Saururi Herba

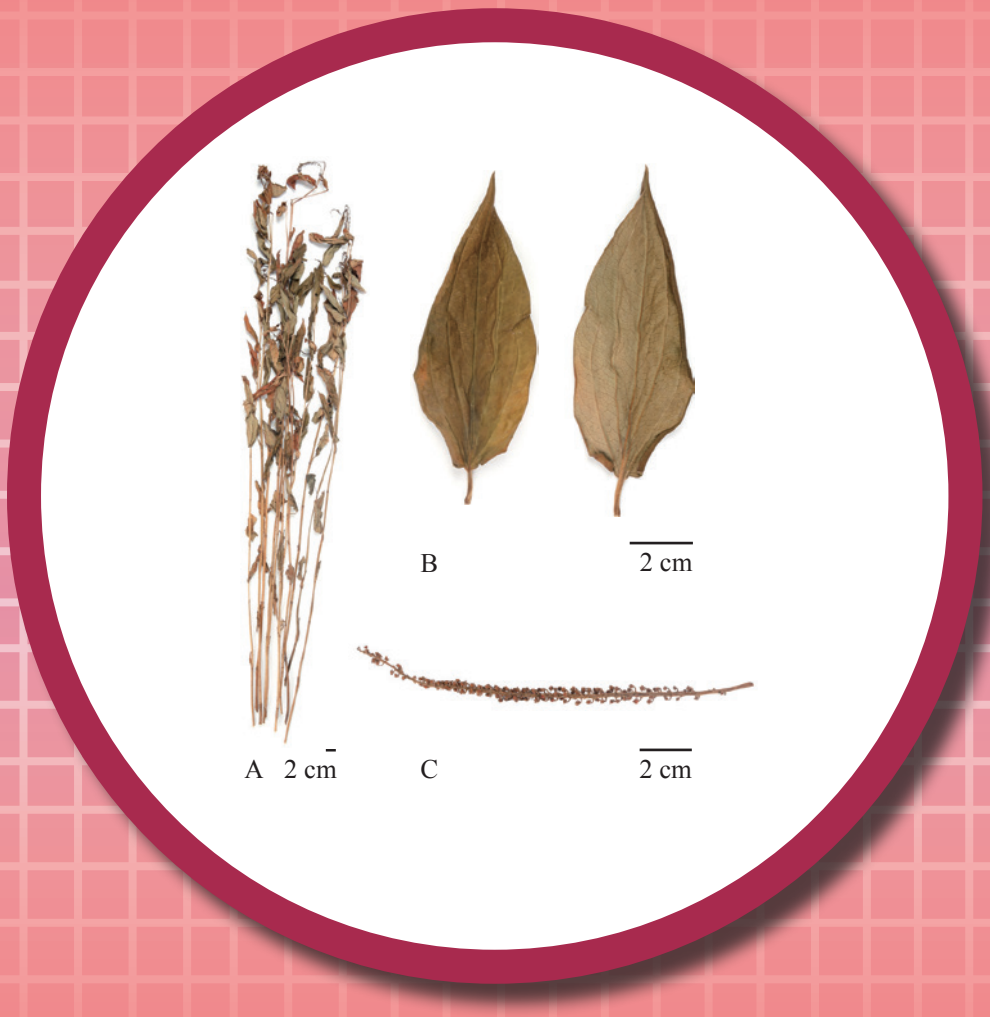

Figure 1 A photograph of Saururi Herba

A. Saururi Herba

B. Magnified image of leaves (left: upper surface, right: lower surface)

C. Racemes

1. NAMES

Official Name: Saururi Herba

Chinese Name: 三白草

Chinese Phonetic Name: Sanbaicao

2. SOURCE

Saururi Herba is the dried aerial part of *Saururus chinensis* (Lour.) Baill. (Saururaceae). The aerial part is collected in summer and autumn, washed clean, then dried under the sun to obtain Saururi Herba.

3. DESCRIPTION

Stems cylindrical, with 4 longitudinal furrows, one of them relatively broad. Fracture yellowish-brown to brown, fibrous and hollow. Leaves simple, alternate, lamina ovate to ovate-lanceolate, 3-12 cm long, 2-12 cm wide; acuminate at apex, cordate at base, margins entire, 5-basinerved; petiole relatively long, with longitudinal wrinkles. Racemes at the apex of branch, opposite to leaves, 6-12 cm long, flowers small, brown. Capsules subspheroidal. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of subsquare cells. Subrounded parenchymatous cells located beneath the epidermis, arranged in reticulate pattern with large intercellular spaces, form the cortex, with large cellular space. Oil cells and secretory ducts scattered in cortex, oil cells contain yellow oil droplets, while secretory ducts contain pale brown contents. Pericyclic fibre 3-7 rows, arranged in a ring or an interrupted ring lining the outerside of the vascular bundle. Vascular bundles collateral, fibre bundle located beneath the xylem. Pith broad, scattered with oil cells and clusters of calcium oxalate, aerenchyma occasionally found [Fig. 2 (i)].

Leaf: Upper and lower epidermis each consists of 1 layer of cells. 1 layer of large parenchymatous cells located beneath the upper epidermis. Palisade tissue consists of 1-2 layers of cells, containing brown contents. Cells of spongy tissue arranged loosely. Vascular bundle of midvein bicollateral, with the xylem made up of vessels and fibres. Vascular bundle sheath consists of several layers of sclerenchymatous cells, forming a ring enclosing the bicollateral vascular bundle. Several layers of collenchymatous cells exist between the midrib and lower epidermis. Glandular hairs present on the surface of lower epidermis. Oil cells and clusters of calcium oxalate scattered in parenchyma [Fig. 2 (ii)].

Powder

Colour green. Secretory ducts 35-42 μm wide, containing brown or reddish-brown substances, brown substances 5-40 μm in diameter, surrounding by parenchymatous cells with beaded-thickened walls occasionally, some with visible pits. Oil cells contain yellow oil droplets. Clusters of calcium oxalate numerous, scattered in parenchymatous cells, 10-20 μm in diameter; polychromatic under the polarized microscope. Fibres straight or curved, 12-25 μm in diameter, walls relatively thick, some with visible pits and pit canals. Upper and lower epidermal cells slightly polygonal, stomata absent in upper epidermis and numerous in lower epidermis. Glandular hairs 2-3 celled. Vessels mainly reticulate, scalariform and spiral vessels also visible (Fig. 3).

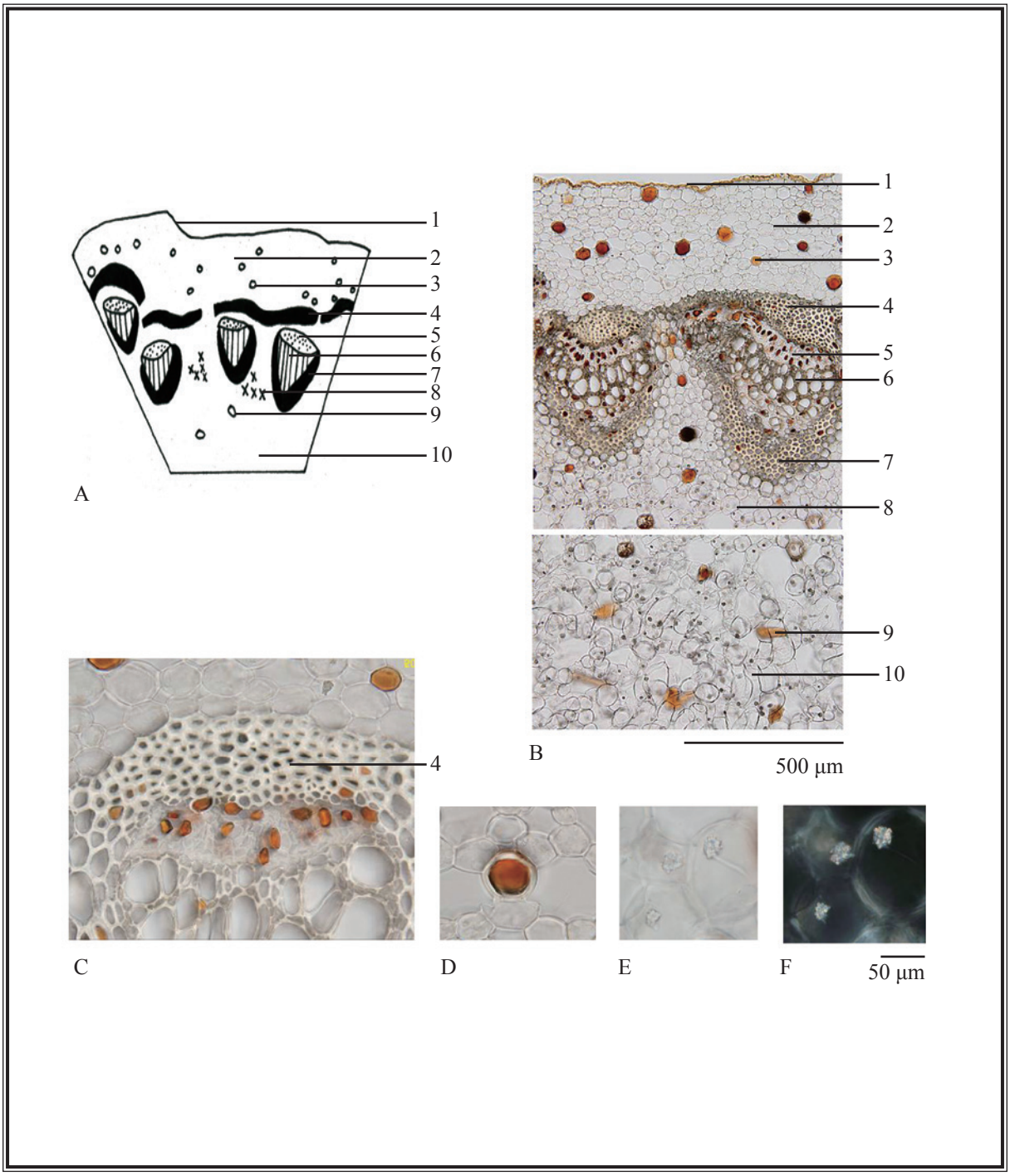

Figure 2 (i) Microscopic features of transverse section of stem of Saururi Herba

A. Sketch B. Section illustration C. Pericyclic fibres D. Oil cell
E. Clusters of calcium oxalate F. Clusters of calcium oxalate (under the polarized microscope)

1. Epidermis 2. Cortex 3. Oil cell 4. Pericyclic fibre 5. Phloem
6. Xylem 7. Fibre bundle 8. Cluster of calcium oxalate 9. Secretory duct 10. Pith

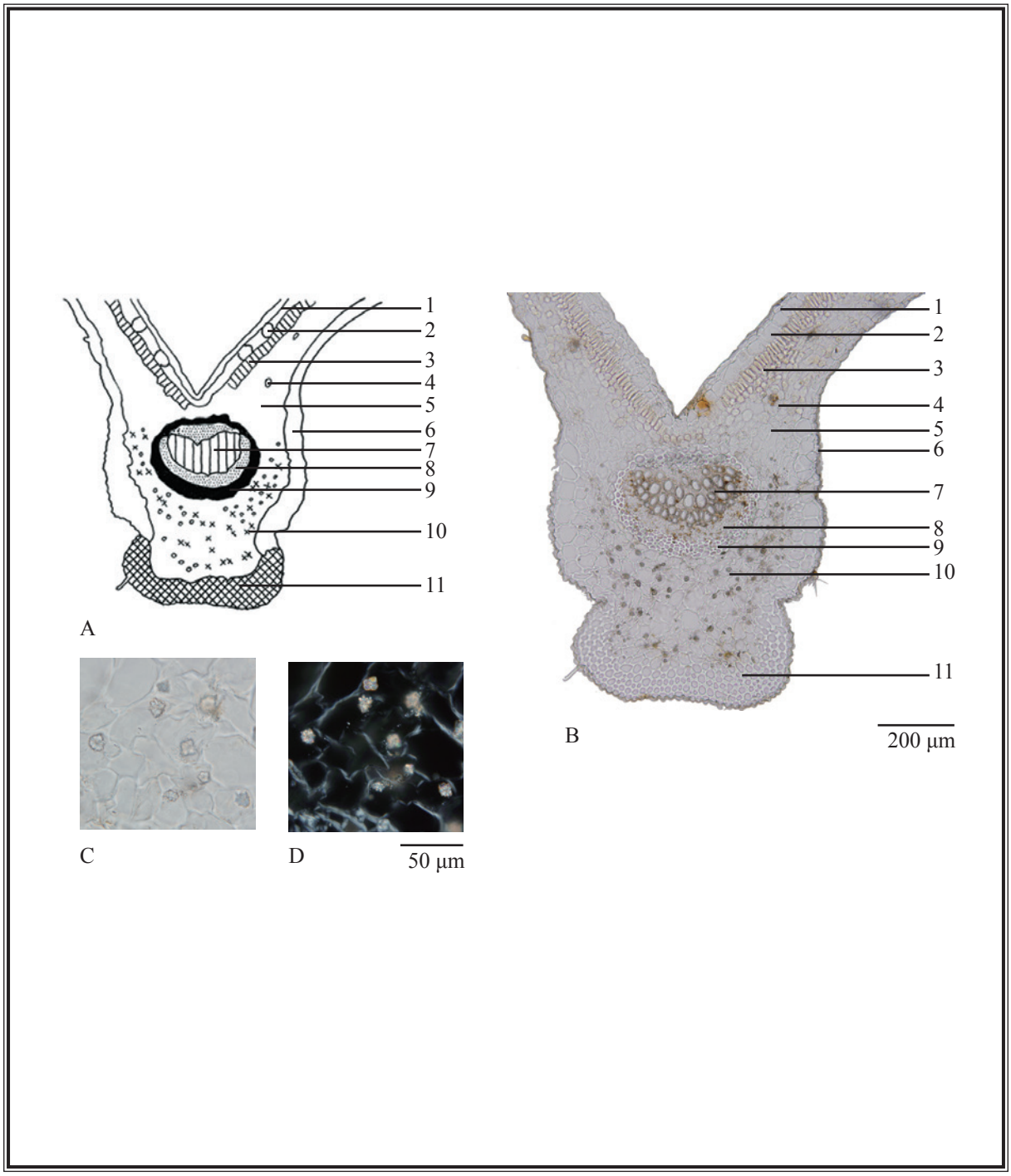

Figure 2 (ii) Microscopic features of transverse section of leaf of Saururi Herba

A. Sketch B. Section illustration C. Clusters of calcium oxalate
D. Clusters of calcium oxalate (under the polarized microscope)

1. Upper epidermis 2. Large parenchymatous cell 3. Palisade tissue 4. Oil cell
5. Spongy tissue 6. Lower epidermis 7. Xylem 8. Phloem 9. Vascular bundle sheath
10. Cluster of calcium oxalate 11. Collenchyma

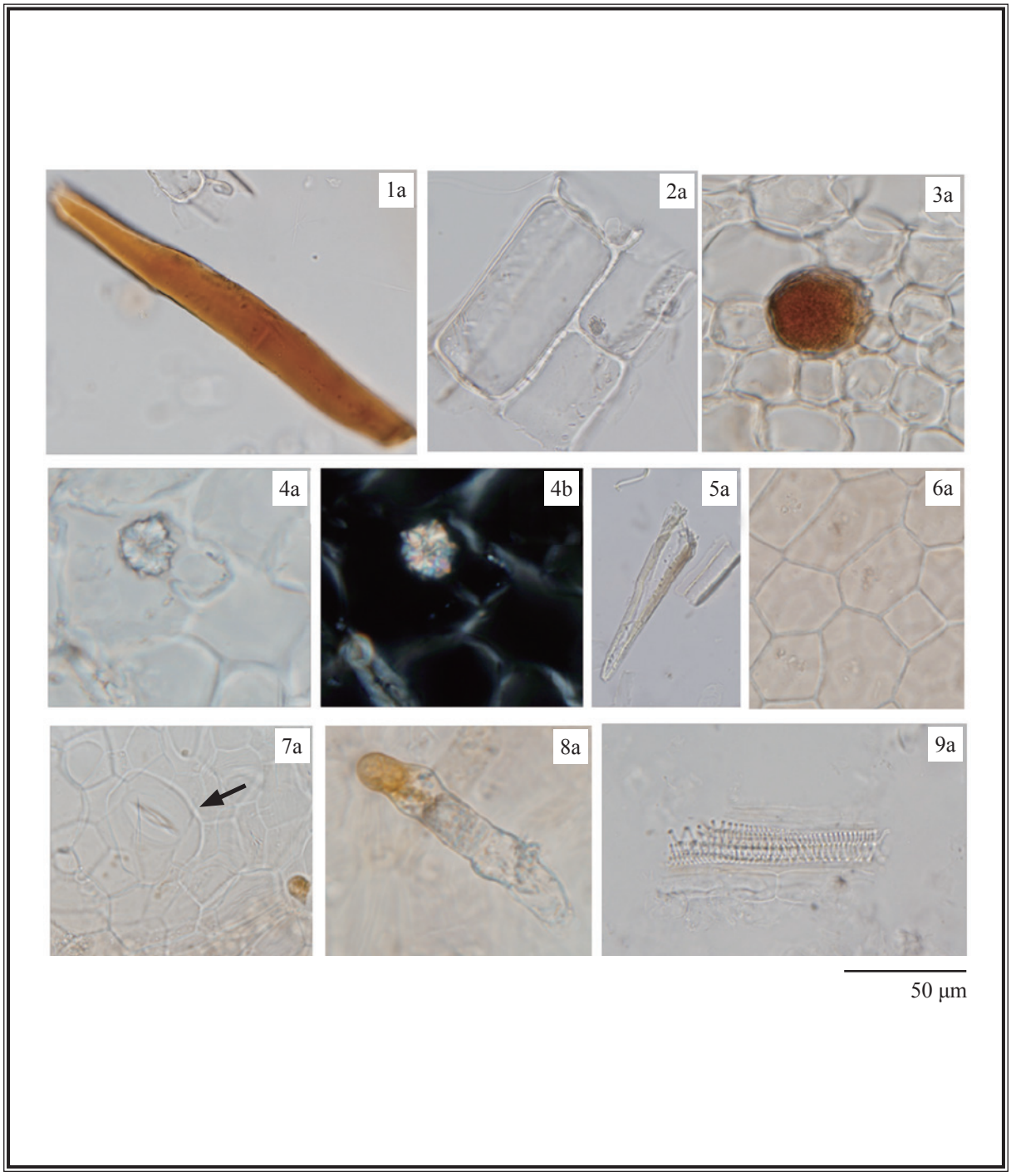

Figure 3 Microscopic features of powder of Saururi Herba

1. Secretory duct (containing brown or reddish-brown substances) 2. Parenchymatous cells
3. Oil cell 4. Cluster of calcium oxalate 5. Fibres 6.Upper epidermal cells
7. Lower epidermal cells with stoma (⟶) 8. Glandular hair 9. Vessels

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

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

Standard solution

Sauchinone standard solution

Weigh 1.0 mg of sauchinone CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), acetone and ethyl acetate (5:2:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 150-mL conical flask, then add 30 mL of methanol. Sonicate (250 W) the mixture for 20 min. Filter and transfer the filtrate to a 150-mL round-bottomed flask. Concentrate the filtrate to about 2 mL at reduced pressure in a rotary evaporator. Transfer the solution to the neutral alumina and activated charcoal column [a glass column with internal diameter of 1.0 cm, dry packing the column with mixture of 0.4 g of activated charcoal (mesh number of 20-40) and 4 g of neutral alumina (mesh number of 100-200)]. Add 60 mL of methanol to the column. Collect the eluant and transfer to a 150-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethyl acetate.

Procedure

Carry out the method by using a HPTLC silica gel G plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately sauchinone standard solution and the test solution (10 µL each) 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. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 6 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

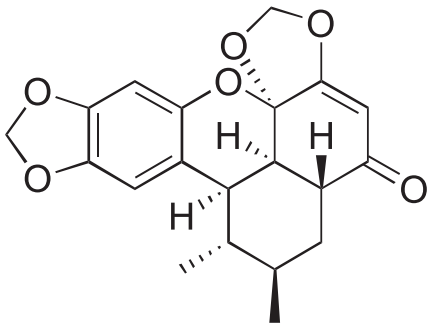

Figure 4 Chemical structure of sauchinone

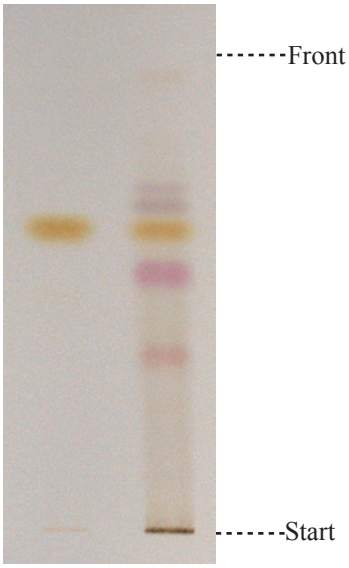

Figure 5 A reference HPTLC chromatogram of Saururi Herba extract observed under visible light after staining

1. Sauchinone 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 sauchinone (Fig. 5).

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

Standard solution

Sauchinone standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.5 mg of sauchinone CRS and dissolve in 25 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 150-mL conical flask, then add 25 mL of methanol. Reflux the mixture in a boiling water bath for 1 h. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	Water (%, v/v)	Elution
0 – 5	17	83	isocratic
5 – 25	17 → 26	83 → 74	linear gradient
25 – 45	26 → 60	74 → 40	linear gradient
45 – 60	60	40	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of sauchinone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of sauchinone should not be more than 5.0%; the RSD of the retention time of sauchinone peak should not be more than 2.0%; the column efficiency determined from sauchinone peak should not be less than 100000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.26	± 0.03
2	0.32	± 0.03
3 (marker, sauchinone)	1.00	-
4	1.05	± 0.03

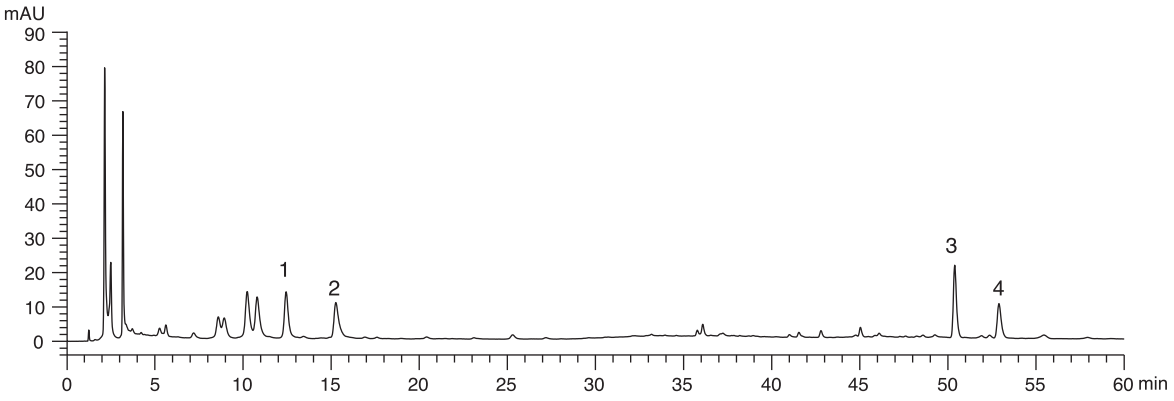

Figure 6 A reference fingerprint chromatogram of Saururi Herba extract

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 – Aflatoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 11.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 11.0%.
Ethanol-soluble extractives (hot extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Sauchinone standard stock solution, Std-Stock (400 mg/L)
Weigh accurately 4.0 mg of sauchinone CRS and dissolve in 10 mL of methanol.
Sauchinone standard solution for assay, Std-AS
Measure accurately the volume of the sauchinone Std-Stock, dilute with methanol to produce a series of solutions of 2.5, 5, 10, 20, 40 mg/L for sauchinone.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 150-mL conical flask, then add 25 mL of methanol. Reflux the mixture in a boiling water bath for 1 h. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	Water (%, v/v)	Elution
0 – 5	17	83	isocratic
5 – 25	17 → 26	83 → 74	linear gradient
25 – 45	26 → 60	74 → 40	linear gradient
45 – 60	60	40	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of sauchinone Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of sauchinone should not be more than 5.0%; the RSD of the retention time of sauchinone peak should not be more than 2.0%; the column efficiency determined from sauchinone peak should not be less than 100000 theoretical plates.

The *R* value between sauchinone 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 sauchinone Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of sauchinone against the corresponding concentrations of sauchinone 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 sauchinone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sauchinone Std-AS. The retention times of sauchinone 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 sauchinone in the test solution, and calculate the percentage content of sauchinone in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.11% of sauchinone ($C_{20}H_{20}O_6$), calculated with reference to the dried substance.

Saururi Herba（三白草）

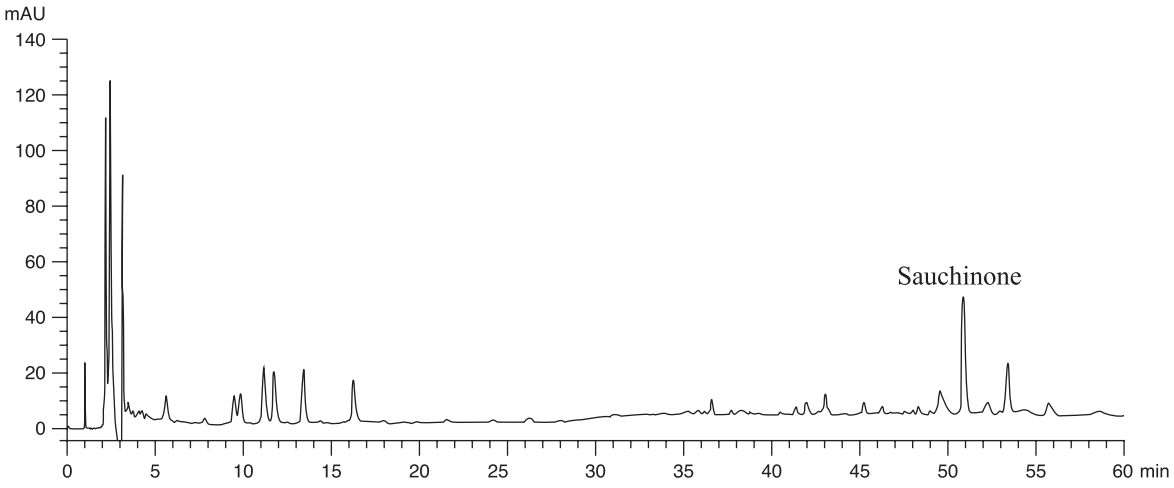

Figure 1 A reference assay chromatogram of Saururi Herba extract