Euphorbiae Hirtae Herba

Figure 1 A photograph of Euphorbiae Hirtae Herba

A. Euphorbiae Hirtae Herba (root ⟶)
B. Upper surface of leaves and flowers on an intact stem
C. Lower surface of leaves and flowers on an intact stem
D. Magnified image of capitulum
E. Magnified image of seeds

1. NAMES

Official name: Euphorbiae Hirtae Herba

Chinese name: 飛揚草

Chinese phonetic name: Feiyangcao

2. SOURCE

Euphorbiae Hirtae Herba is the dried whole plant of *Euphorbia hirta* L. (Euphorbiaceae). The whole plant is collected in summer and autumn, washed clean, then dried under the sun to obtain Euphorbiae Hirtae Herba.

3. DESCRIPTION

Main root slightly curved, bearing numerous rootlets. Stem subcylindrical, 15-55 cm long, 1-3 mm in diameter. Externally yellowish to brown, texture fragile, easily broken, hollow in the centre; aerial part covered by long hairs. Leaves opposite, crumpled, elliptic ovate to sub-rhombic when flattened out, 1-4 cm long, 0.5-1.3 cm wide; the upper surface greenish-brown, the lower surface pale greenish-brown, apex acute, base oblique, margin serrulate, 3 veins relatively distinct. Cymes numerous, head-like, axillary. Seeds ovate-triangle. Odour slight; taste slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of 2-8 layers of cells, containing reddish-brown contents and microcrystals of calcium oxalate. Cortex relatively narrow, few cells containing microcrystals of calcium oxalate. Phloem consists of several layers of cells. Laticiferous tubes scattered around the phloem. Cambium indistinct. Xylem occuping the majority of root, completely lignified, rays indistinct [Fig. 2 (i)].

Stem: Epidermis consists of 1 layer of cells, arranged in a ring, with multicellular non-glandular hairs on the surface. Cortex wide, scattered with laticiferous tubes. Laticiferous tubes scattered or aggregated, mainly observed beside phloem, sometimes scattered in cortex. Phloem narrow. Phloem fibres and laticiferous tubes found on the lateral part of phloem, arranged in an interrupt ring. Cambium indistinct. Xylem vessels lignified, linked up in a ring. Pith visible, parenchymatous cells subrounded and large [Fig. 2 (ii)].

Leaf: Upper epidermis consists of 1 layer of cells, rectangular to polygonal, containing pale brown to brown pigment granules (before staining). Palisade tissue consists of 1-2 layers of cells. Spongy tissue consists of 2-3 layers of cells. Vascular bundle of midrib has large bundles sheaths, collateral. Laticiferous tubes scattered around the vascular bundle and phloem. Microcrystals of calcium oxalate scattered in parenchymatous cells. Multicellular non-glandular hairs raised from the upper and lower epidermis. Lower epidermal cells relatively small [Fig. 2 (iii)].

Powder

Colour pale yellow. Multicellular non-glandular hair consists of 2-6 cells, apical 2 cells relatively long with protuberance on the surface, 2-30 μm in diameter. Pollen grains pale yellow, subrounded, 5-26 μm in diameter, smooth surface. Vessels 2-76 μm in diameter, mainly spiral vessels; reticulate and scalariform vessels also visible. Epidermal cells of stem polygonal, some with yellow and brown contents. Phloem fibres mostly singly scattered, long fusiform, 2-27 μm in diameter, with thick and lignified walls, pits indistinct; polychromatic under the polarized microscope. Cork cells colourless to pale brown, subpolygonal to subsquare on surface view, cork cells wall 1-10 μm in thickness. Microcrystals of calcium oxalate scattered, 2-31 μm in diameter; polychromatic under the polarized microscope. Palisade cells of testa in groups, colourless in the lateral view; 1-2 rows, slender cylindrical, arranged densely. Lower epidermal cells of leaf wavy and curved around the anticlinal wall, stomata mostly anisocytic (Fig. 3).

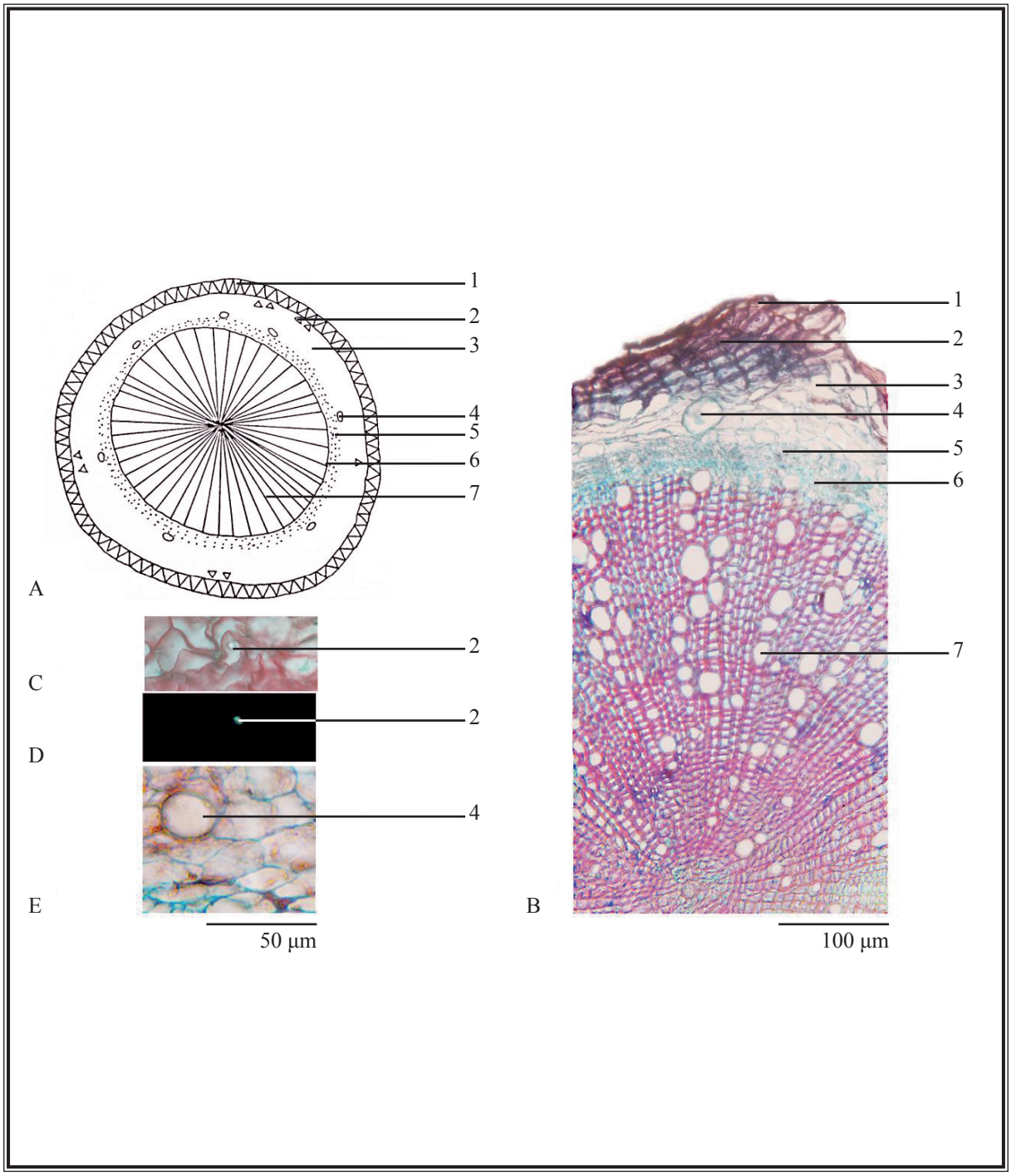


Figure 2 (i) Microscopic features of transverse section of root of Euphorbiae Hirtae Herba

A. Sketch B. Section illustration

C. Microcrystal of calcium oxalate (under the light microscope)

D. Microcrystal of calcium oxalate (under the polarized microscope) E. Laticiferous tube

1. Cork 2. Microcrystal of calcium oxalate 3. Cortex 4. Laticiferous tube

5. Phloem 6. Cambium 7. Xylem

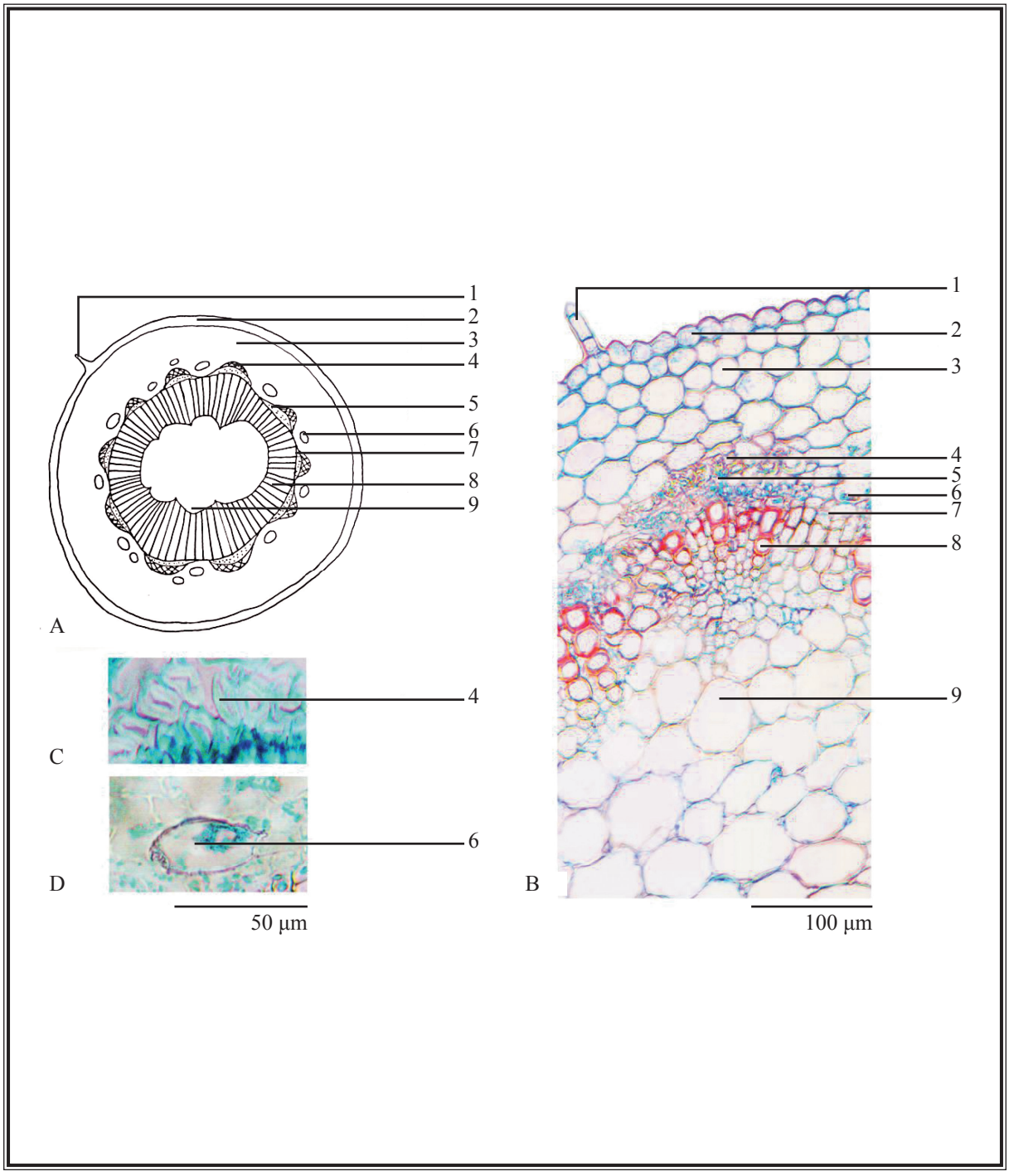


Figure 2 (ii) Microscopic features of transverse section of stem of Euphorbiae Hirtae Herba

A. Sketch B. Section illustration C. Phloem fibres D. Laticiferous tube

1. Multicellular non-glandular hair 2. Epidermis 3. Cortex 4. Phloem fibre
5. Phloem 6. Laticiferous tube 7. Cambium 8. Xylem 9. Pith

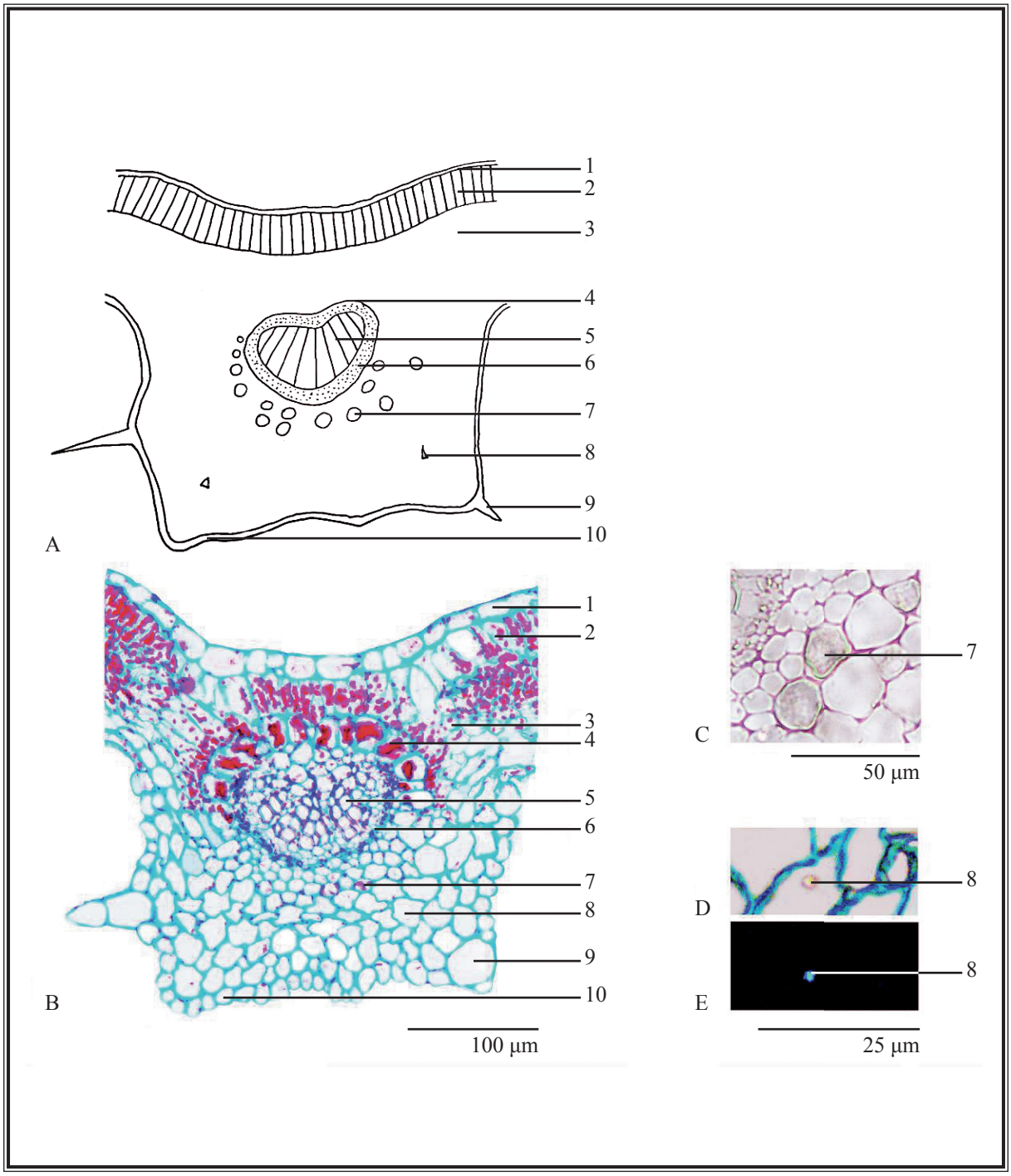


Figure 2 (iii) Microscopic features of transverse section of leaf of Euphorbiae Hirtae Herba

A. Sketch B. Section illustration C. Laticiferous tubes
D. Microcrystal of calcium oxalate (under the light microscope)
E. Microcrystal of calcium oxalate (under the polarized microscope)

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Vascular bundle sheath
5. Xylem 6. Phloem 7. Laticiferous tube 8. Microcrystal of calcium oxalate
9. Multicellular non-glandular hair 10. Lower epidermis

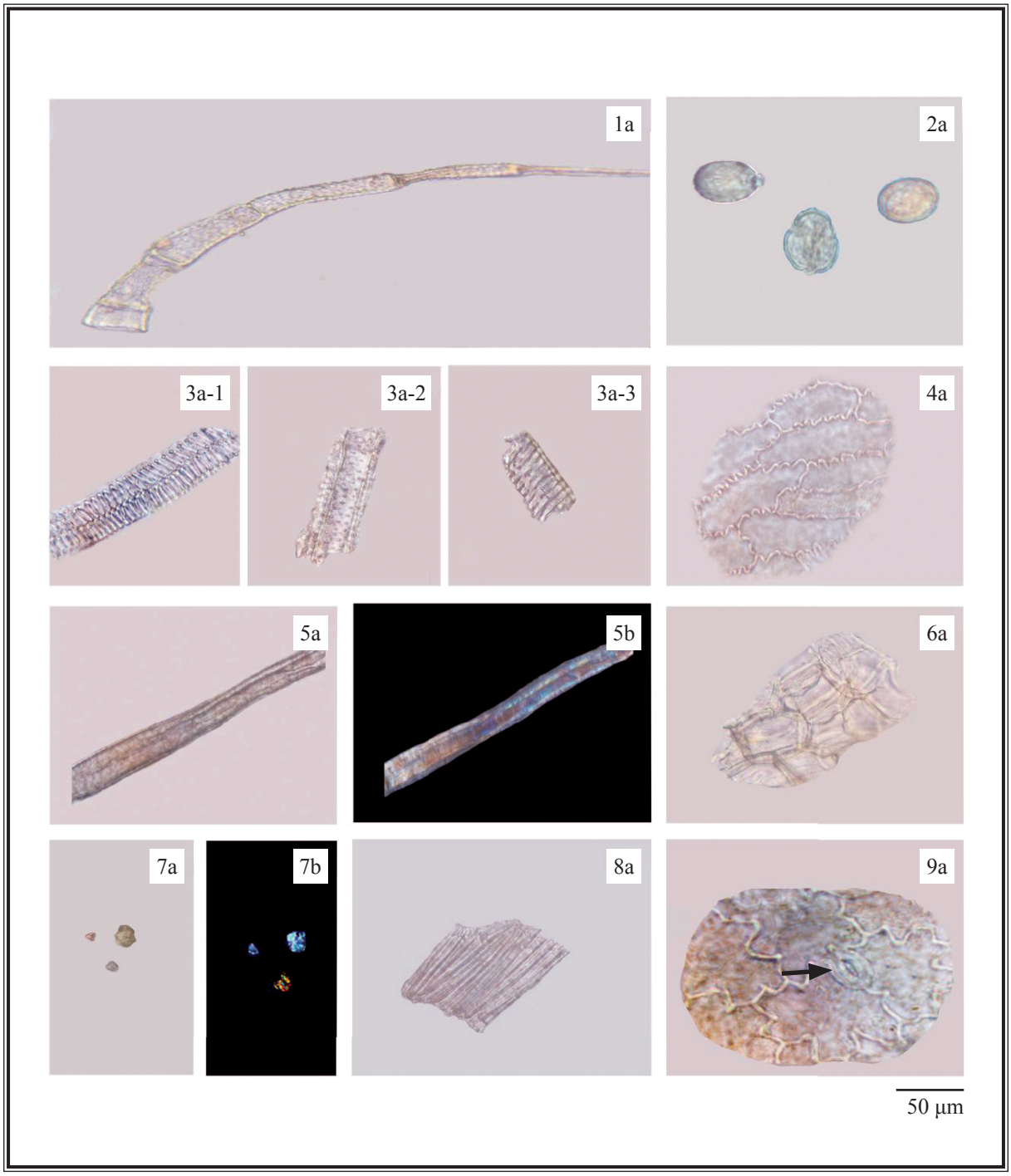


Figure 3 Microscopic features of powder of Euphorbiac Hirtae Herba

1. Multicellular non-glandular hair 2. Pollen grains
3. Vessels (3-1 spiral vessel, 3-2 reticulate vessel, 3-3 scalariform vessel)
4. Epidermal cells of stem 5. Phloem fibre
6. Cork cells 7. Microcrystals of calcium oxalate 8. Palisade cells of testa
9. Lower epidermal cells of leaf with stoma (→)

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

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

Standard solutions

β-Amyrin standard solution

Weigh 1.0 mg of β-amyrin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Lupeol standard solution

Weigh 0.5 mg of lupeol CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol and formic acid (6:3:3, v/v).

Spray reagent

Add slowly 5 mL of sulphuric acid to 95 mL of ethanol and dissolve 5 g of vanillin.

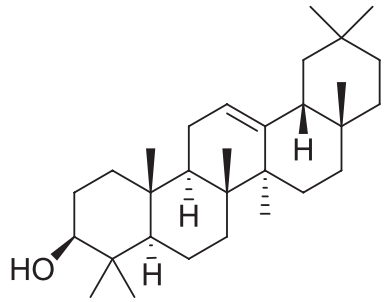
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol. Sonicate (200 W) the mixture for 15 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of ethanol. Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC RP-18 F_{254S} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately β-amyrin standard solution (6 μL), lupeol standard solution (3 μL) and the test solution (4 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 10 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 and bands become visible (about 10 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

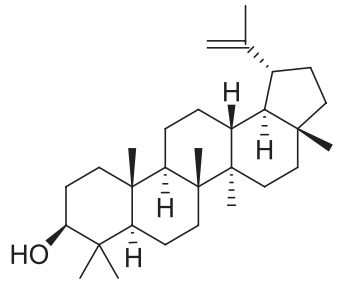


Figure 4 Chemical structures of (i) β-amyrin and (ii) lupeol

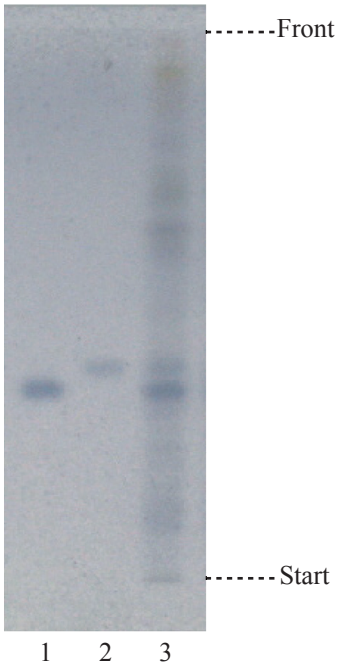


Figure 5 A reference HPTLC chromatogram of Euphorbiae Hirtae Herba extract observed under visible light after staining

1. β-Amyrin standard solution 2. Lupeol 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 β-amyrin and lupeol (Fig. 5).

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

Standard solutions

β-Amyrin standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of β-amyrin CRS and dissolve in 10 mL of ethanol.

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

Weigh 0.5 mg of lupeol CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (200 W) the mixture for 45 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction two more times each with 7 mL of ethanol. Combine the supernatants and make up to the mark with ethanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (202 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (3.5 μm particle size, 130 Å pore size and 185 m^2/g surface area). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.7 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.01% Phosphoric acid (%, v/v)	Elution
0 – 15	71 → 76	29 → 24	linear gradient
15 – 60	76 → 62	24 → 38	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of β-amyrin Std-FP and lupeol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of β-amyrin and lupeol should not be more than 5.0%; the RSD of the retention times of β-amyrin and lupeol peaks should not be more than 2.0%; the column efficiencies determined from β-amyrin and lupeol peaks should not be less than 4000 and 9000 theoretical plates respectively.

The *R* value between peak 3 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 β-amyrin Std-FP, lupeol Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of β-amyrin and lupeol peaks in the chromatograms of β-amyrin Std-FP, lupeol Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify β-amyrin and lupeol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of β-amyrin Std-FP and lupeol Std-FP. The retention times of β-amyrin and lupeol 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 Euphorbiae Hirtae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.33	± 0.03
2	0.39	± 0.03
3 (lupeol)	0.71	± 0.03
4 (marker, β-amyrin)	1.00	-

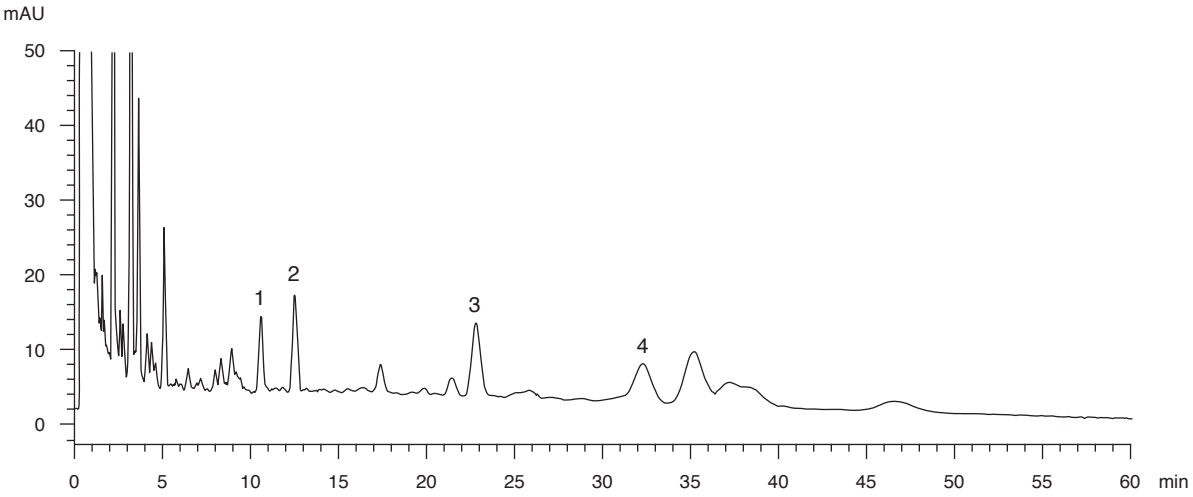


Figure 6 A reference fingerprint chromatogram of Euphorbiae Hirtae Herba extract

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)*: 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 XVI)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 9.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

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

Mixed β-amyrin and lupeol standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 5.0 mg of β-amyrin CRS and 5.0 mg of lupeol CRS, and dissolve in 5 mL of ethanol.

Mixed β-amyrin and lupeol standard solution for assay, Std-AS

Measure accurately the volume of the mixed β-amyrin and lupeol Std-Stock, dilute with ethanol to produce a series of solutions of 5, 25, 50, 100, 200 mg/L for both β-amyrin and lupeol.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (200 W) the mixture for 45 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction two more times each with 7 mL of ethanol. Combine the supernatants and make up to the mark with ethanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (202 nm) and a column (2.1 × 100 mm) packed with ODS bonded silica gel (3.5 μm particle size, 130 Å pore size and 185 m^2/g surface area). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.7 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.01% Phosphoric acid (%, v/v)	Elution
0 – 15	71 → 76	29 → 24	linear gradient
15 – 60	76 → 62	24 → 38	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed β-amyrin and lupeol Std-AS (50 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of β-amyrin and lupeol should not be more than 5.0%; the RSD of the retention times of β-amyrin and lupeol should not be more than 2.0%; the column efficiencies determined from β-amyrin and lupeol peaks should not be less than 4000 and 9000 theoretical plates respectively.

The *R* value between β-amyrin peak and the closest peak; and the *R* value between lupeol 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 β-amyrin and lupeol Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of β-amyrin and lupeol against the corresponding concentrations of the mixed β-amyrin and lupeol Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify β-amyrin and lupeol peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed β-amyrin and lupeol Std-AS. The retention times of β-amyrin and lupeol 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 β-amyrin and lupeol in the test solution, and calculate the percentage contents of β-amyrin and lupeol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.094% of total content of β-amyrin ($C_{30}H_{50}O$) and lupeol ($C_{30}H_{50}O$), calculated with reference to the dried substance.

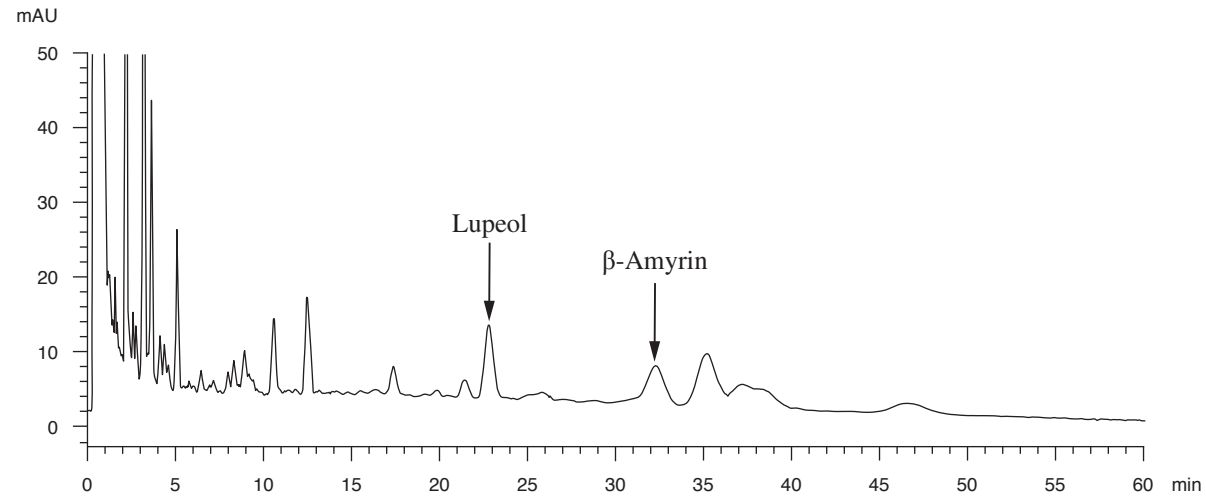


Figure 7 A reference assay chromatogram of Euphorbiae Hirtae Herba extract