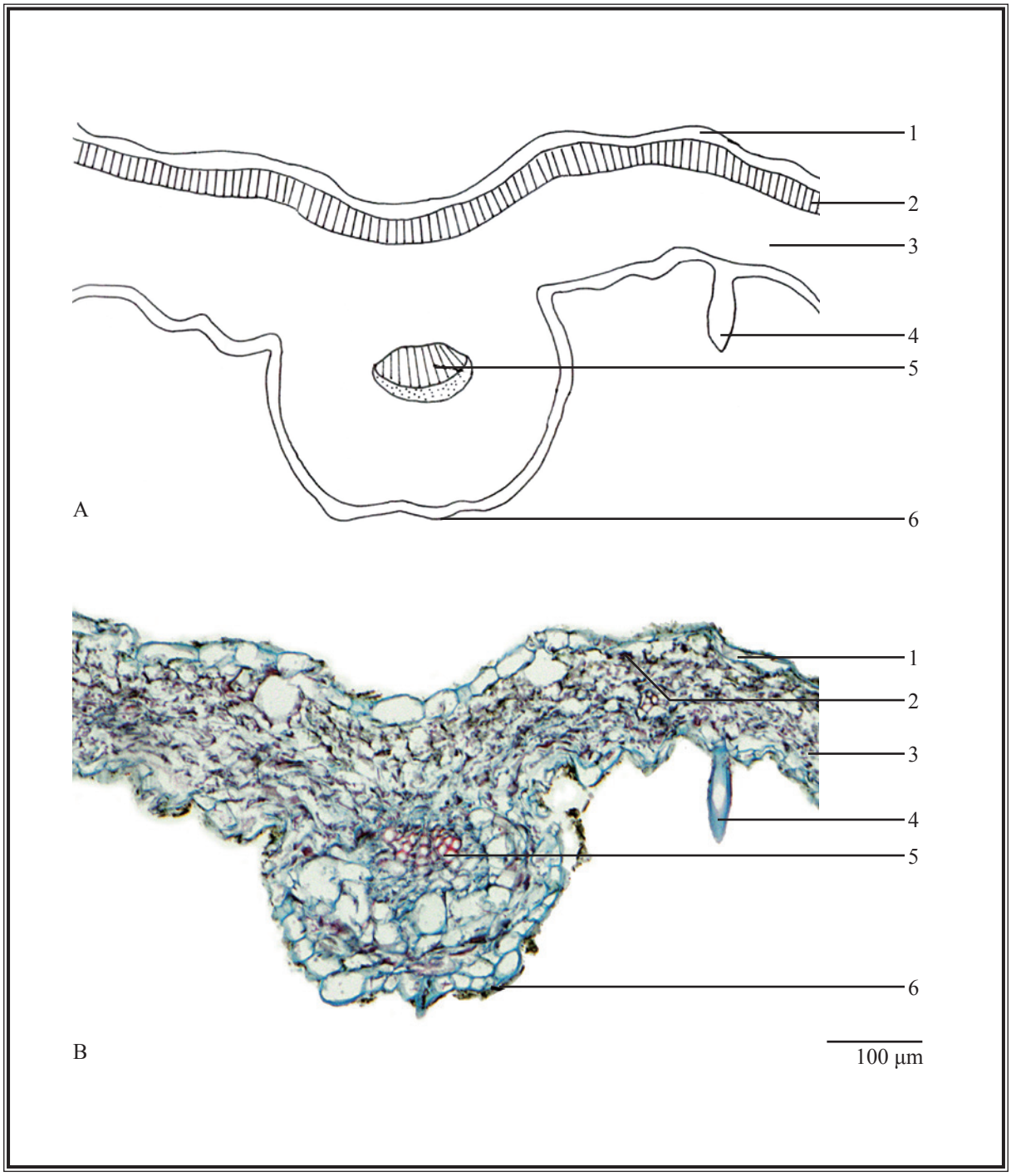

Figure 2 (iii) Microscopic features of the transverse section of leaf of Lobeliae Chinensis Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Unicellular non-glandular hair
5. Vascular bundle 6. Lower epidermis

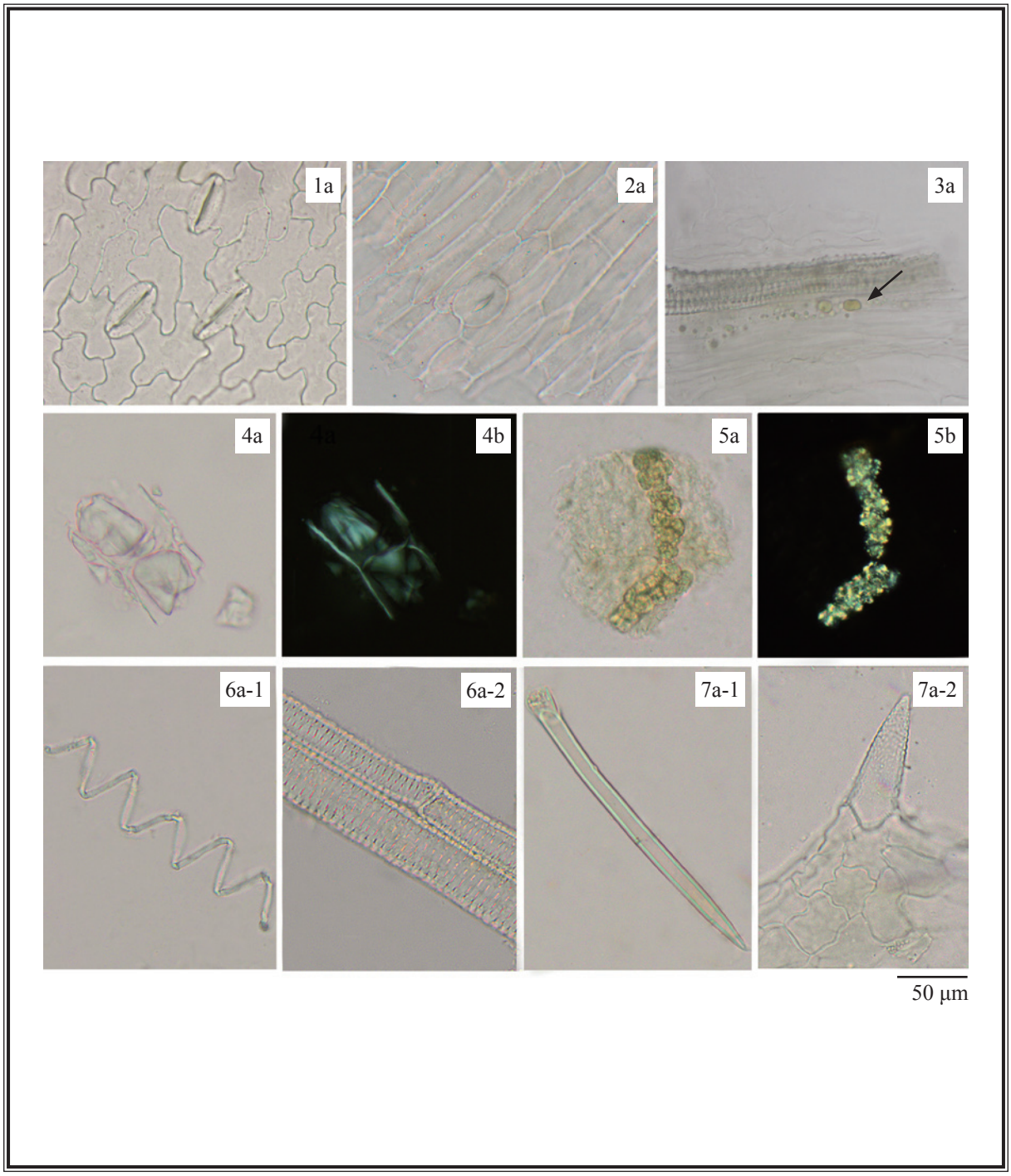

Figure 3 Microscopic features of powder of Lobeilae Chinensis Herba

1. Epidermal cells and stomata of leaf 2. Epidermal cells and stoma of stem
3. Laticiferous tube (⟶) 4. Inulins 5. Crystals of calcium oxalate
6. Vessels (6-1 spiral vessel, 6-2 reticulate vessels)
7. Non-glandular hairs (7-1 slender shape, 7-2 short conical)

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

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

Standard solutions

Diosmin standard solution

Weigh 2.0 mg of diosmin CRS (Fig. 4) and dissolve in 0.1 mL of dimethyl sulphoxide. Pipette 0.02 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol.

Linarin (buddleoside) standard solution

Weigh 8.0 mg of linarin CRS (Fig. 4) and dissolve in 0.1 mL of dimethyl sulphoxide. Pipette 0.01 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol.

Developing solvent system

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

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of methanol. Sonicate (350 W) the mixture for 15 min. Centrifuge at about 6000 × *g* for 5 min. Filter through a 0.45-μm nylon 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 diosmin standard solution (3 μL), linarin standard solution (1 μL) and the test solution (5 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 7.5 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 heat at about 105ºC (about 3 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

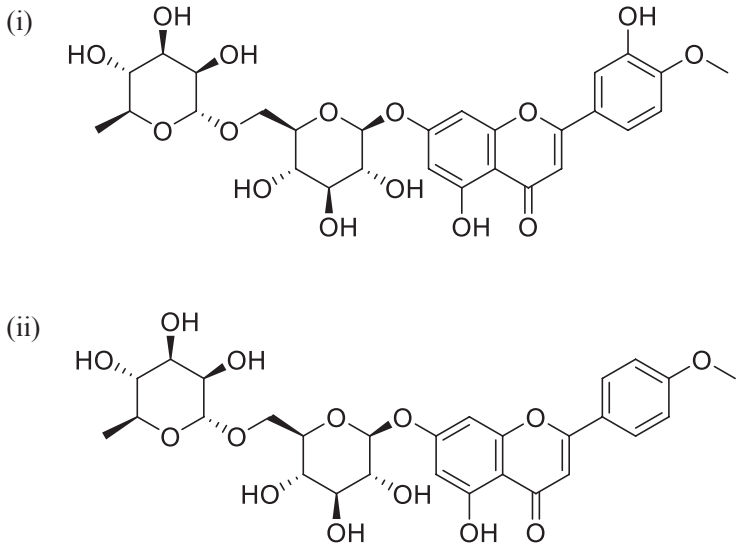

Figure 4 Chemical structures of (i) diosmin and (ii) linarin (buddleoside)

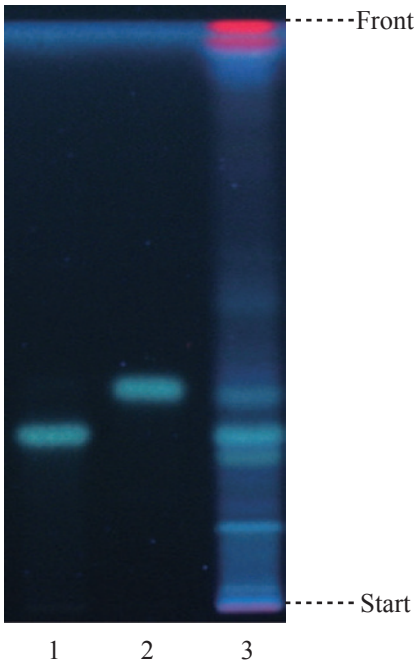

Figure 5 A reference HPTLC chromatogram of Lobeliae Chinensis Herba extract observed under UV light (366 nm) after staining

1. Diosmin standard solution 2. Linarin standard solution
3. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of diosmin and linarin (Fig. 5).

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

Standard solutions

Diosmin standard solution for fingerprinting, Std-FP (80 mg/L)

Weigh 8.0 mg of diosmin CRS and dissolve in 10 mL of dimethyl sulphoxide. Pipette 0.2 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol.

Linarin (buddleoside) standard solution for fingerprinting, Std-FP (18 mg/L)

Weigh 1.8 mg of linarin CRS and dissolve in 10 mL of dimethyl sulphoxide. Pipette 0.2 mL of the solution to a 2-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of methanol. Sonicate (350 W) the mixture for 15 min. Centrifuge at about 6000 × *g* for 5 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (267 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	83	17	isocratic
35 – 60	83 → 70	17 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of diosmin Std-FP and linarin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of diosmin and linarin should not be more than 5.0%; the RSD of the retention times of diosmin and linarin peaks should not be more than 2.0%; the column efficiencies determined from diosmin and linarin peaks should not be less than 10000 and 300000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and 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. 6).

Procedure

Separately inject diosmin Std-FP, linarin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of diosmin and linarin peaks in the chromatograms of diosmin Std-FP, linarin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify diosmin and linarin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of diosmin Std-FP and linarin Std-FP. The retention times of diosmin and linarin peaks in the chromatograms of the test solution and the corresponding Std-FP 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 Lobeliae Chinensis Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.38	± 0.03
2 (diosmin)	0.60	± 0.07
3	0.71	± 0.04
4 (marker, linarin)	1.00	-

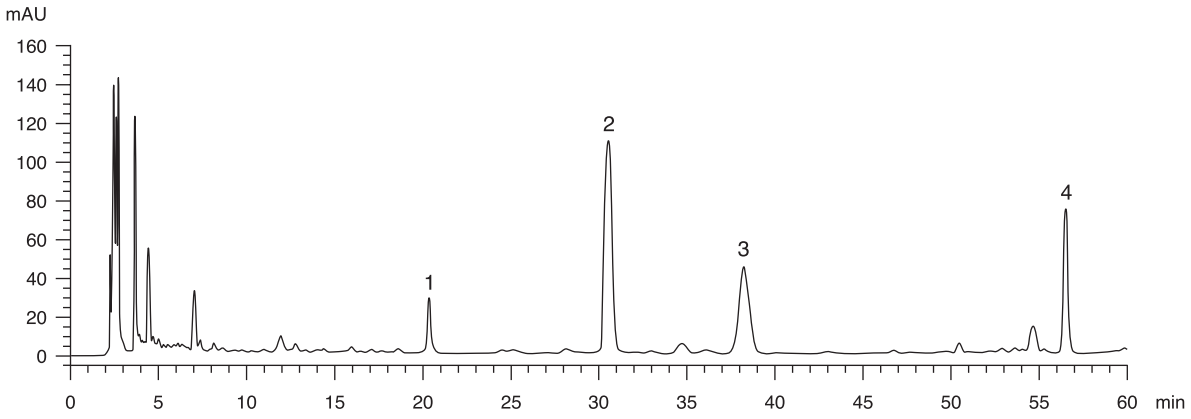

Figure 6 A reference fingerprint chromatogram of Lobeliae Chinensis Herba extract

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)*: The CMM shall meet the requirements for arsenic and mercury as specified in Appendix V. For cadmium and lead, Lobeliae Chinensis Herba should meet the specified limits of not more than 3.0 mg/kg and 12.0 mg/kg, respectively, when the CMM will be processed as a decoction in the final consumption form; otherwise, the limits for cadmium and lead specified in Appendix V shall be applied.

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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 14.0%.
Acid-insoluble ash: not more than 6.0%.

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 40.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

Mixed diosmin and linarin (buddleoside) standard stock solution, Std-Stock (540 mg/L for diosmin and 520 mg/L for linarin)

Weigh accurately 5.4 mg of diosmin CRS and 5.2 mg of linarin CRS, and dissolve in 10 mL of dimethyl sulphoxide.

Mixed diosmin and linarin standard solution for assay, Std-AS

Measure accurately the volume of the mixed diosmin and linarin Std-Stock, dilute with methanol to produce a series of solutions of 4, 8, 16, 32.5, 65 mg/L for diosmin and 1, 2, 4, 8, 16 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 5 mL of dimethyl sulphoxide. Sonicate (350 W) the mixture for 15 min. Centrifuge at about 6000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	80	20	isocratic
15 – 30	80 → 70	20 → 30	linear gradient
30 – 34	70	30	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed diosmin and linarin Std-AS (16 mg/L for diosmin and 4 mg/L for linarin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of diosmin and linarin should not be more than 5.0%; the RSD of the retention times of diosmin and linarin peaks should not be more than 2.0%; the column efficiencies determined from diosmin and linarin peaks should not be less than 8000 and 100000 theoretical plates respectively.

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

Calibration curves

Inject a series of the mixed diosmin and linarin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of diosmin and linarin against the corresponding concentrations of the mixed diosmin and linarin Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify diosmin and linarin peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed diosmin and linarin Std-AS. The retention times of diosmin and linarin peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of diosmin and linarin in the test solution, and calculate the percentage contents of diosmin and linarin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.31% of the total content of diosmin ($C_{28}H_{32}O_{15}$) and linarin ($C_{28}H_{32}O_{14}$), calculated with reference to the dried substance.

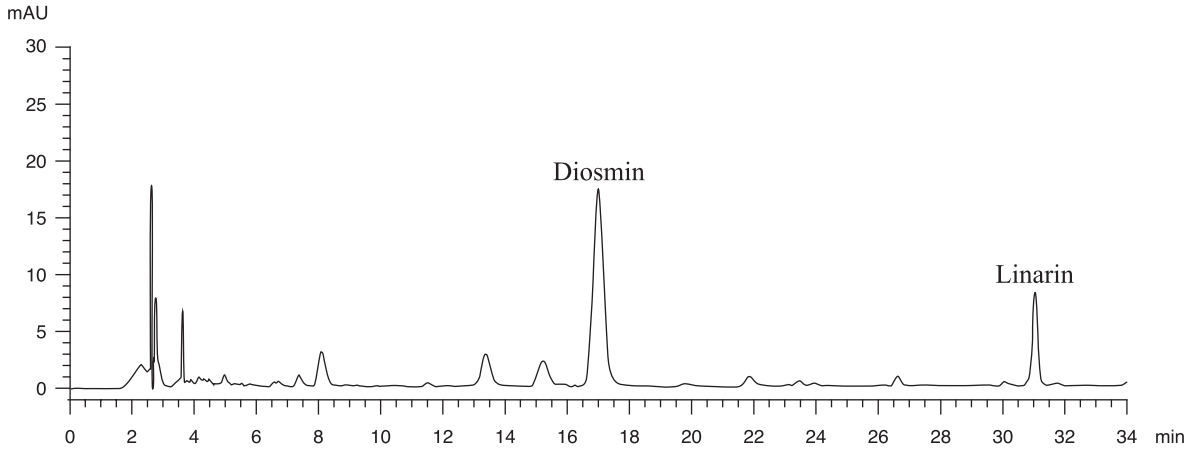

Figure 7 A reference assay chromatogram of Lobeliae Chinensis Herba extract

8. CAUTION

This CMM should be used after proper processing (such as decoction).