Ecliptae Herba

Figure 1 A photograph of Ecliptae Herba

1. NAMES

Official Name: Ecliptae Herba

Chinese Name: 墨旱蓮

Chinese Phonetic Name: Mohanlian

2. SOURCE

Ecliptae Herba is the dried aerial part of *Eclipta prostrata* L. (Asteraceae). The aerial part of herb is collected at flowering stage, foreign matter removed, then dried under the sun to obtain Ecliptae Herba.

3. DESCRIPTION

White-pubescent throughout. Stem cylindrical, with longitudinal ridges, 1-5 mm in diameter; externally greenish-brown to dark green. Leaves simple, opposite, almost sessile; blade crumpled and rolled or broken; when intact and flattened in whole, dark green, long-lanceolate, apex acute, base narrowly cuneate, margins entire or shallowly dentate. Capitula 2-8 mm in diameter. Achenes elliptic and flattened, 2-3 mm long, pale brown to brown. Odour slight; taste slightly bitter and salty (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of cells with thick wall and non-glandular hairs. Sclerenchymatous cells in 2-4 layers. Cortex parenchymatous cells arranged loose, containing pigment masses. Fibre bundles are located on the outside of vascular bundles. Collateral vascular bundles 12-20, phloem narrow, cambium forms an interrupted ring. Pith broad (Fig. 2).

Leaf: Upper and lower epidermis consist of 1 layer of cells with stomata, non-glandular hairs, occasionally with glandular hairs. Sclerenchymatous cells of 1-3 layers are located on the inside of the upper and lower epidermis. Palisade tissue and spongy tissue consist of 1 layer of palisade-shaped parenchymatous cells and 4-6 layers of subrounded parenchymatous cells, respectively. Collateral vascular bundles, 3-5, line the middle vein (Fig. 2).

Powder

Colour greenish-brown. Non-glandular hairs, frequently 3-celled, with walls thickened with distinct prominent warts, 260-800 µm long. Pollen grains spherical, 15-20 µm in diameter, with warts. Pigment masses are scattered; polychromatic under the polarized microscope. Vessels mainly spiral and reticulate, 13-100 µm in diameter. Epidermal cells with wavy and sinuous anticlinal walls; anomocytic stomata are surrounded by 3-4 subsidiary cells (Fig. 3).

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

Standard solution

Wedelolactone standard solution

Weigh 1.0 mg of wedelolactone CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of n-hexane, ethyl acetate and formic acid (10:7:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (240 W) the mixture for 30 min. Filter the mixture.

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 wedelolactone standard solution (1.5 µL) and the test solution (3 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 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 (366 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 wedelolactone.

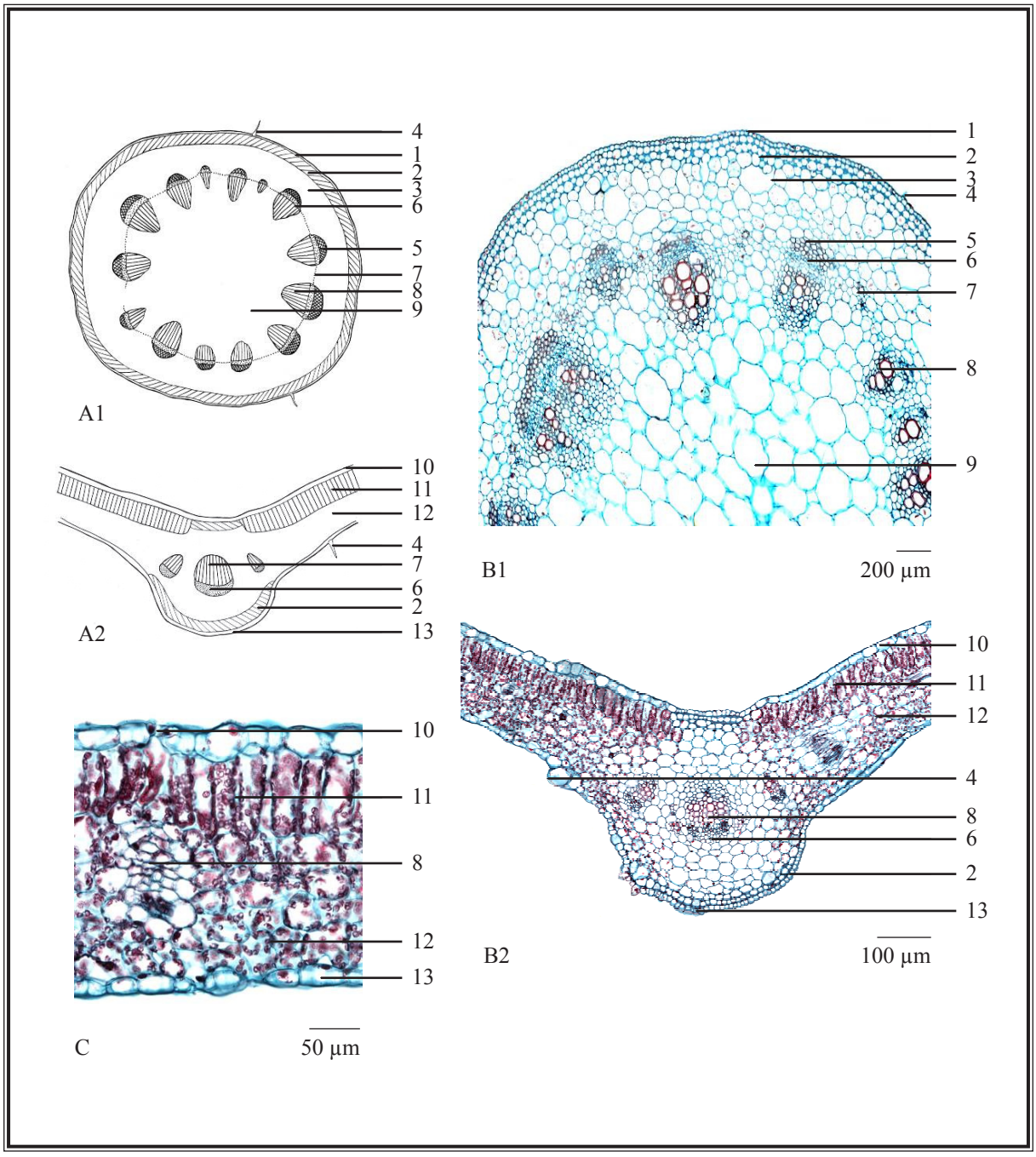

Figure 2 Microscopic features of transverse section of Ecliptae Herba

A. Sketch (A1: Stem, A2: Leaf) B. Section illustration (B1: Stem, B2: Leaf) C. Lamina

1. Epidermis 2. Sclerenchyma 3. Cortex 4. Non-glandular hair 5. Fibre bundle 6. Phloem 7. Interfascicular cambium 8. Xylem 9. Pith 10. Upper epidermis 11. Palisade tissue 12. Spongy tissue 13. Lower epidermis

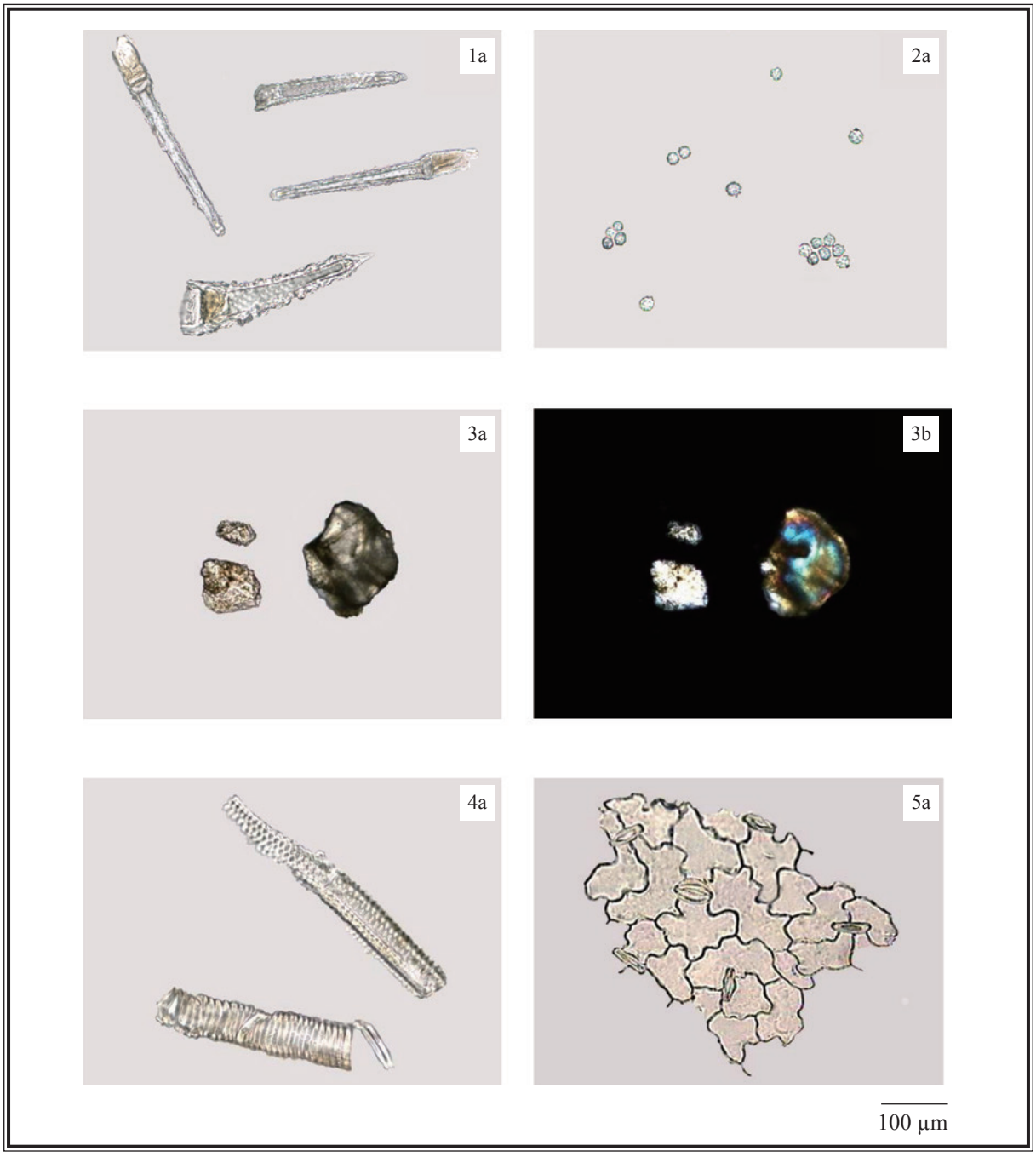

Figure 3 Microscopic features of powder of Ecliptae Herba

1. Non-glandular hairs 2. Pollen grains 3. Pigment masses 4. Spiral and reticulate vessels 5. Anomocytic stomata

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

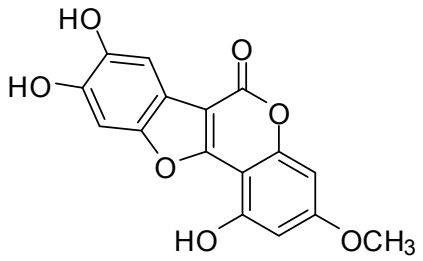

Figure 4 Chemical structure of wedelolactone

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

Standard solution

Wedelolactone standard solution for fingerprinting, Std-FP (16 mg/L)

Weigh 0.4 mg of wedelolactone CRS and dissolve in 25 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (50%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask 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. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.5% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	85 → 80	15 → 20	linear gradient
15 – 40	80 → 70	20 → 30	linear gradient
40 – 60	70 → 60	30 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of wedelolactone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of wedelolactone should not be more than 5.0%; the RSD of the retention time of wedelolactone

peak should not be more than 2.0%; the column efficiency determined from wedelolactone peak should not be less than 100000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.45	± 0.04
2	0.52	± 0.03
3	0.57	± 0.03
4 (marker, wedelolactone)	1.00	-

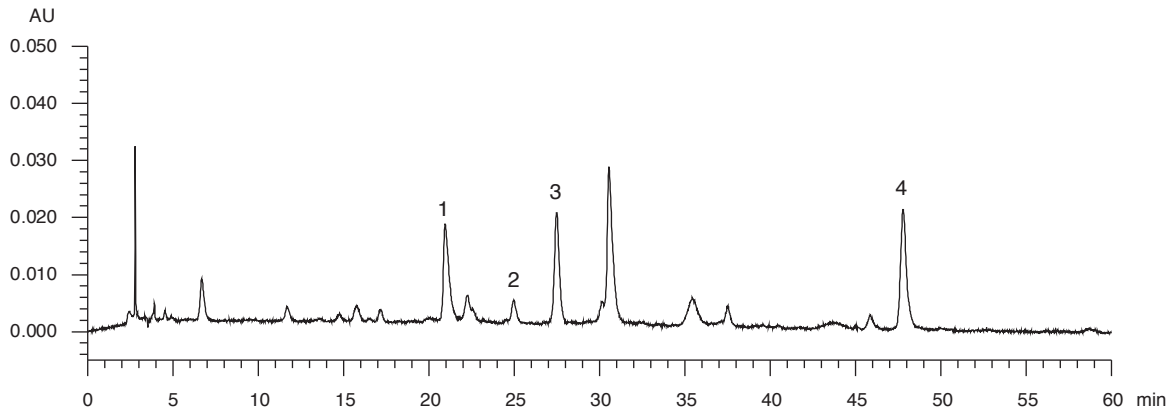

Figure 5 A reference fingerprint chromatogram of Ecliptae Herba extract

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. 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 XVIII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 18.0%.
Acid-insoluble ash: not more than 7.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 2.0%.

7. ASSAY

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

Standard solution

Wedelolactone standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of wedelolactone CRS and dissolve in 10 mL of methanol.

Wedelolactone standard solution for assay, Std-AS

Measure accurately the volume of the wedelolactone Std-Stock, dilute with methanol to produce a series of solutions of 4, 8, 16, 24, 32 mg/L for wedelolactone.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 25 mL of methanol. Reflux the mixture at 65°C for 3 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask 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 (351 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 0.5% acetic acid and acetonitrile (70:30, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of wedelolactone Std-AS (16 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of wedelolactone should not be more than 5.0%; the RSD of the retention time of wedelolactone peak should not be more than 2.0%; the column efficiency determined from wedelolactone peak should not be less than 10000 theoretical plates.

The *R* value between wedelolactone 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 wedelolactone Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of wedelolactone against the corresponding concentrations of wedelolactone 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 wedelolactone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of wedelolactone Std-AS. The retention times of wedelolactone peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of wedelolactone in the test solution, and calculate the percentage content of wedelolactone in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.043% of wedelolactone ($C_{16}H_{10}O_7$), calculated with reference to the dried substance.