

# Herba Leonuri

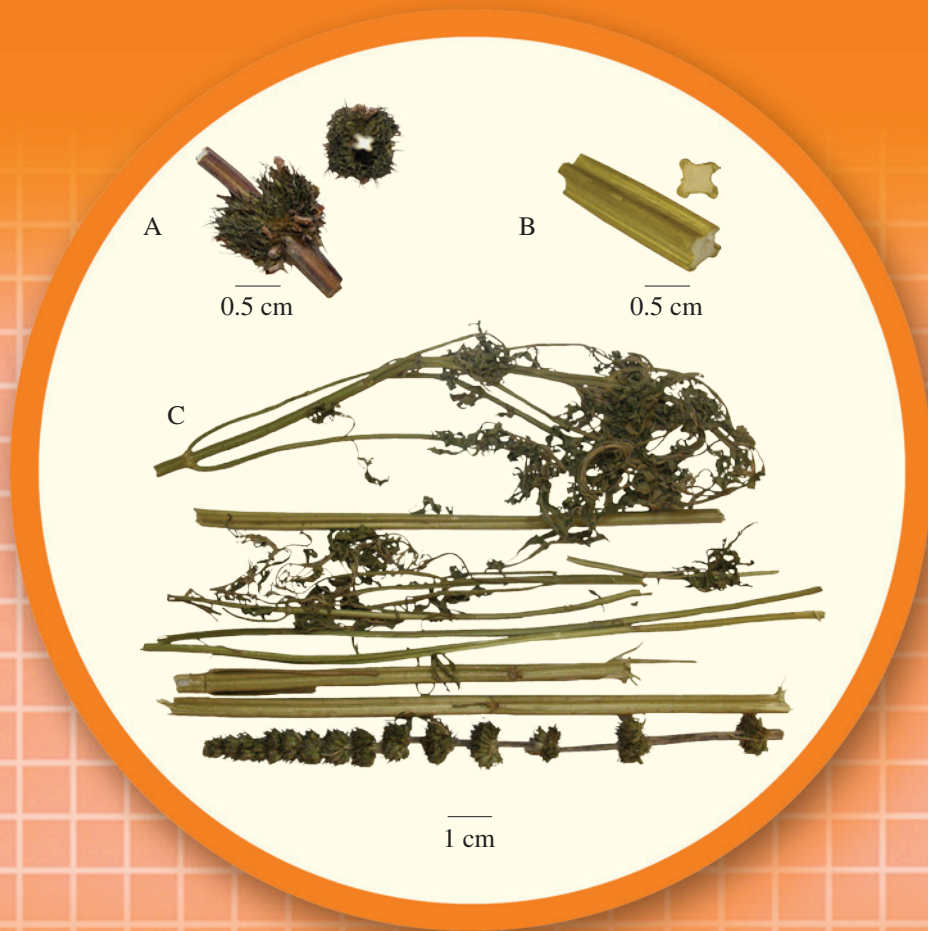


Figure 1 A photograph of Herba Leonuri

A. Stem node

B. Internode

C. Aerial part

## 1. NAMES

Official Name: Herba Leonuri

Chinese Name: 益母草

Chinese Phonetic Name: Yimucao

## 2. SOURCE

Herba Leonuri is the dried aerial parts of *Leonurus japonicus* Houtt. (Lamiaceae). The dried herb is collected in summer before flowering and dried under the sun to obtain Herba Leonuri.

## 3. DESCRIPTION

Stem quadrangular, 2-10 mm in diameter. Externally greyish-green or yellowish-green, the upper part ramose, furrowed longitudinally on 4 sides, texture light and pliable, broken stem shows pith in the centre. Leaves opposite, blades greyish-green when dry, mostly shrivelled and in fragments, fallen off easily. Verticillaster axillary, flowers pale purple, calyx tubular, corolla bilabiate. Odour slight; taste slightly bitter (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification (Appendix III)

#### Transverse section

**Stem:** Epidermal cells are covered with cuticle and trichomes. Glandular scales with a 4-8-celled head and a unicellular stalk, non-glandular hairs 1-4-celled. Hypodermal collenchyma cells abundant in the angular region. Cortex consists of several layers of parenchyma cells, fine crystals of calcium oxalate can be observed occasionally. Endodermis distinct. Pericyclic fibre bundles slightly lignified. Phloem relatively narrow. Cambium indistinct. Xylem is well developed in the angular region, with medullary parenchyma cells relatively large, some cells containing raphides of calcium oxalate [Fig. 2(i)].

**Leaf:** Upper and lower epidermis consist of one layer of cells, with trichomes. Palisade and spongy tissues consist of palisade-shaped parenchyma cells and irregular or subround parenchyma cells, respectively [Fig. 2(ii)].

## Powder

Colour greyish-green. Non-glandular hairs are easily visible, composed of 1-4-cells, slightly curved, with a thick wall and fine warty protuberance on the surface. Glandular scales consists of 4-8-celled head, subrounded or elliptical in top view, stalk very short. Diacytic and anomocytic stomata are clearly visible in pieces of scattered epidermal tissue of the disintegrated leaves, 12-30 µm long, 10-20 µm in diameter. Extremely fine crystals of calcium oxalate are found occasionally. Vessels are mainly bordered-pitted, scalariform or spiral vessels are also observed, 6-55 µm in diameter (Fig. 3).

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

### Standard solution

*Stachydrine hydrochloride standard solution*

Weigh 5.0 mg of stachydrine hydrochloride CRS (Fig. 4) and dissolve in 0.5 mL of methanol.

### Developing solvent system

Prepare a mixture of n-butanol, hydrochloric acid and ethyl acetate (8:2:0.5, v/v).

### Spray reagent

Dissolve 0.85 g of bismuth subnitrate in 50 mL of acetic acid (20%, v/v) and then add 20 mL of potassium iodide solution (40%, w/v) to obtain potassium iodobismuthate solution. Transfer 1 mL of potassium iodobismuthate solution to a 10-mL volumetric flask. Add 2 mL of 0.6 M hydrochloric acid and make up to the mark with water. Freshly prepare the reagent.

### Test solution

Weigh 2.0 g of the powdered sample and place it in a cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 300 mL of ethyl acetate to a 500-mL round-bottomed flask. Perform the soxhlet extraction for about 12 h. Collect and air dry the residue. Transfer the residue to a 50-mL conical flask. Add 25 mL of absolute ethanol and 150 µL of 0.1 M hydrochloric acid. Sonicate (220 W) the mixture for 30 min. Filter and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 0.5 mL of absolute ethanol.

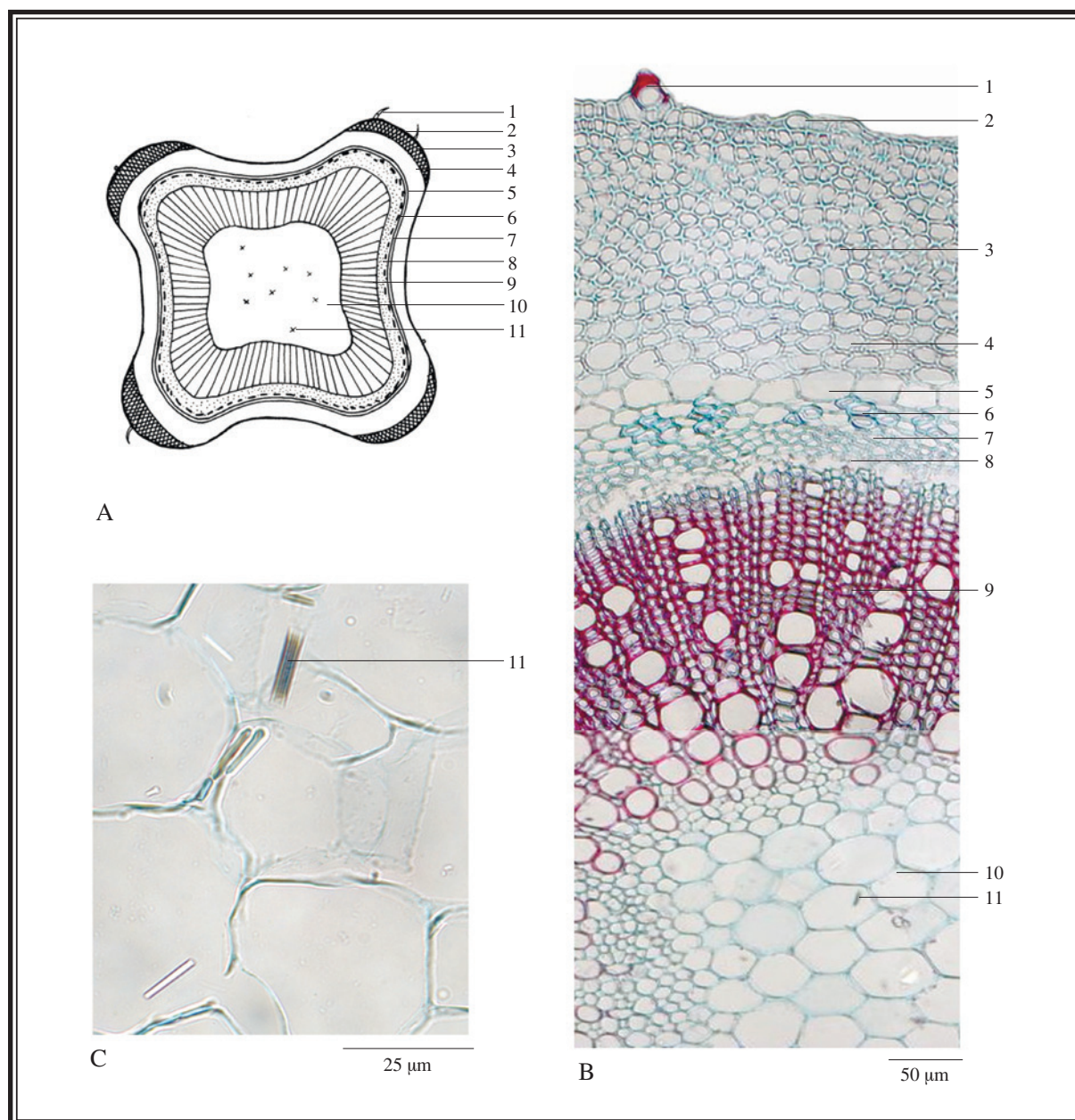


Figure 2(i) Microscopic features of transverse section of the stem of Herba Leonuri

A. Sketch B. Section illustration C. Raphides of calcium oxalate in the pith

1. Non-glandular hair 2. Epidermis 3. Hypodermal collenchyma 4. Cortex
5. Endodermis 6. Pericyclic fibre bundles 7. Phloem 8. Cambium 9. Xylem
10. Pith 11. Raphides of calcium oxalate

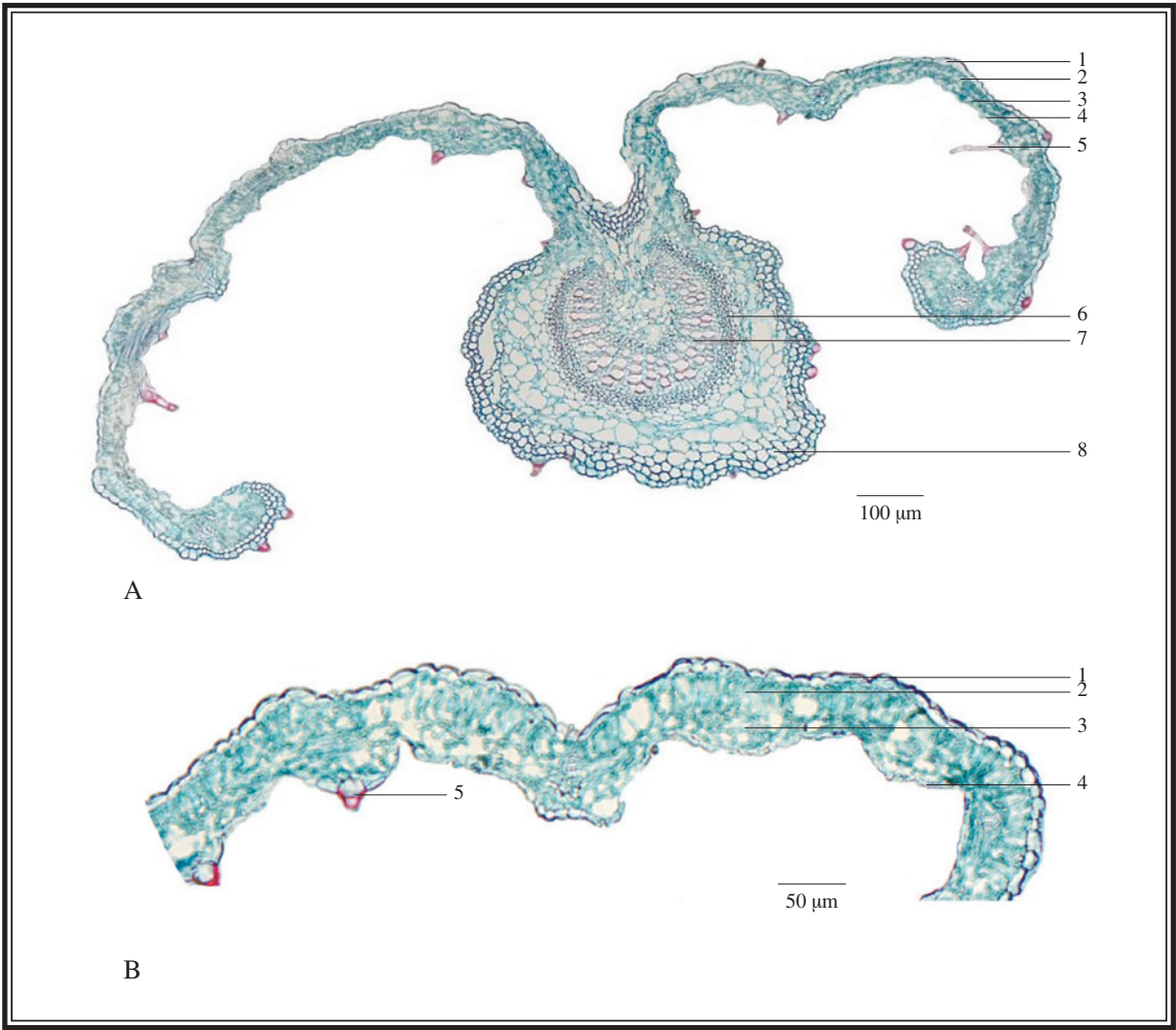


Figure 2(ii) Microscopic features of the transverse section of the leaf of Herba Leonuri

A. Section illustration of leaf blade    B. Section illustration of leaf blade farther away from midvein

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

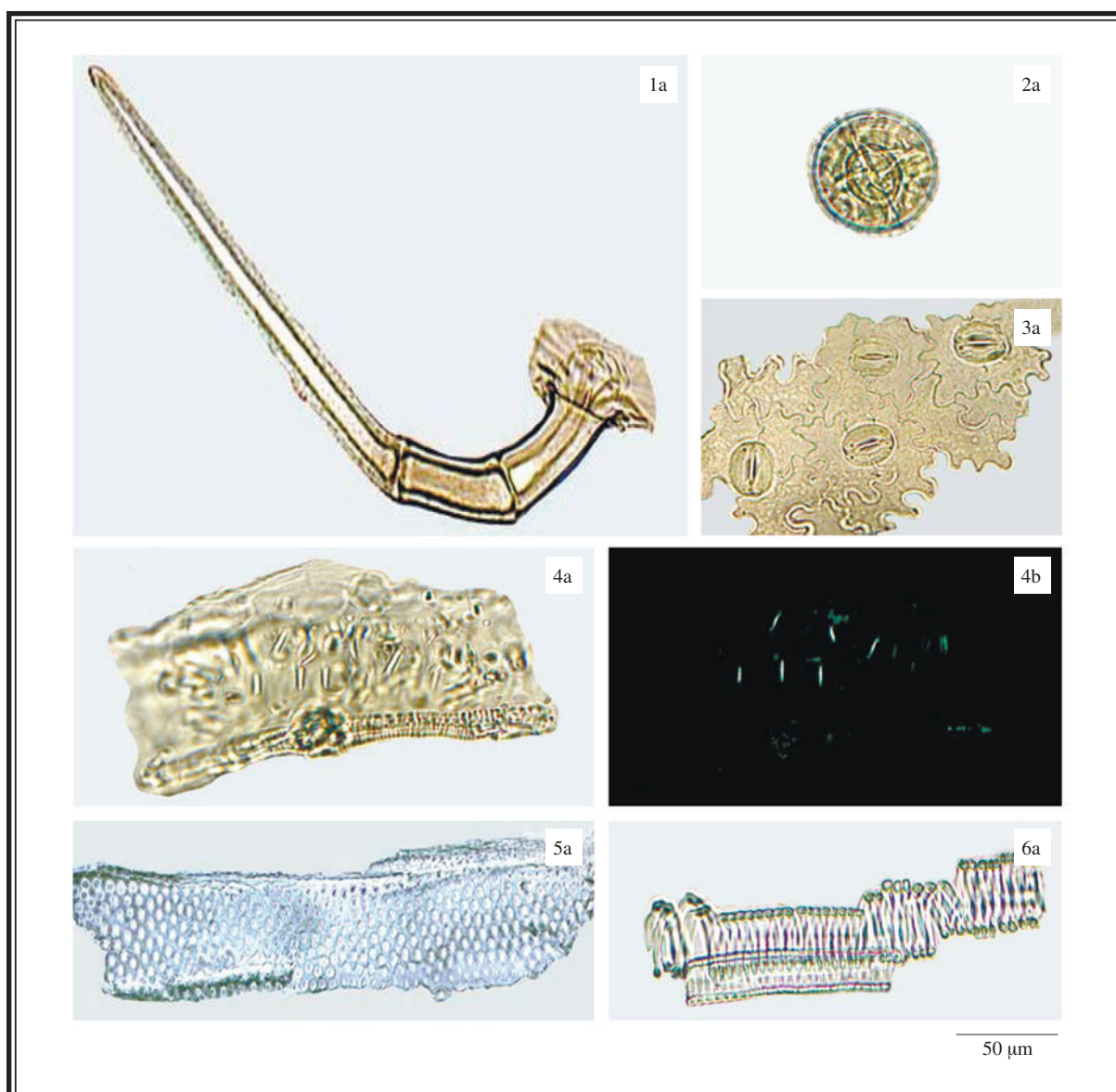


Figure 3 Microscopic features of powder of the aerial parts of Herba Leonuri

1. Non-glandular hair 2. Glandular scale 3. Diacytic stomata
4. Crystals of calcium oxalate 5. Bordered-pitted vessels 6. Spiral vessels

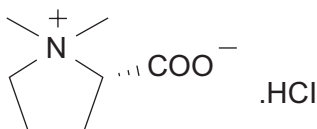
a. Features under the light microscope b. Features under the polarized microscope

## Procedure

Carry out the method by using a HPTLC silica gel F<sub>254</sub> plate and a freshly prepared developing solvent system as described above. Apply separately stachydrine hydrochloride standard solution (3 µL) and the test solution (5 µL) to the plate. Develop over a path of about 5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in an oven at 105°C (about 15 min). Spray the plate evenly with the spray reagent. Examine the plate under visible light. Calculate the  $R_f$  value by using the equation as indicated in Appendix IV(A).

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 stachydrine hydrochloride.

(i)



(ii)

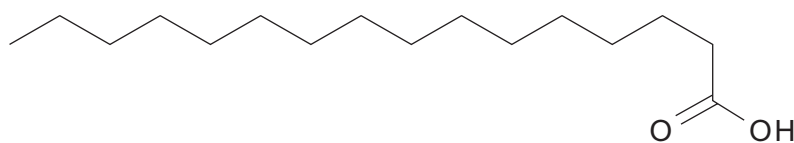


Figure 4 Chemical structures of (i) stachydrine hydrochloride and (ii) palmitic acid

## 4.3 Gas Chromatographic Fingerprinting (Appendix XII)

### Standard solution

*Palmitic acid standard solution for fingerprinting, Std-FP (400 mg/L)*

Weigh 4.0 mg of palmitic acid CRS (Fig. 4) and dissolve in 10 mL of methanol.

### Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL conical flask. Filter through a 0.45-µm PTFE filter.

### Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-5, 0.32 mm × 30 m) of which the internal wall is covered with (5%-phenyl)-methylpolysiloxane in a layer about 0.25 µm thick. The injection temperature is at 210°C. The detector temperature is at 300°C. The split injection mode at a ratio of 15:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C/min)
0–2	70	-
2–27	70 → 220	6
27–37	220	-
37–40	220 → 250	10
40–60	250	-

System suitability requirements

Perform at least five replicate injections, each using 2 μL of palmitic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of palmitic acid should not be more than 5.0%; the RSD of the retention time of palmitic acid peak should not be more than 2.0%; the column efficiency determined from palmitic acid peak should not be less than 500000 theoretical plates.

The *R* value between peak 4 and peak 5 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject palmitic acid Std-FP and the test solution (2 μL each) into the GC system and record the chromatograms. Measure the retention time of palmitic acid peak in the chromatogram of palmitic acid Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify palmitic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of palmitic acid Std-FP. The retention times of palmitic 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 Herba Leonuri extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.29	±0.03
2	0.35	±0.03
3	0.51	±0.03
4 (marker, palmitic acid)	1.00	-
5	1.13	±0.03

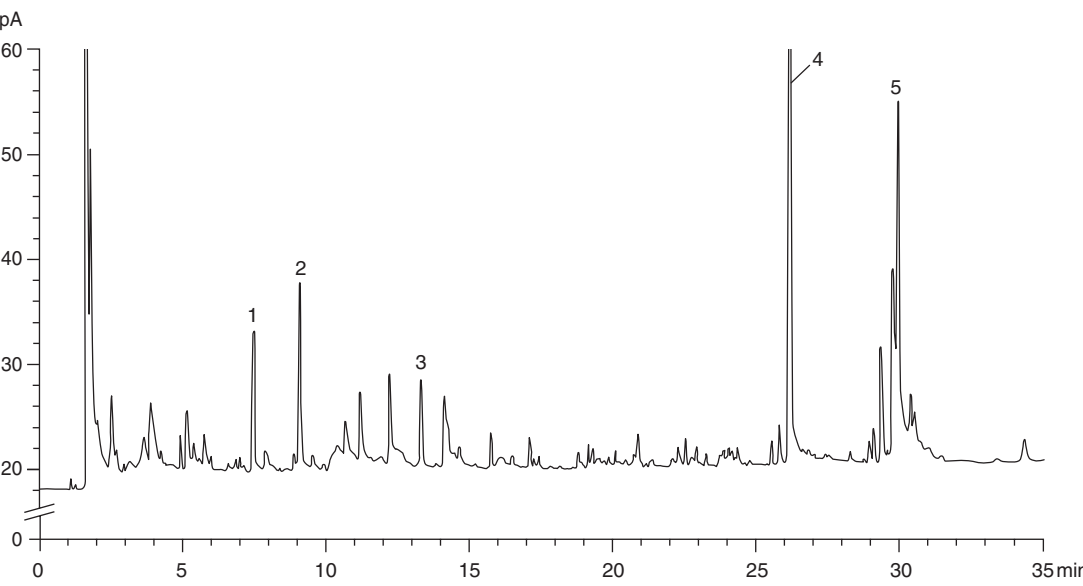


Figure 5 A reference fingerprint chromatogram of Herba Leonuri extract

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. 5).

## 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 XV): meet the requirements.
- 5.5
Foreign Matter
(Appendix VIII): not more than 2.0%.

## 5.6 Ash (Appendix IX)

Total ash: not more than 13.5%.

Acid-insoluble ash: not more than 2.0%.

## 5.7 Water Content (Appendix X): not more than 12.0%.

## 6. EXTRACTIVES (Appendix XI)

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

Ethanol-soluble extractives (hot extraction method): not less than 16.0%.

## 7. ASSAY

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

### Standard solution

*Stachydrine hydrochloride standard stock solution, Std-Stock (1000 mg/L)*

Weigh accurately 5.0 mg of stachydrine hydrochloride CRS and dissolve in 5 mL of absolute ethanol.

*Stachydrine hydrochloride standard solution for assay, Std-AS*

Measure accurately the volume of the stachydrine hydrochloride Std-Stock, dilute with absolute ethanol to produce a series of solutions of 100, 300, 500, 700, 900 mg/L for stachydrine hydrochloride.

### Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 300 mL of absolute ethanol to a 500-mL round-bottomed flask. Perform the soxhlet extraction for about 15 h. Concentrate the filtrate to about 5 mL at reduced pressure in a rotary evaporator. Transfer the solution to the aluminum oxide (mesh number of 70-230, 10 g) clean-up column (a glass column with internal diameter of 1.5-1.8 cm, wet packing the column with homogeneous mixture of aluminum oxide and 95% ethanol up to 4 cm height). Add 200 mL of absolute ethanol to the clean-up column. Collect the eluant and transfer to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in a mixture of 0.1% acetic acid and acetonitrile (2:8, v/v). Transfer the solution to a 10-mL volumetric flask and make up to the mark with a mixture of 0.1% acetic acid and acetonitrile (2:8, v/v). Filter through a 0.45-μm PTFE filter.

### Chromatographic system

The liquid chromatograph is equipped with a DAD (203 nm) and a column (4.6 × 250 mm) packed with aminopropyl group (5 µm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of acetonitrile and water (80:20, v/v). The elution time is about 60 min.

### System suitability requirements

Perform at least five replicate injections, each using 20 µL of stachydrine hydrochloride Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of stachydrine hydrochloride should not be more than 5.0%; the RSD of the retention time of stachydrine hydrochloride peak should not be more than 5.0%; the column efficiency determined from stachydrine hydrochloride peak should not be less than 3000 theoretical plates.

The *R* value between stachydrine hydrochloride peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

### Calibration curve

Inject a series of stachydrine hydrochloride Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of stachydrine hydrochloride against the corresponding concentrations of stachydrine hydrochloride Std-AS. Obtain the slope, y-intercept and the *r*<sup>2</sup> value from the 5-point calibration curve.

### Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify stachydrine hydrochloride peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of stachydrine hydrochloride Std-AS. The retention times of stachydrine hydrochloride 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 stachydrine hydrochloride in the test solution, and calculate the percentage content of stachydrine hydrochloride in the sample by using the equations indicated in Appendix IV(B).

### Limits

The sample contains not less than 0.61% of stachydrine [calculated as stachydrine hydrochloride (C<sub>7</sub>H<sub>13</sub>NO<sub>2</sub>·HCl)], calculated with reference to the dried substance.

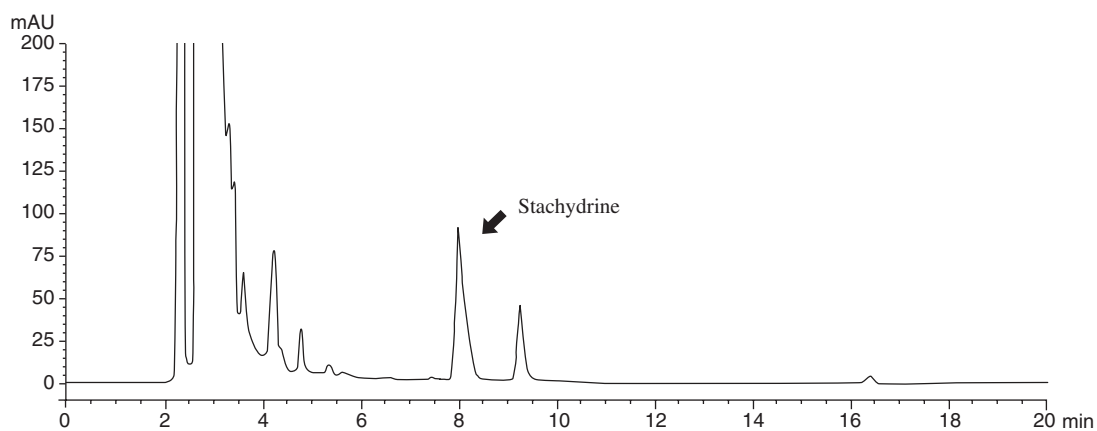
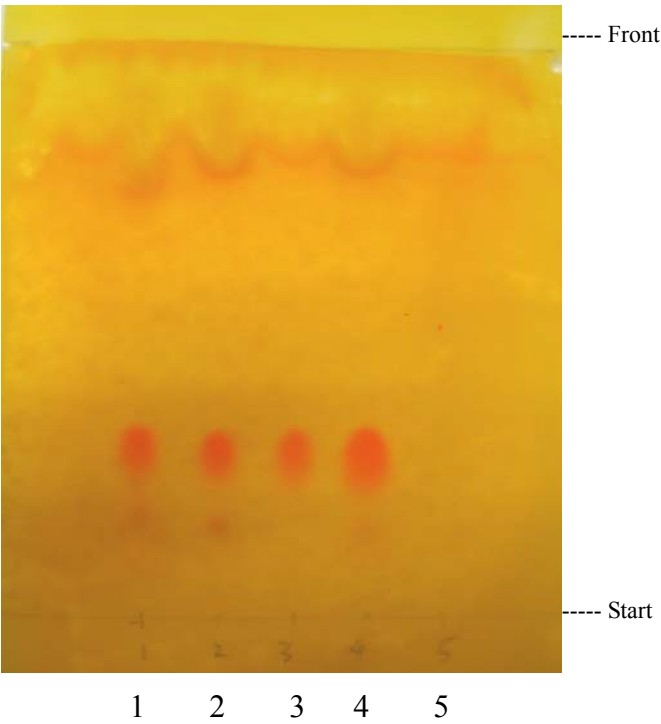


Figure 6 A reference assay chromatogram of Herba Leonuri extract

**Herba Leonuri (益母草)**



Lane	Sample	Results
1	Sample (Herba Leonuri)	Stachydrine hydrochloride positive
2	Sample duplicate (Herba Leonuri)	Stachydrine hydrochloride positive
3	Standard (Stachydrine hydrochloride)	Stachydrine hydrochloride positive
4	Spiked sample (Sample plus stachydrine hydrochloride)	Stachydrine hydrochloride positive
5	Blank (Ethanol)	Negative

Figure 1 TLC results of Herba Leonuri extract observed under visible light after staining