Lycopi Herba

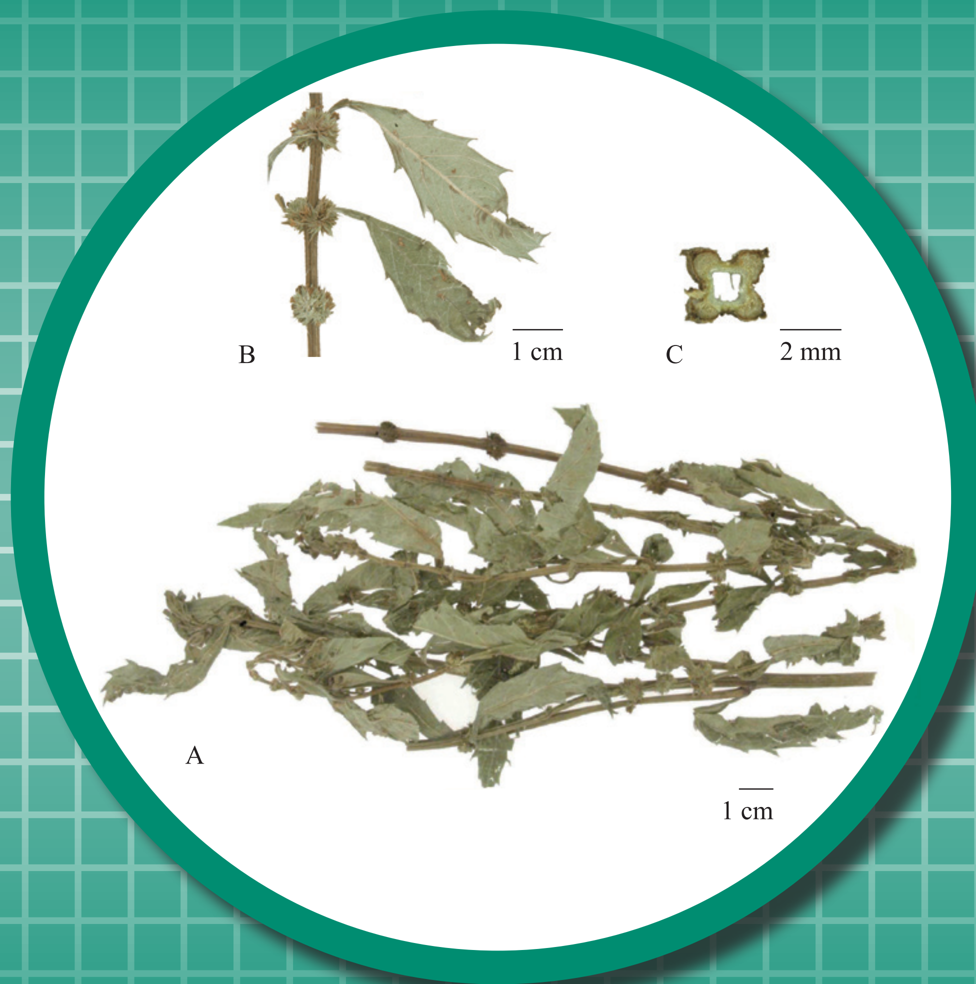

Figure 1 A photograph of Lycopi Herba

A. Lycopi Herba B. Magnified aerial part C. Magnified transverse section of stem

1. NAMES

Official Name: Lycopi Herba

Chinese Name: 澤蘭

Chinese Phonetic Name: Zelan

2. SOURCE

Lycopi Herba is the dried aerial part of *Lycopus lucidus* Turcz. var. *hirtus* Regel (Lamiaceae). The aerial part is collected in summer and autumn when foliage branch growing luxuriantly, then dried under the sun to obtain Lycopi Herba.

3. DESCRIPTION

Stem square-cylindrical, rarely branched, shallow and longitudinal furrows located on the four sides, 50-100 cm long, 2-6 mm in diameter. Externally yellowish-green to purplish-red, nodes appearing purplish-red, white-tomentose. Texture fragile; fracture yellowish-white, pith hollow. Leaves opposite, petiole short or nearly sessile; lamina mostly crumpled, when intact flattened out, lanceolate to oblong, 5-10 cm long; the upper surface green, the lower surface greyish-green and densely glandular-dotted, pubescent on both surfaces; apex acute, base attenuate, margin serrate. Verticillaster axillary, corolla mostly fallen off, bracts and calyx persistent, bracteole lanceolate with hairs on margin, calyx campanulate, with 5-toothed. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of cells. Non-glandular hairs sometimes visible. 8-10 layers of collenchymatous cells located at angular regions and 2-3 layers of collenchymatous cells present beneath epidermis. Pericyclic fibre bundles arranged in an interrupted ring. Cortex cells subrounded, size varies. Vascular bundle collateral. Xylem vessels scattered singly or in groups, arranged radially. Pith mainly consists of parenchymatous cells [Fig. 2 (i)].

Leaf: Non-glandular hairs located at upper epidermis and lower epidermis. Palisade tissue consists of 2 layers of cells. Collenchyma mainly located at the inner sides of upper and lower epidermis of midrib. Vascular bundle collateral. Xylem vessels subrounded, arranged radially. Lower epidermal cells polygonal or irregular shaped [Fig. 2 (ii)].

Powder

Colour yellowish-white to greyish-green. Non-glandular hairs abundant, consisting of 1-5 cells, with warty protrusions. Glandular scales consist of 8-celled head, subrounded, 60-98 µm in diameter. Anticlinal walls of lower epidermal cells wavy curved, stomata diacytic, occasionally anomocytic. Vessels mainly pitted, scalariform and spiral, 9-40 µm in diameter (Fig. 3).

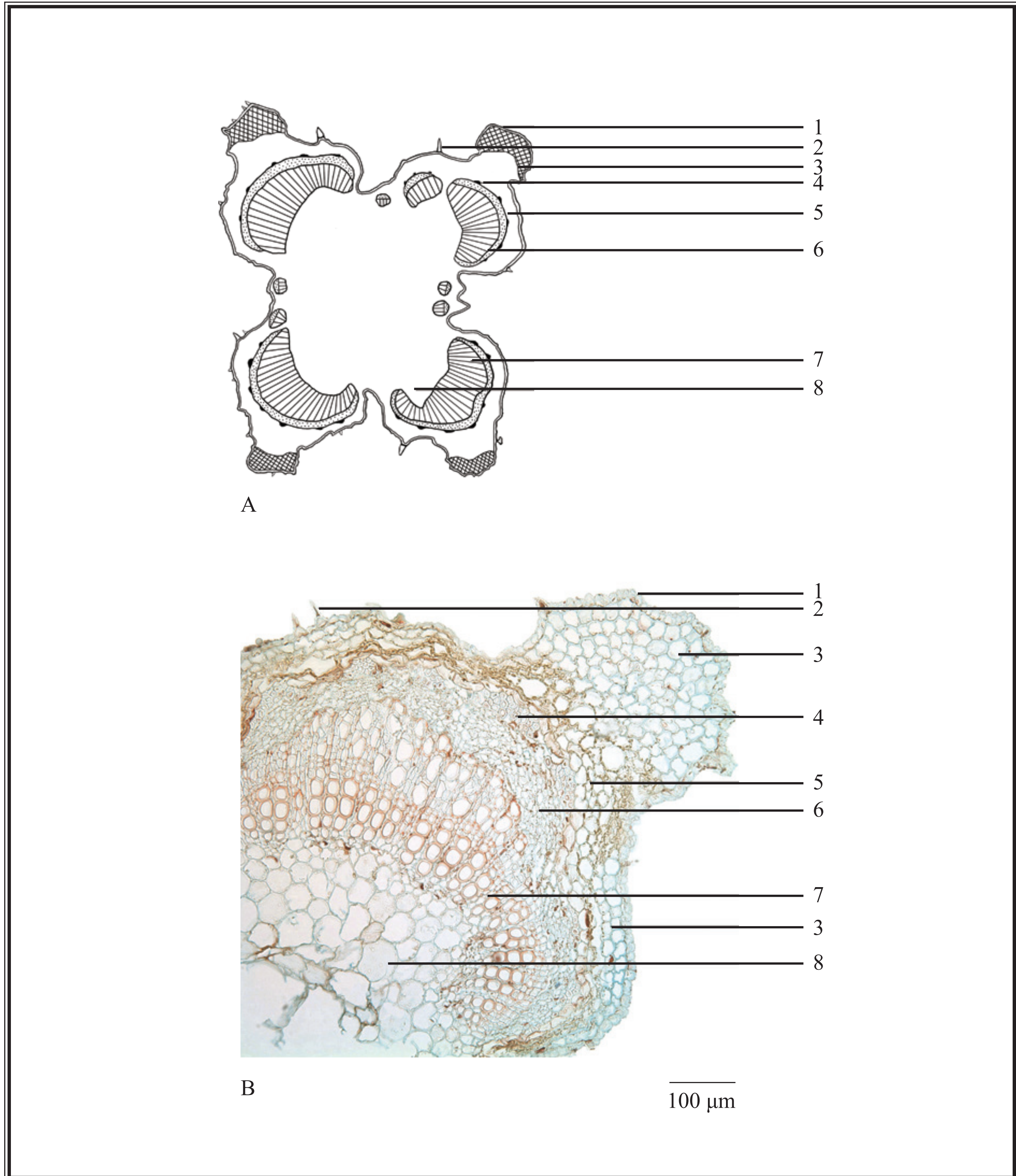

Figure 2 (i) Microscopic features of transverse section of stem of Lycopi Herba

A. Sketch B. Section illustration

1. Epidermis 2. Non-glandular hairs 3. Collenchyma
4. Pericyclic fibre bundles 5. Cortex 6. Phloem 7. Xylem 8. Pith

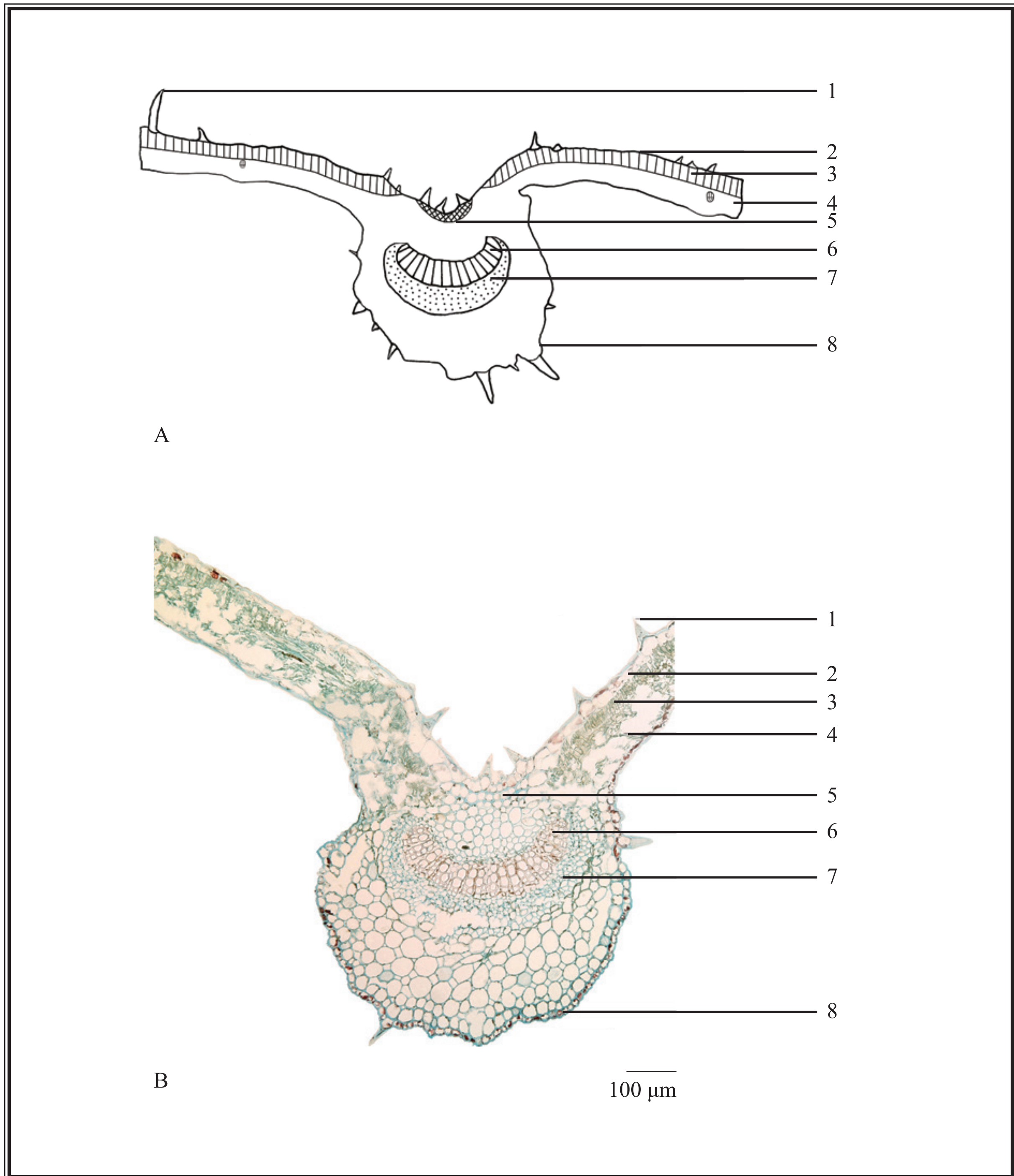

Figure 2 (ii) Microscopic features of transverse section leaf of Lycopi Herba

A. Sketch B. Section illustration

1. Non-glandular hairs 2. Upper epidermis 3. Palisade tissue
4. Spongy tissue 5. Collenchyma 6. Xylem 7. Phloem 8. Lower epidermis

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

1. Non-glandular hairs 2. Glandular scales
3. Lower epidermal cells with stomata 4. Vessels

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

Standard solution

Ursolic acid standard solution

Weigh 1.0 mg of ursolic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and formic acid (3:1:0.1, v/v).

Spray reagent

Add slowly 5 mL of sulphuric acid to 45 mL of ethanol and dissolve 0.5 g of vanillin.

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. 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 1 mL of ethanol.

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 ursolic acid standard solution and the test solution (1 μL each) to the plate. Develop over a path of about 6 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 until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

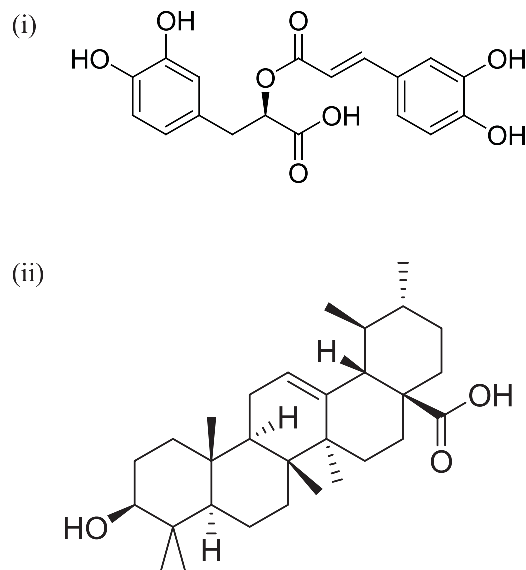

Figure 4 Chemical structures of (i) rosmarinic acid and (ii) ursolic acid

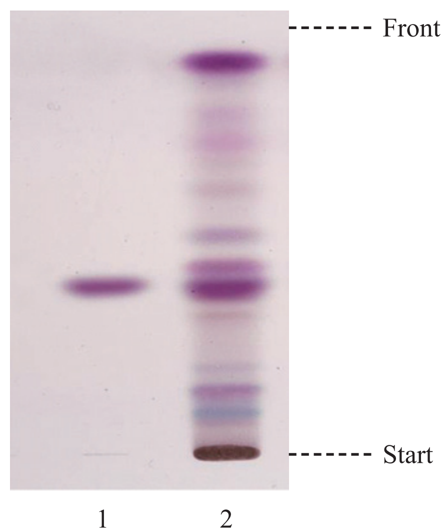

Figure 5 A reference HPTLC chromatogram of Lycopi Herba extract observed under visible light after staining

1. Ursolic 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 ursolic acid (Fig 5).

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

Standard solution

Rosmarinic acid standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 1.0 mg of rosmarinic acid CRS (Fig. 4) and dissolve in 20 mL of methanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (50%). Filter through a 0.45-µm RC 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)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	80 → 55	20 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of rosmarinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should not be more than 5.0%; the RSD of the retention time of rosmarinic acid peak should not be more than 2.0%; the column efficiency determined from rosmarinic acid peak should not be less than 20000 theoretical plates.

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 rosmarinic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of rosmarinic acid peak in the chromatogram of rosmarinic acid Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify rosmarinic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rosmarinic acid Std-FP. The retention times of rosmarinic 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 five characteristic peaks of Lycopi Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Lycopi Herba extract

Peak No.	RRT	Acceptable Range
1 (caffeic acid)	0.53	± 0.03
2	0.74	± 0.03
3	0.83	± 0.03
4 (marker, rosmarinic acid)	1.00	-
5	1.45	± 0.03

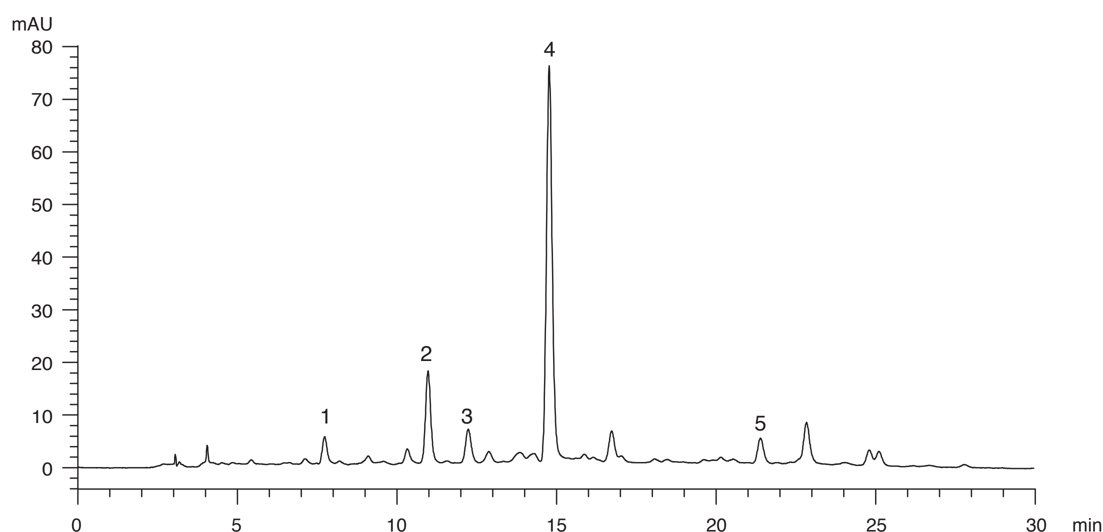

Figure 6 A reference fingerprint chromatogram of Lycopi Herba extract

For positive identification, the sample must give the above five 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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 9.0%.
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
Rosmarinic acid standard stock solution, Std-Stock (500 mg/L)
Weigh accurately 5.0 mg of rosmarinic acid CRS and dissolve in 10 mL of methanol (50%).
Rosmarinic acid standard solution for assay, Std-AS
Measure accurately the volume of the rosmarinic acid Std-Stock, dilute with methanol (50%) to produce a series of solutions of 1, 25, 50, 75, 100 mg/L for rosmarinic acid.

Test solution
Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol (50%). Filter through a 0.45-µm RC 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	80 → 55	20 → 45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of rosmarinic acid Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should not be more than 5.0%; the RSD of the retention time of rosmarinic acid peak should not be more than 2.0%; the column efficiency determined from rosmarinic acid peak should not be less than 20000 theoretical plates.

The *R* value between rosmarinic 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 rosmarinic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of rosmarinic acid against the corresponding concentrations of rosmarinic acid Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify rosmarinic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rosmarinic acid Std-AS. The retention times of rosmarinic 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 rosmarinic acid in the test solution, and calculate the percentage content of rosmarinic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.18% of rosmarinic acid ($C_{18}H_{16}O_8$), calculated with reference to the dried substance.