# Eupatorii Herba


**Figure 1** A photograph of Eupatorii Herba

A. Eupatorii Herba B. Leaves attached to stem

## 1. NAMES

Official Name: Eupatorii Herba

Chinese Name: 佩蘭

Chinese Phonetic Name: Peilan

## 2. SOURCE

Eupatorii Herba is the dried aerial part of *Eupatorium fortunei* Turcz. (Asteraceae). The aerial part is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Eupatorii Herba.

## 3. DESCRIPTION

Stem cylindrical, up to 100 cm long, 2-6 mm in diameter. Externally yellowish-brown or yellowish-green, sometimes purple, with distinct nodes and longitudinal ridges; texture fragile; fracture white or hollow in pith. Leaves opposite, with petiole, lamina mostly crumpled and broken, green to brownish-green. Lamina trifid or non-divided when intact and flattened; trifid lamina middle lobe larger, lobes lanceolate or oblong-lanceolate, base narrow, margin serrate; non-divided lamina ovate, ovate-lanceolate or elliptical. Odour aromatic; taste slightly bitter (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification *(Appendix III)*

**Transverse section**

**Stem:** Epidermis consists of 1 layer of rectangular-shaped cells, arranged orderly. Collenchyma underneath the epidermis, consisting of 2-3 layers of cells. Cortex consists of 3-11 layers of parenchymatous cells. Collateral vascular bundles 20-30, arranged in ring. Phloem fibres located outside the phloem arranged in a half-moon shape, lignified. Phloem narrow. Cambium consists of 2-3 layers of cells, arranged in a ring. Xylem broad. Pith large, occupied about 2/3 of stem [Fig. 2 (i)].

**Petiole:** Half-moon shaped. Vascular bundles 10-20, varied in size, arranged in a V-shaped. Non-glandular hair 3- to 10-celled, presents occasionally. Epidermis consists of 1 layer of subrectangular cells, arranged orderly. Collenchyma underneath epidermis, consisting of 2-4 layers of cells. Ground parenchyma consists of parenchymatous cells. Xylem broad. Phloem narrow [Fig 2 (ii)].

**Leaf:** Thin, tissue weakly differentiated. Upper epidermis consists of 1 layer of subrectangular cells, arranged orderly. Palisade tissue consists of 1 layer of short cylindrical cells. Xylem broad. Phloem narrow. Spongy tissue loosely arranged. Lower epidermis consists of 1 layer subrectangular cells, arranged orderly [Fig 2 (iii)].

**Powder**

Colour yellowish-green to greenish-brown. Upper epidermal cells slightly sinuous in surface view. Lower epidermal cells sinuous in surface view, accompany with stomata, stomata anomocytic, 21-42 μm long and 15-29 μm wide. Non-glandular hairs 3- to 10-celled, occasionally found, mostly fragmented. Fibres scattered singly or in bundles, 9-26 μm in diameter; polychromatic under the polarized microscope. Stem vessels spiral, scalariform, reticulate and bordered-pitted (Fig. 3).

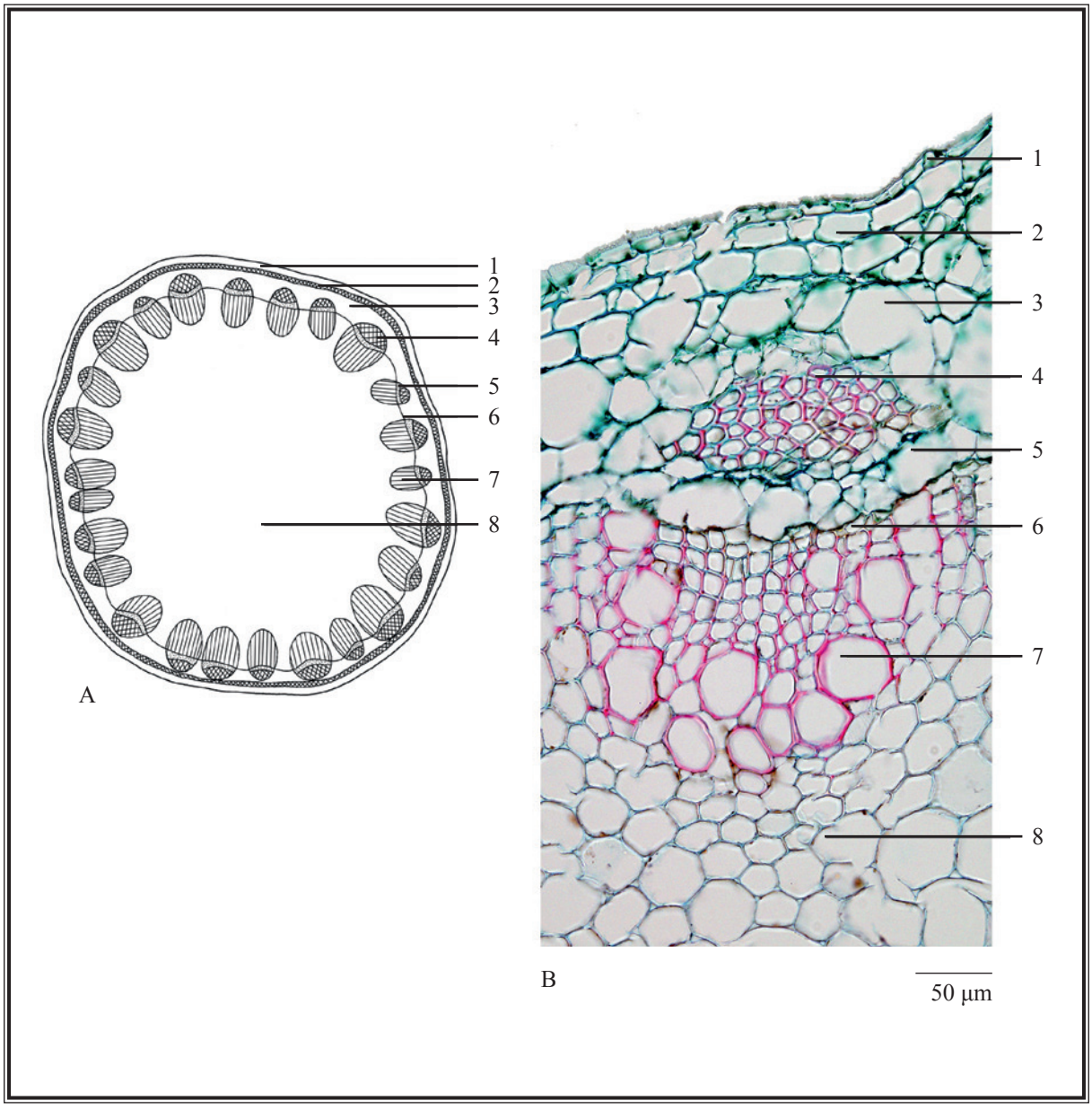


**Figure 2 (i)** Microscopic features of transverse section of stem of Eupatorii Herba

A. Sketch B. Section illustration

1. Epidermis 2. Collenchyma 3. Cortex 4. Phloem fibres
5. Phloem 6. Cambium 7. Xylem 8. Pith

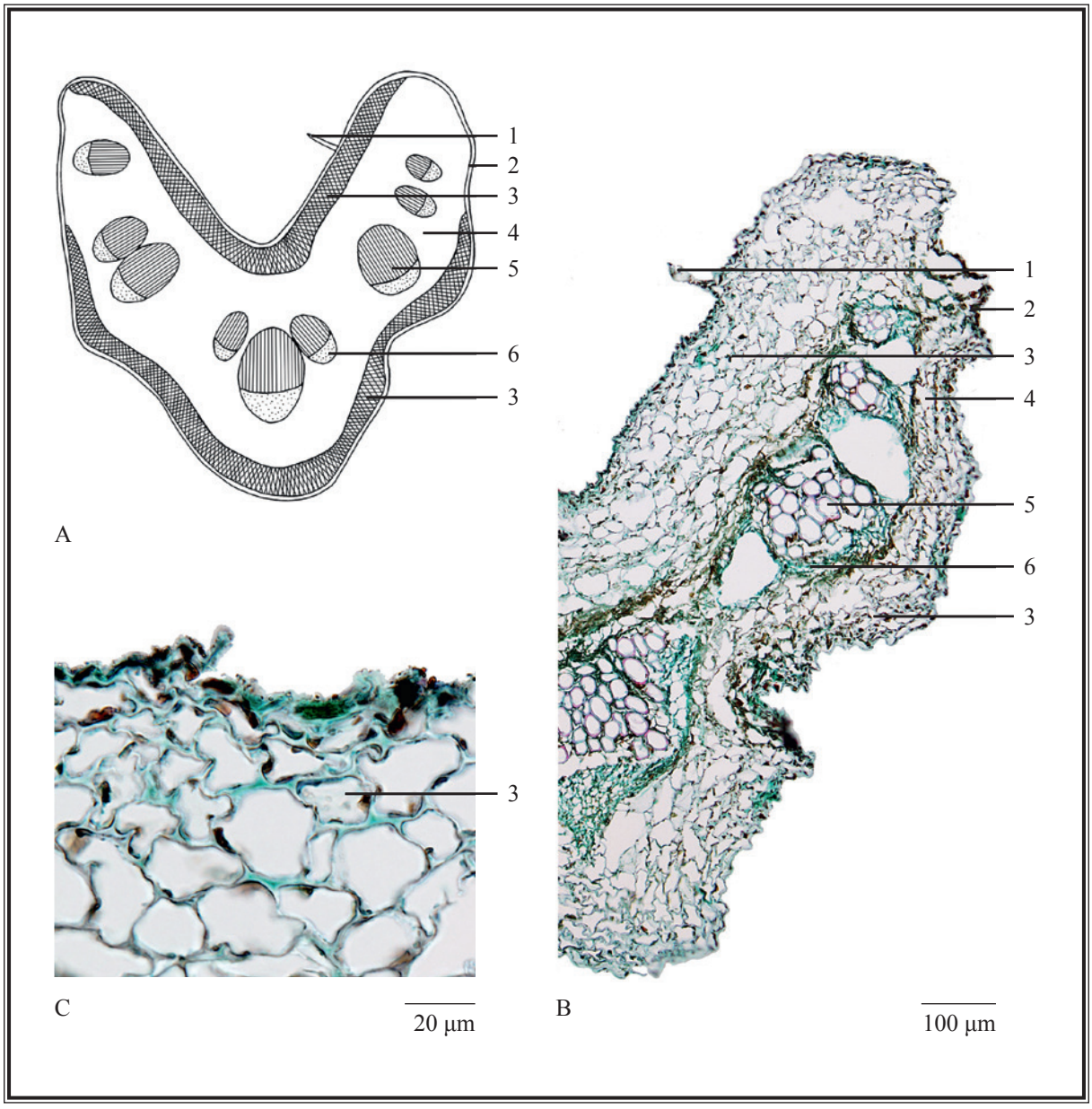


**Figure 2 (ii)** Microscopic features of transverse section of petiole of Eupatorii Herba

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Epidermis 3. Collenchyma 4. Ground parenchyma
5. Xylem 6. Phloem

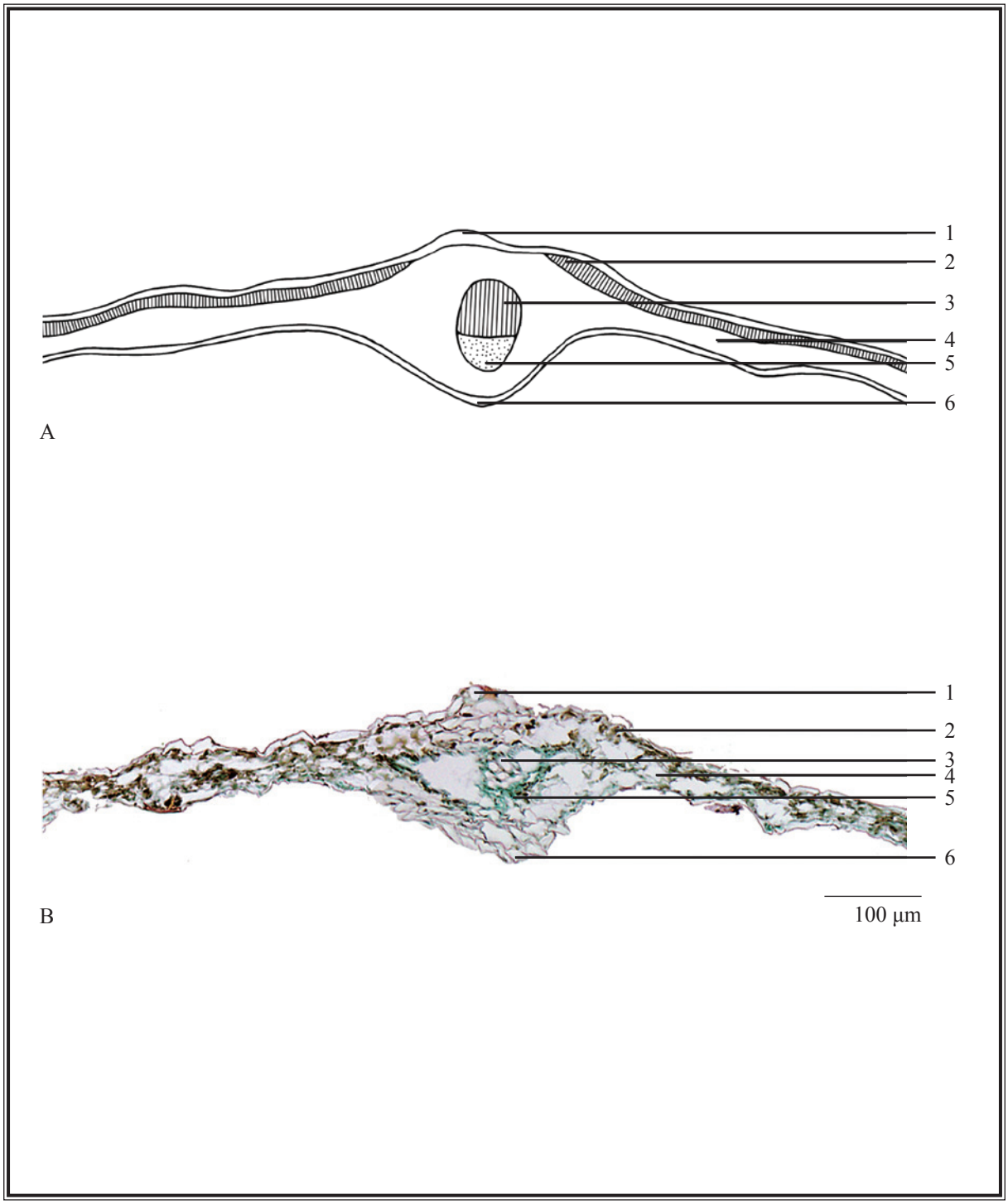


**Figure 2 (iii)** Microscopic features of transverse section of leaf of Eupatorii Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Xylem 4. Spongy tissue 5. Phloem 6. Lower epidermis

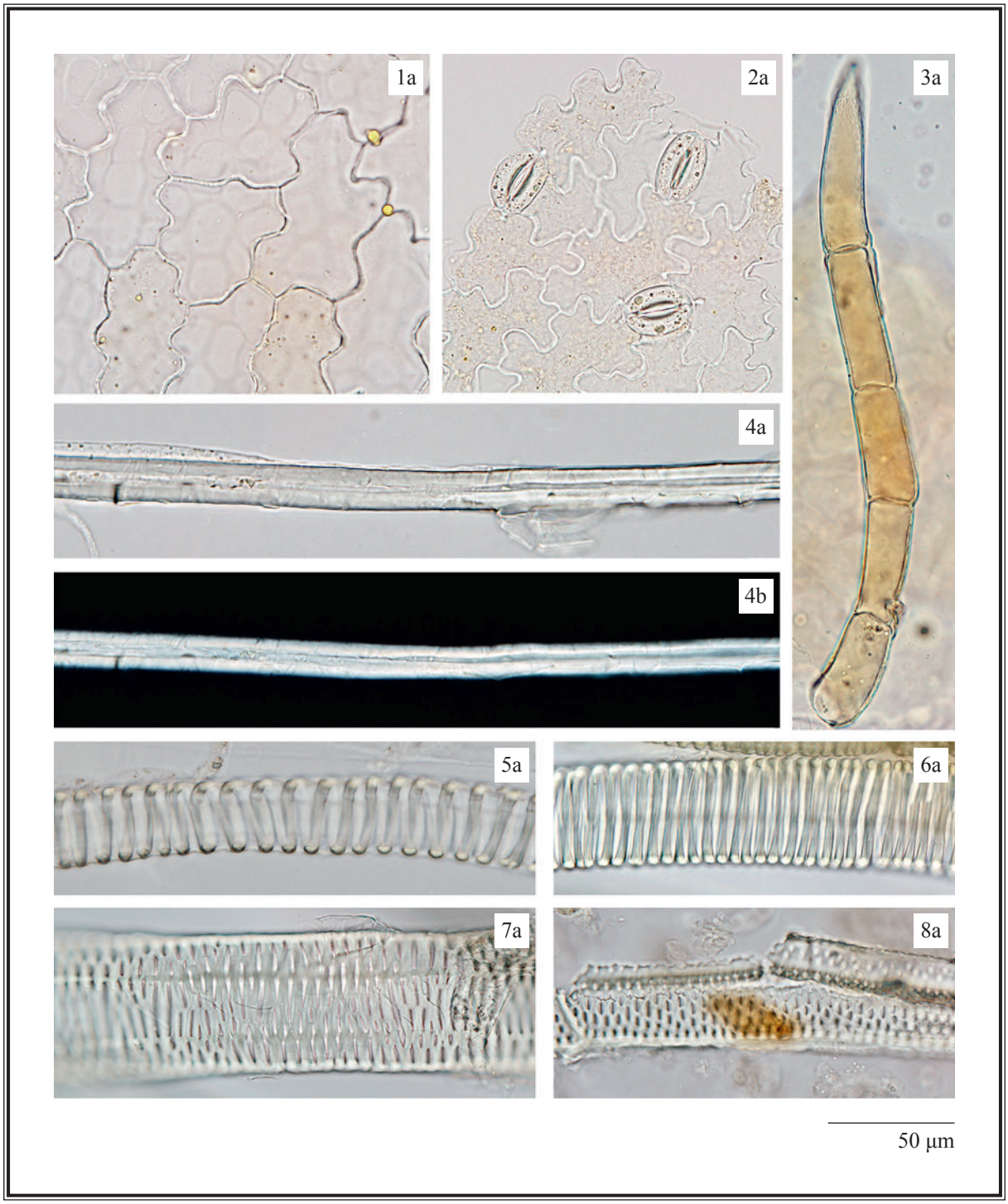


**Figure 3** Microscopic features of powder of Eupatorii Herba

1. Upper epidermal cells of leaf 2. Lower epidermal cells of leaf with stomata
3. Non-glandular hair 4. Fibre 5. Spiral vessel of stem 6. Scalariform vessel of stem
7. Reticulate vessel of stem 8. Bordered-pitted vessels of stem

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

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

**Standard solution**

*Coumarin standard solution*

Weigh 1.0 mg of coumarin CRS (Fig. 4) and dissolve in 1 mL of methanol.

**Developing solvent system**

Prepare a mixture of n-hexane, ethyl acetate and formic acid (6:3:0.1, v/v).

**Test solution**

Weigh 3.0 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of methanol. Sonicate (180 W) the mixture for 15 min. Filter and transfer the filtrate to a 100-mL separating funnel. Extract for two times each with 20 mL of n-hexane and discard the n-hexane layer. Combine the methanol extracts.

**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 coumarin standard solution (4 µL) and the test solution (9 µL) to the plate. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). 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 coumarin.

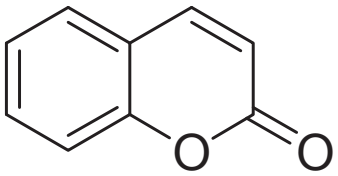


**Figure 4** Chemical structure of coumarin

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

**Standard solution**

*Coumarin standard solution for fingerprinting, Std-FP (100 mg/L)*

Weigh 1.0 mg of coumarin CRS 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 20 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. 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 (275 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) | 0.5% Phosphoric acid (%, v/v) | Methanol (%, v/v) | Elution |
|---|---|---|---|
| 0 – 50 | 80 → 10 | 20 → 90 | linear gradient |
| 50 – 60 | 10 | 90 | isocratic |

**System suitability requirements**

Perform at least five replicate injections, each using 10 μL of coumarin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of coumarin should not be more than 5.0%; the RSD of the retention time of coumarin peak should not be more than 2.0%; the column efficiency determined from coumarin peak should not be less than 40000 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. 5).

**Procedure**

Separately inject coumarin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of coumarin peak in the chromatogram of coumarin Std-FP and the retention times of the three characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify coumarin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of coumarin Std-FP. The retention times of coumarin 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 Eupatorii Herba extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the three characteristic peaks of Eupatorii Herba extract

| Peak No. | RRT | Acceptable Range |
|---|---|---|
| 1 | 0.75 | ± 0.03 |
| 2 (marker, coumarin) | 1.00 | - |
| 3 | 1.06 | ± 0.03 |

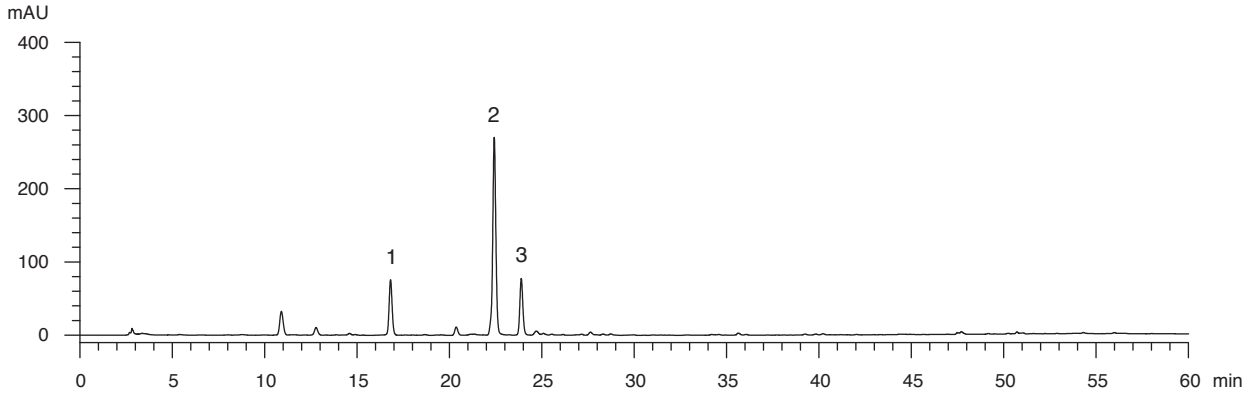


**Figure 5** A reference fingerprint chromatogram of Eupatorii Herba extract

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. 5).

## 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 10.5%.
Acid-insoluble ash: not more than 2.0%.

**5.7 Water Content** *(Appendix X)*

Oven dried method: not more than 12.0%.

## 6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 22.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

## 7. ASSAY

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

**Standard solution**

*Coumarin standard stock solution, Std-Stock (20 mg/L)*

Weigh accurately 1.0 mg of coumarin CRS and dissolve in 50 mL of methanol.

*Coumarin standard solution for assay, Std-AS*

Measure accurately the volume of the coumarin Std-Stock, dilute with methanol to produce a series of solutions of 1, 5, 10, 15, 20 mg/L for coumarin.

**Test solution**

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (220 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 15 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction for two more times. Combine the filtrates 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 (275 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

**Table 3** Chromatographic system conditions

| Time (min) | 0.5% Phosphoric acid (%, v/v) | Methanol (%, v/v) | Elution |
|---|---|---|---|
| 0 – 40 | 75 → 60 | 25 → 40 | linear gradient |

**System suitability requirements**

Perform at least five replicate injections, each using 20 μL of coumarin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of coumarin should not be more than 5.0%; the RSD of the retention time of coumarin peak should not be more than 2.0%; the column efficiency determined from coumarin peak should not be less than 15000 theoretical plates.

The *R* value between coumarin 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 coumarin Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of coumarin against the corresponding concentrations of coumarin Std-AS. Obtain the slope, y-intercept and the $r^2$ value from the 5-point calibration curve.

**Procedure**

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

**Limits**

The sample contains not less than 0.12% of coumarin ($C_9H_6O_2$), calculated with reference to the dried substance.