Salviae Plebeiae Herba

Figure 1 A photograph of Salviae Plebeiae Herba

A. Salviae Plebeiae Herba B. Branch and leaves
C. Magnified image of inflorescence D. Magnified image of leaves
E. Magnified image of lower surface of leaf F. Magnified image of flowers

1. NAMES

Official name: Salviae Plebeiae Herba

Chinese name: 荔枝草

Chinese phonetic name: Lizhicao

2. SOURCE

Salviae Plebeiae Herba is the dried aerial part of *Salvia plebeia* R. Br. (Lamiaceae). The aerial part is collected in summer and autumn while flowering and leaf being green, foreign matter removed, then dried under the sun to obtain Salviae Plebeiae Herba.

3. DESCRIPTION

Stem square-columnar, frequently branched, 22-97 cm long, 1-7 mm in diameter; externally greyish-green to brown, covered with short pubescence; fracture pale white, hollow in the centre. Leaves opposite, lamina usually crumpled, when whole, elliptic-ovate to elliptic-lanceolate, 1.5-9.7 cm long, 0.4-3.5 cm wide, margin dentate, both surfaces pubescent; petiole 0.4-6.8 cm long. Verticillaster spicate, apical or altar, corolla easily dropped; calyx campanulate, persistent, around 2-5.5 mm long, greyish-green to pale brown. Texture fragile, slight. Odour aromatic; taste bitter, pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of 1 layer of subsquare or rectangular cells; remnants of glandular hairs and non-glandular hairs sometimes present on epidermis. Collenchyma locate on the outer side of cortex in arrises, cells subpolygonal or irregular polygonal, thickened at corners. Cortex very narrow, sometimes indistinct. Pericycle fibre bundles locate in cortex, arranged in an interrupted ring, abundant near arrises. Phloem very narrow, cells relatively small. Xylem radially arranged, broad in arrises; medullary rays distinct. Pith large, usually hollow in the centre, consisting of large parenchymatous cells [Fig. 2 (i)].

Leaf: Upper epidermis consists of 1 layer of flat rectangular cells. Non-glandular hair frequently observed on the surface of upper and lower epidermis. Palisade tissue consists of 1-2 layers of cells. Spongy tissue consists of loosely arranged irregular cells, extended into both sides of the main vein. Collenchyma locate underneath the upper and lower epidermis of midrib, cells thickened at corners. Vascular bundles of midrib collateral; vessels 2-6 cells, radially arranged. Phloem parenchymatous cells polygonal. Lower epidermis consists of 1 layer of flat rounded or irregular cells, cells relativelly small [Fig. 2 (ii)].

Powder

Colour yellowish-brown. Non-glandular hairs numerous, composed of 2-5 cells, 7-59 μm in diameter; basal cells usually enlarged, apical cells acute. Head of glandular hairs unicellular, ovate or spherical, 7-27 μm in diameter, sometimes with yellowish-brown contents, stalk unicellular or multicellular. Glandular scales numerous, greyish, yellowish-brown or colourless, composed of 4 cells, subglobose, 18-64 μm in diameter. Upper epidermal cells irregular polygonal in surface view, anticlinal walls nearly straight, stomata diacytic. Lower epidermal cells irregular polygonal in surface view, anticlinal walls sinuous, stomata diacytic or anomocytic. Pericycle fibres mostly in bundles, septate, lignified. Vessels numerous, mainly bordered-pitted and reticulate type, 6-60 μm in diameter. Raphides of calcium oxalates scattered in mesophyll cells, fine, 2-11 μm in diameter; bright white under the polarized microscope. Prisms of calcium oxalates abundantly exist in tracts, scattered in lower epidermal cells, 2-8 μm in diameter; polychromatic or bright white under the polarized microscope. Pollen grains occasionally observed, 20-39 μm in diameter; two types in shape, one subspherical, with spiny exine and germinal pores; another elliptic, with furrows, surface bearing reticulate striations (Fig. 3).

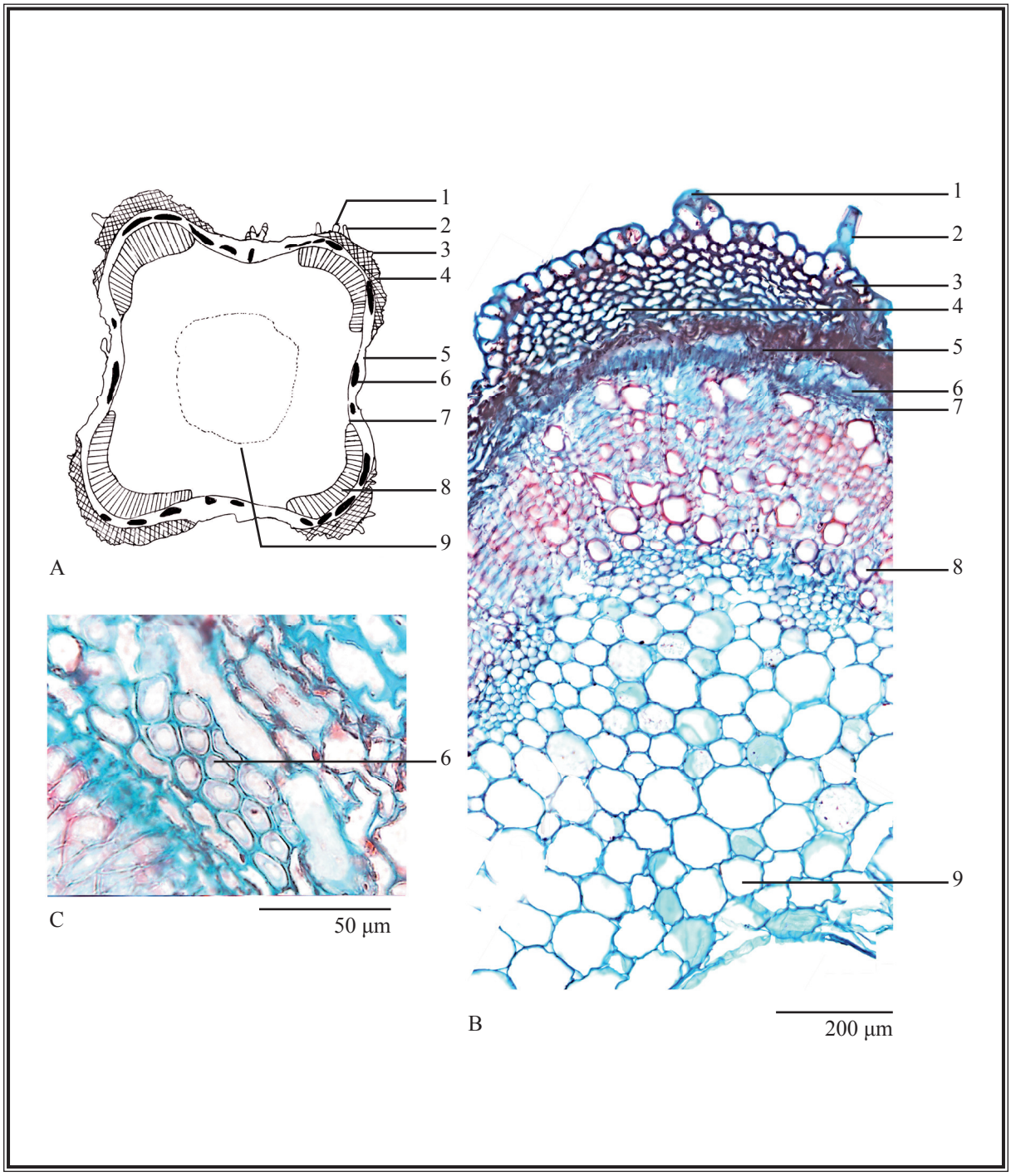


Figure 2 (i) Microscopic features of transverse section of stem of Salviae Plebeiae Herba

A. Sketch B. Section illustration
C. Pericycle fibre bundle

1. Glandular hair 2. Non-glandular hair 3. Epidermis 4. Collenchyma
5. Cortex 6. Pericycle fibre bundle 7. Phloem 8. Xylem 9. Pith

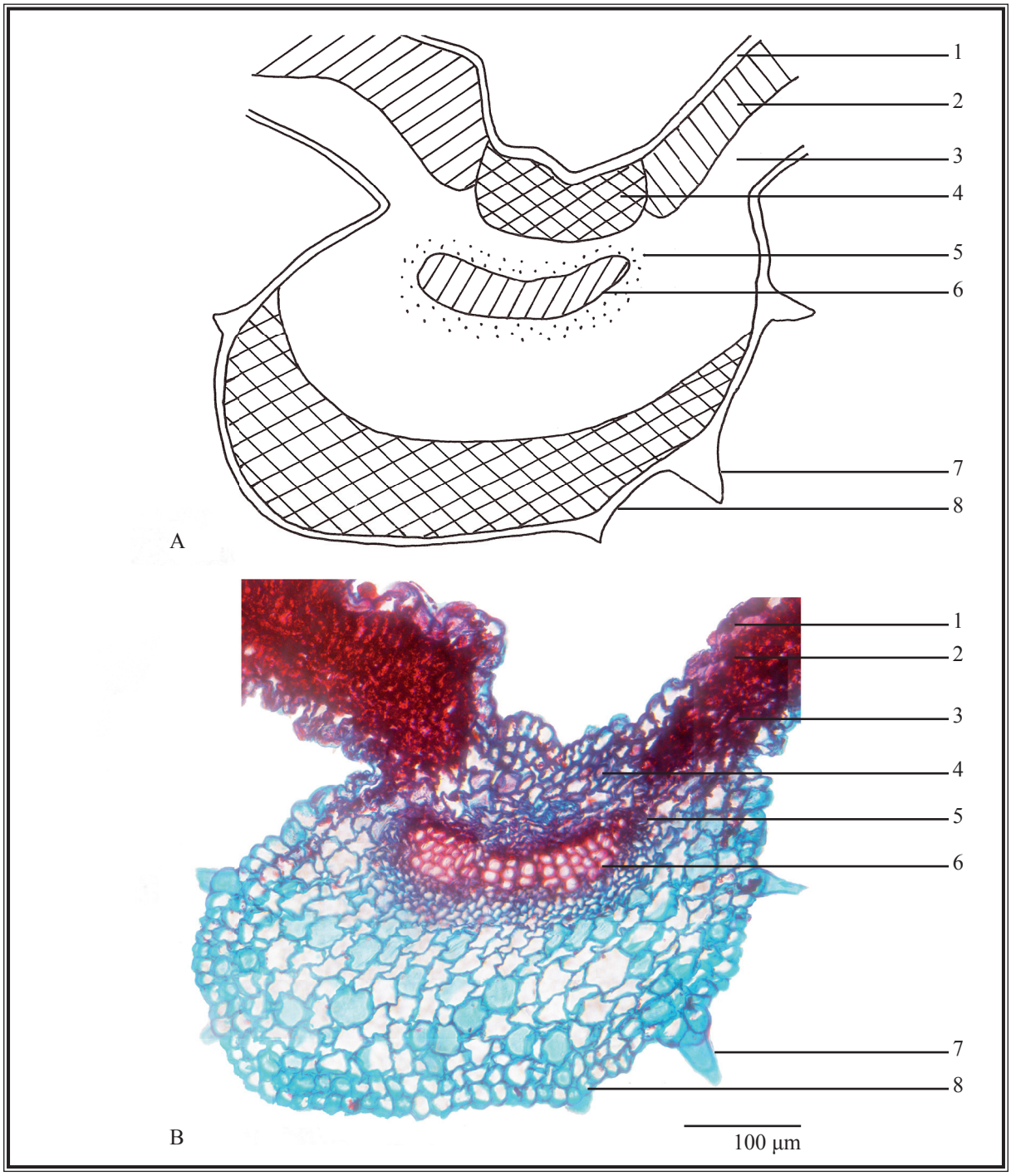


Figure 2 (ii) Microscopic features of transverse section of leaf of Salviae Plebeiae Herba

A. Sketch B. Section illustration

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

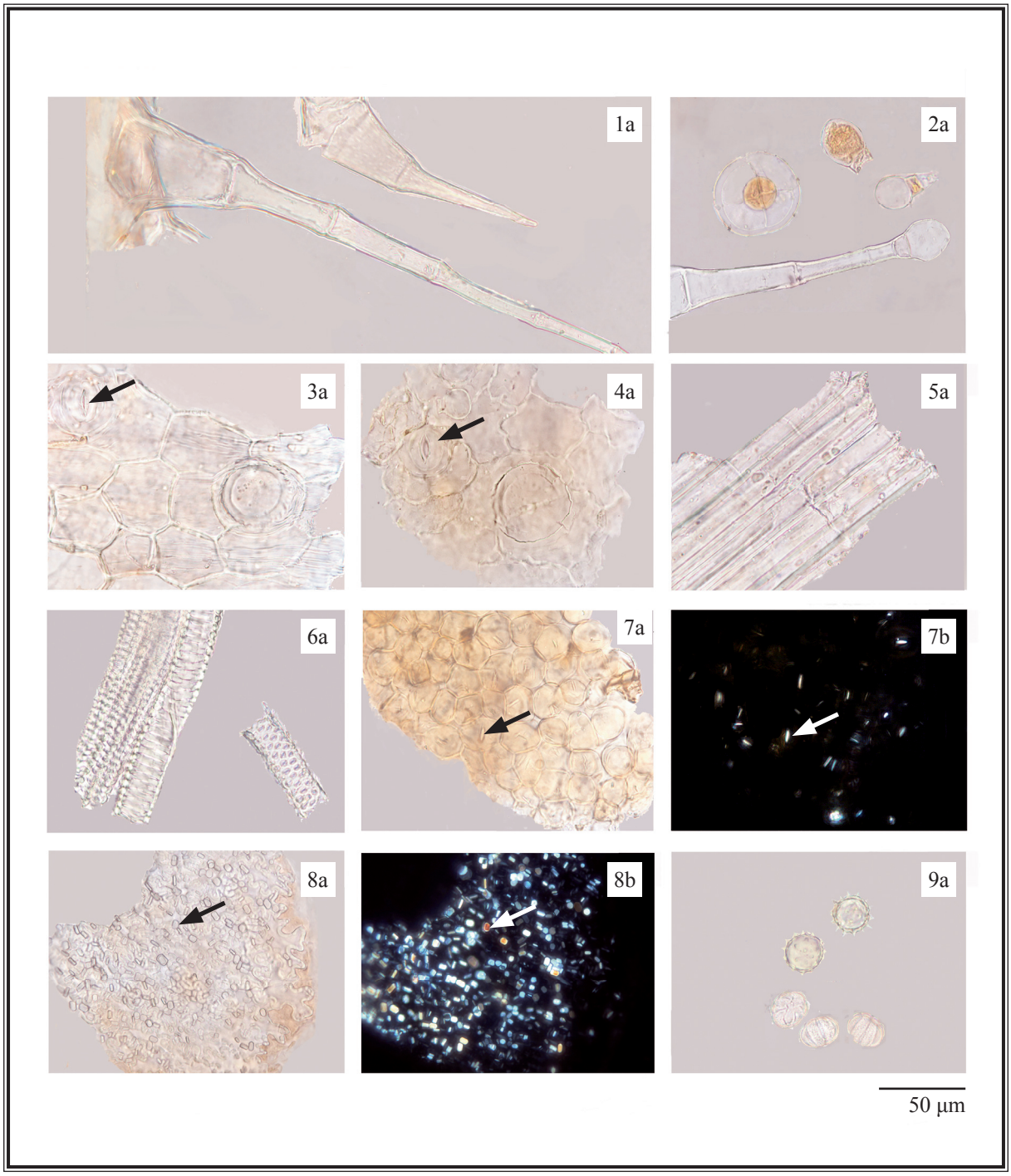


Figure 3 Microscopic features of powder of Salviae Plebeiae Herba

1. Non-glandular hairs 2. Glandular hairs and glandular scale
3. Upper epidermal cells with diacytic stoma (⟶)
4. Lower epidermal cells with anomocytic stoma (⟶) 5. Fibres
6. Reticulate and bordered-pitted vessels 7. Raphides of calcium oxalate (⟶) in mesophyll cells
8. Prisms of calcium oxalate (⟶) in lower epidermal cells 9. Pollen grains

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

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

Standard solution

Homoplantaginin standard solution

Weigh 0.5 mg of homoplantaginin CRS (Fig. 4) and dissolve in 2 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of dichloromethane, methanol, formic acid and water (11:1.4:0.1:0.1, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

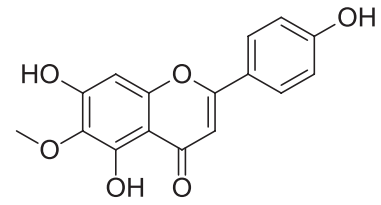
Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (50%). Sonicate (400 W) the mixture for 10 min. Filter the mixture.

Procedure

Carry out the method by using a TLC polyamide plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately homoplantaginin standard solution (2 μL) and the test solution (5 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC 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 70ºC (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

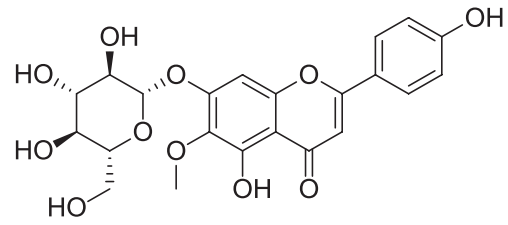


Figure 4 Chemical structures of (i) hispidulin and (ii) homoplantaginin

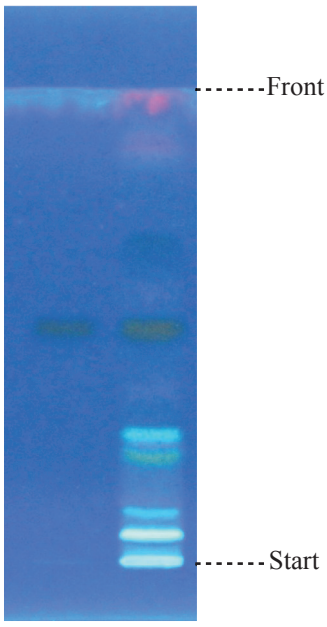


Figure 5 A reference TLC chromatogram of Salviae Plebeiae Herba extract observed under UV light (366 nm) after staining

1. Homoplantaginin 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 homoplantaginin (Fig. 5).

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

Standard solutions

Hispidulin standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.5 mg of hispidulin CRS (Fig. 4) and dissolve in 100 mL of ethanol (50%).

Homoplantaginin standard solution for fingerprinting, Std-FP (15 mg/L)

Weigh 1.5 mg of homoplantaginin CRS and dissolve in 100 mL of ethanol (50%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction two more times. Wash the residue with ethanol (50%). Combine the extracts and make up to the mark with ethanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (342 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)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	65	35	isocratic
10 – 30	65 → 55	35 → 45	linear gradient
30 – 40	55 → 45	45 → 55	linear gradient
40 – 60	45 → 30	55 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL of hispidulin Std-FP and homoplantaginin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hispidulin and homoplantaginin should not be more than 5.0%; the RSD of the retention times of hispidulin and homoplantaginin peaks should not be more than 2.0%; the column efficiencies determined from hispidulin and homoplantaginin peaks should not be less than 140000 and 24000 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 hispidulin Std-FP, homoplantaginin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of hispidulin and homoplantaginin peaks in the chromatograms of hispidulin Std-FP, homoplantaginin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify hispidulin and homoplantaginin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of hispidulin Std-FP and homoplantaginin Std-FP. The retention times of hispidulin and homoplantaginin 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 five characteristic peaks of Salviae Plebeiae Herba extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.76	± 0.03
2	0.92	± 0.03
3 (marker, homoplantaginin)	1.00	-
4 (hispidulin)	1.72	± 0.03
5	1.92	± 0.03

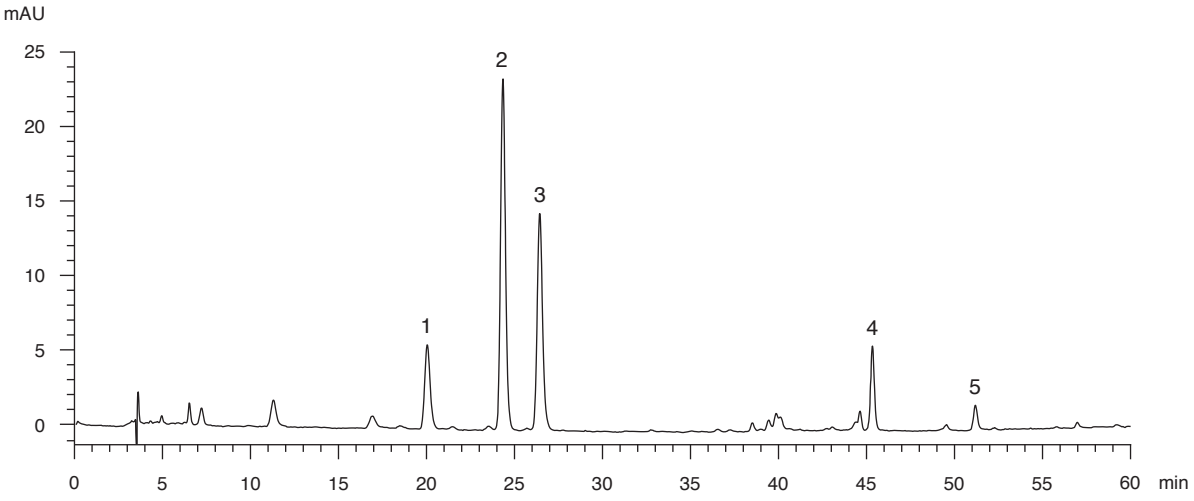


Figure 6 A reference fingerprint chromatogram of Salviae Plebeiae Herba extract

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. 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 13.5%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

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

Standard solution

Mixed hispidulin and homoplantaginin standard stock solution, Std-Stock (100 mg/L for hispidulin and 300 mg/L for homoplantaginin)

Weigh accurately 1.0 mg of hispidulin CRS and 3.0 mg of homoplantaginin CRS, and dissolve in 10 mL of ethanol (50%).

Mixed hispidulin and homoplantaginin standard solution for assay, Std-AS

Measure accurately the volume of the mixed hispidulin and homoplantaginin Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 0.5, 1, 3, 9, 15 mg/L for hispidulin and 1.5, 3, 9, 27, 45 mg/L for homoplantaginin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction two more times. Wash the residue with ethanol (50%). Combine the extracts and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (342 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	65	35	isocratic
10 – 30	65 → 55	35 → 45	linear gradient
30 – 40	55 → 45	45 → 55	linear gradient
40 – 60	45 → 30	55 → 70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed hispidulin and homoplantaginin Std-AS (3 mg/L for hispidulin and 9 mg/L for homoplantaginin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hispidulin and homoplantaginin should not be more than 5.0%; the RSD of the retention times of hispidulin and homoplantaginin peaks should not be more than 2.0%; the column efficiencies determined from hispidulin and homoplantaginin peaks should not be less than 140000 and 24000 theoretical plates respectively.

The *R* value between hispidulin peak and the closest peak; and the *R* value between homoplantaginin 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 hispidulin and homoplantaginin Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of hispidulin and homoplantaginin against the corresponding concentrations of the mixed hispidulin and homoplantaginin 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 hispidulin and homoplantaginin peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed hispidulin and homoplantaginin Std-AS. The retention times of hispidulin and homoplantaginin 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 hispidulin and homoplantaginin in the test solution, and calculate the percentage contents of hispidulin and homoplantaginin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.23% of the total content of hispidulin ($C_{16}H_{12}O_6$) and homoplantaginin ($C_{22}H_{22}O_{11}$), calculated with reference to the dried substance.

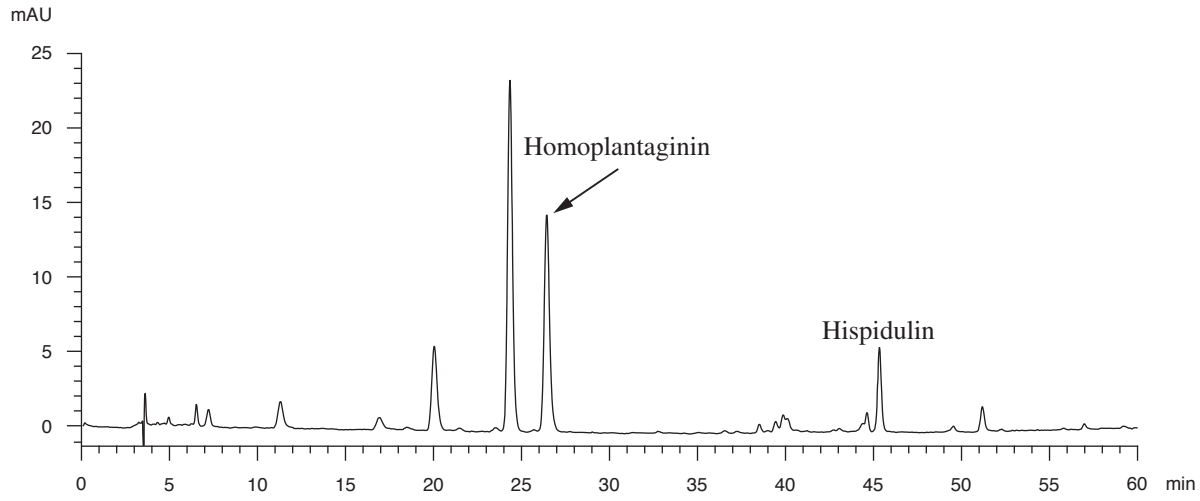


Figure 7 A reference assay chromatogram of Salviae Plebeiae Herba extract