Scutellariae Barbatae Herba

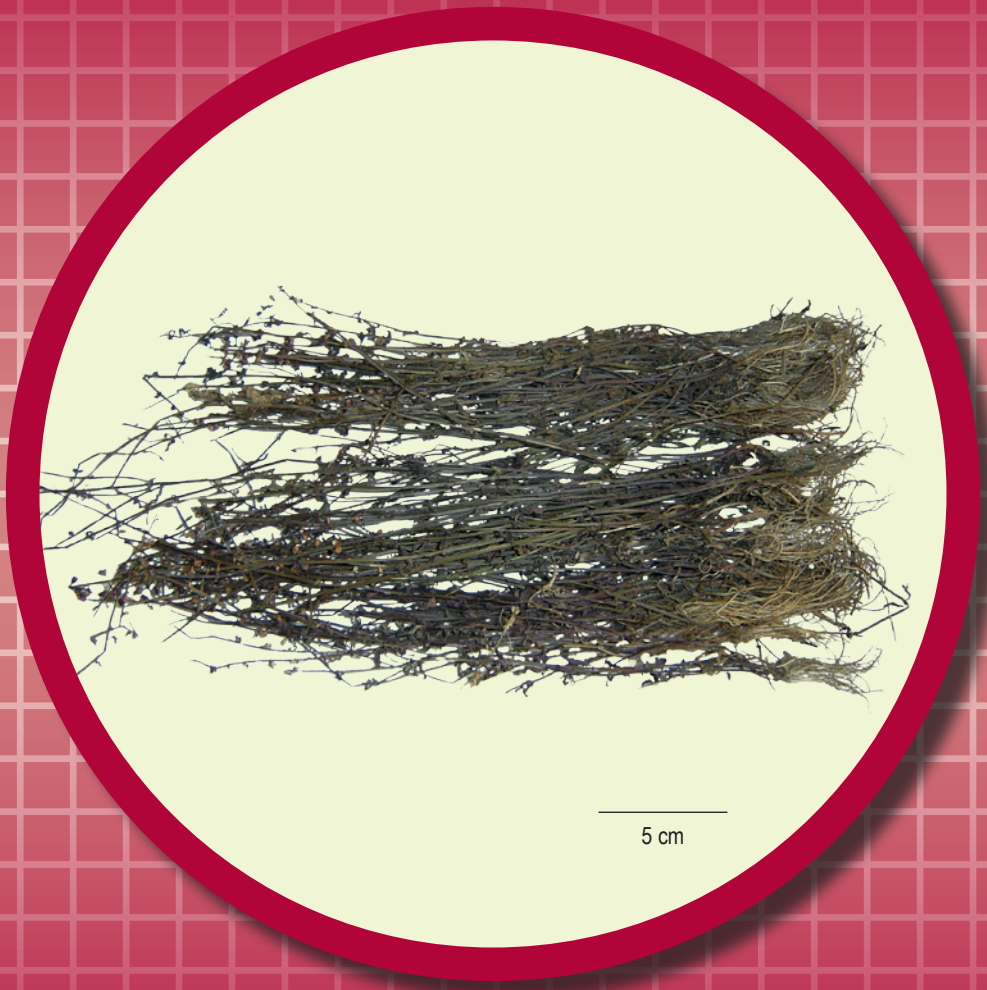


Figure 1 A photograph of Scutellariae Barbatae Herba

1. NAMES

Official Name: Scutellariae Barbatae Herba

Chinese Name: 半枝蓮

Chinese Phonetic Name: Banzhilian

2. SOURCE

Scutellariae Barbatae Herba is the dried whole plant of *Scutellaria barbata* D. Don (Lamiaceae). The whole plant is collected in summer and autumn when foliage branch growing luxuriantly, foreign matter removed, washed clean, then dried under the sun to obtain Scutellariae Barbatae Herba.

3. DESCRIPTION

Root slender. Stem square, dark purple or brownish-green, frequently branched. Leaves opposite, short petiole or near sessile; lamina crumpled and easily broken, when whole, lanceolate or ovate lanceolate, 0.7-3.6 cm long and 0.4-2.5 cm wide, obtuse at the apex, broadly cuneate near the base, entire or indistinctly crenate, the upper surface dark green, the lower surface greyish-green. Flowers solitary in the leaf axils of the upper part of the branches, corolla 2-lipped, brown or pale blue-purple, frequently fallen off, calyx lobes blunt, 1.8-4 mm long, pubescent. Nutlets oblate. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermal cells 1 layer, subrectangular, arranged in order, covered with cuticle, occasionally with glandular hairs. Cortex relatively thin, cell elongated tangentially, collenchyma occurrs at the outside of cortex, more frequently at the edge and corner. Endodermis distinct, 1 layer of cells, subrectangular. Vascular bundles collateral, developed at the four edges. Phloem narrow, cambium distinct, xylem relatively broad, linked up into a ring, vessels radially arranged. Pith broad, parenchymatous cells subrounded, generally hollow in mid-part (Fig. 2).

Root: Epidermis consists of 1 layer of cells, cell subsquare or rectangular. Cortex broad. Endodermis distinct, consisting of 1 layer of cells, cell subrounded, arranged in order. Phloem narrow, cambium in a ring, xylem vessels scattered (Fig. 3).

Leaf: Upper epidermis consists of 1 layer of cells, with glandular scales, non-glandular hairs, glandular hairs and sunken stoma. Palisade tissue consists of 1 layer of palisade cells, arranged in order and spongy tissue consists of subrounded parenchymatous cells, arranged loosely. Lower epidermis consists of 1 layer of cells, subrounded, relatively small. The inner side of the upper and lower epidermis had collenchyma (Fig. 3).

Powder

Colour yellowish-green. Epidermal cells of stem irregular rectangular or ovate in shape, stoma and glandular scale visible, stoma in diacytic type, with 4-7 subsidiary cells. Epidermal cells of leaf walls slightly curved, stoma in diacytic type, with 2-7 subsidiary cells. Glandular scales 4 to 8-celled, subrounded or elliptical, 24-47 μm in diameter. Glandular hairs consist of a few-celled head, stalk 1 cell. Non-glandular hairs consist of 1-4 cells, apical cell relatively long, with fine warty protuberance on the surface. Mainly reticulate vessels, 8-46 μm in diameter, bordered-pitted and spiral vessels occasionally visible. Fibres often in bundles, 8-36 μm in diameter, usually broken, and relative thick walls; polychromatic under the polarized microscope (Fig. 3).

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

Standard solution

Apigenin standard solution

Weigh 0.5 mg of apigenin CRS (Fig. 5) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and formic acid (10:0.5:0.5, v/v).

Spray reagent

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

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 (90 W) the mixture for 15 min. Filter the mixture.

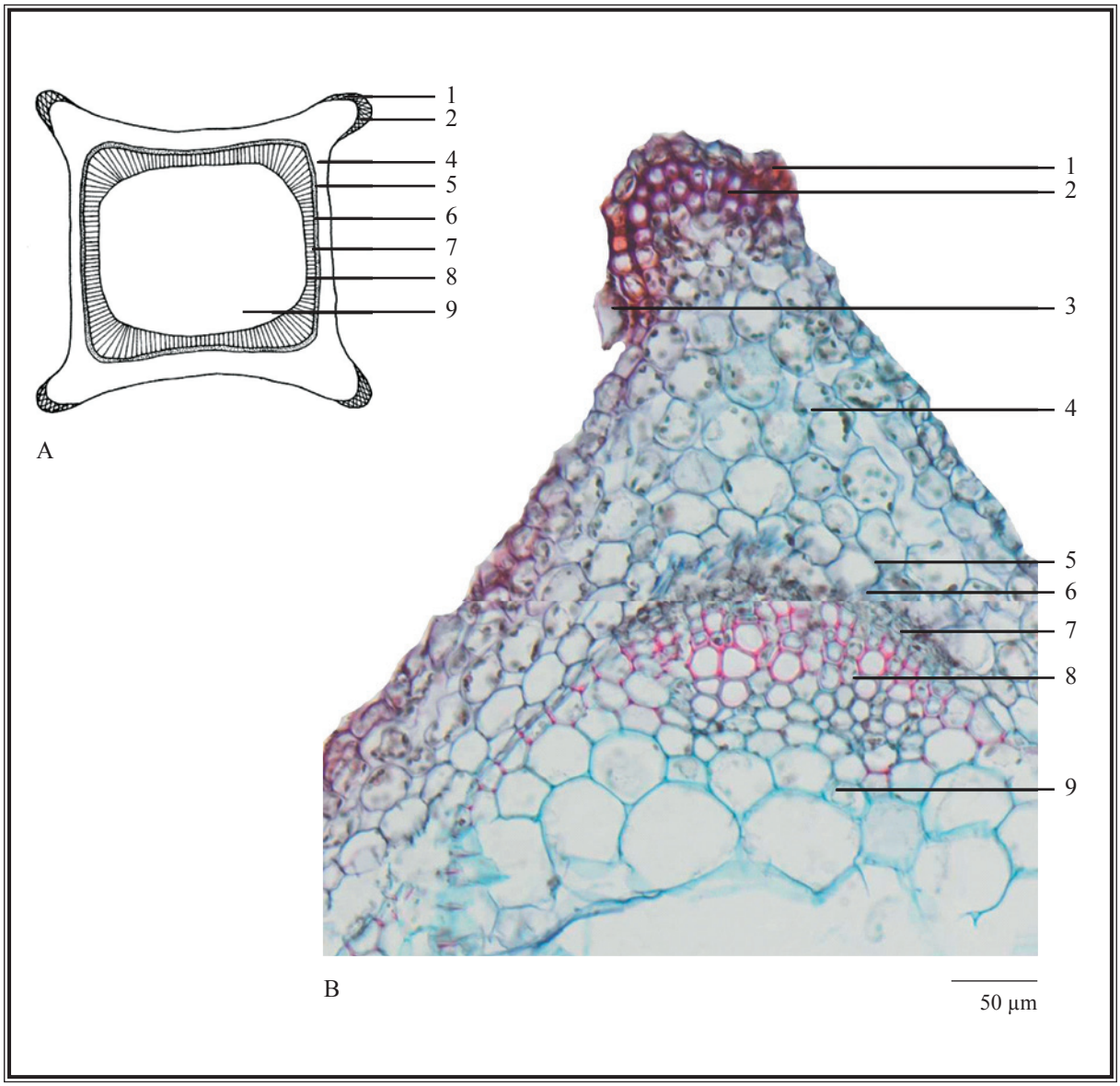


Figure 2 Microscopic features of transverse section of stem of Scutellariae Barbatae Herba

A. Sketch B. Section illustration

1. Epidermis 2. Collenchyma 3. Glandular hair 4. Cortex 5. Endodermis 6. Phloem 7. Cambium 8. Xylem 9. Pith

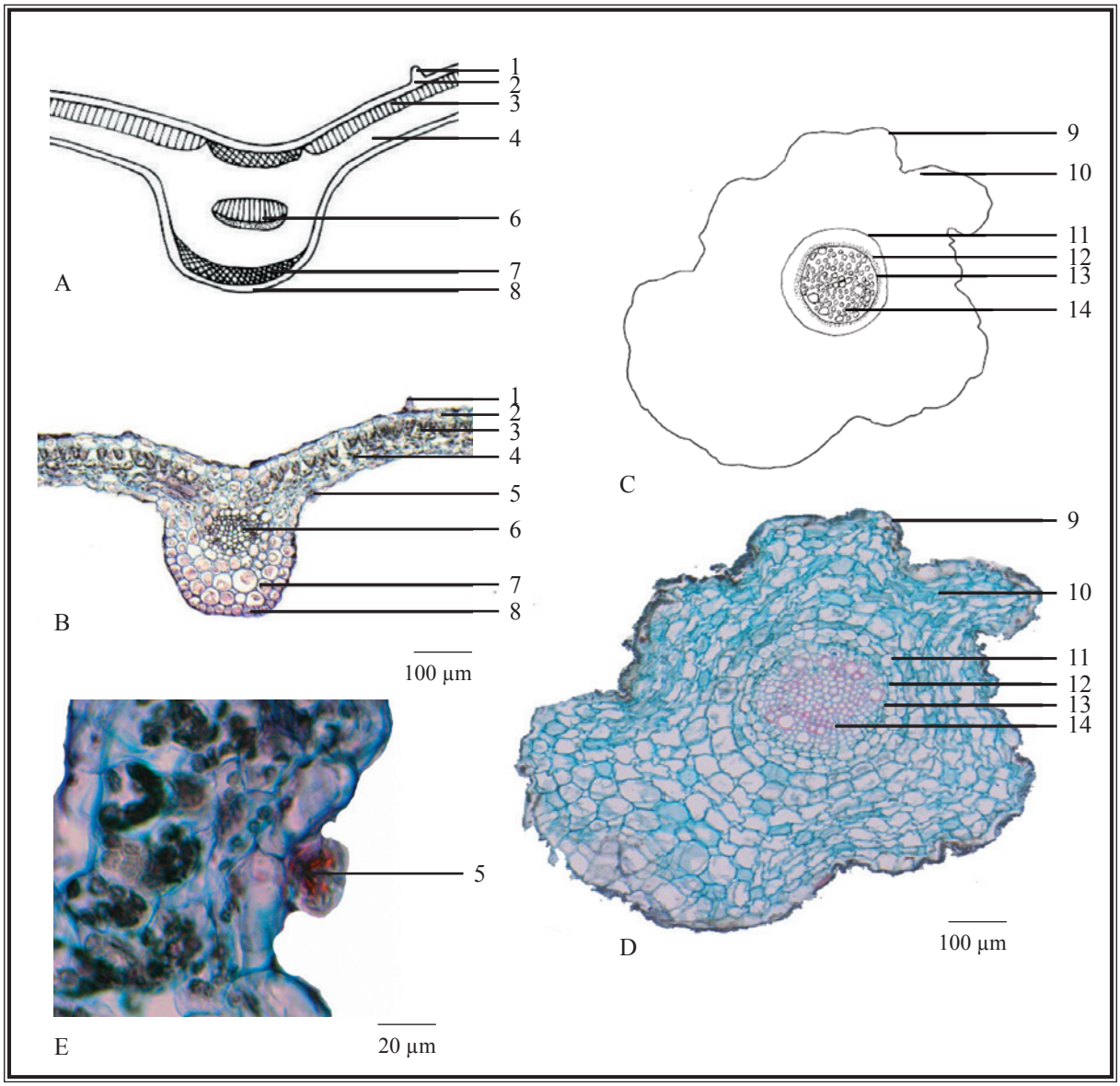


Figure 3 Microscopic features of transverse section of leaf and root of Scutellariae Barbatae Herba

A. Sketch of leaf B. Section illustration of leaf
C. Sketch of root D. Section illustration of root
E. Glandular hair

1. Non-glandular hair 2. Upper epidermis 3. Palisade tissue 4. Spongy tissue 5. Glandular hair
6. Vascular bundle 7. Collenchyma 8. Lower epidermis 9. Epidermis 10. Cortex 11. Endodermis
12. Phloem 13. Cambium 14. Xylem

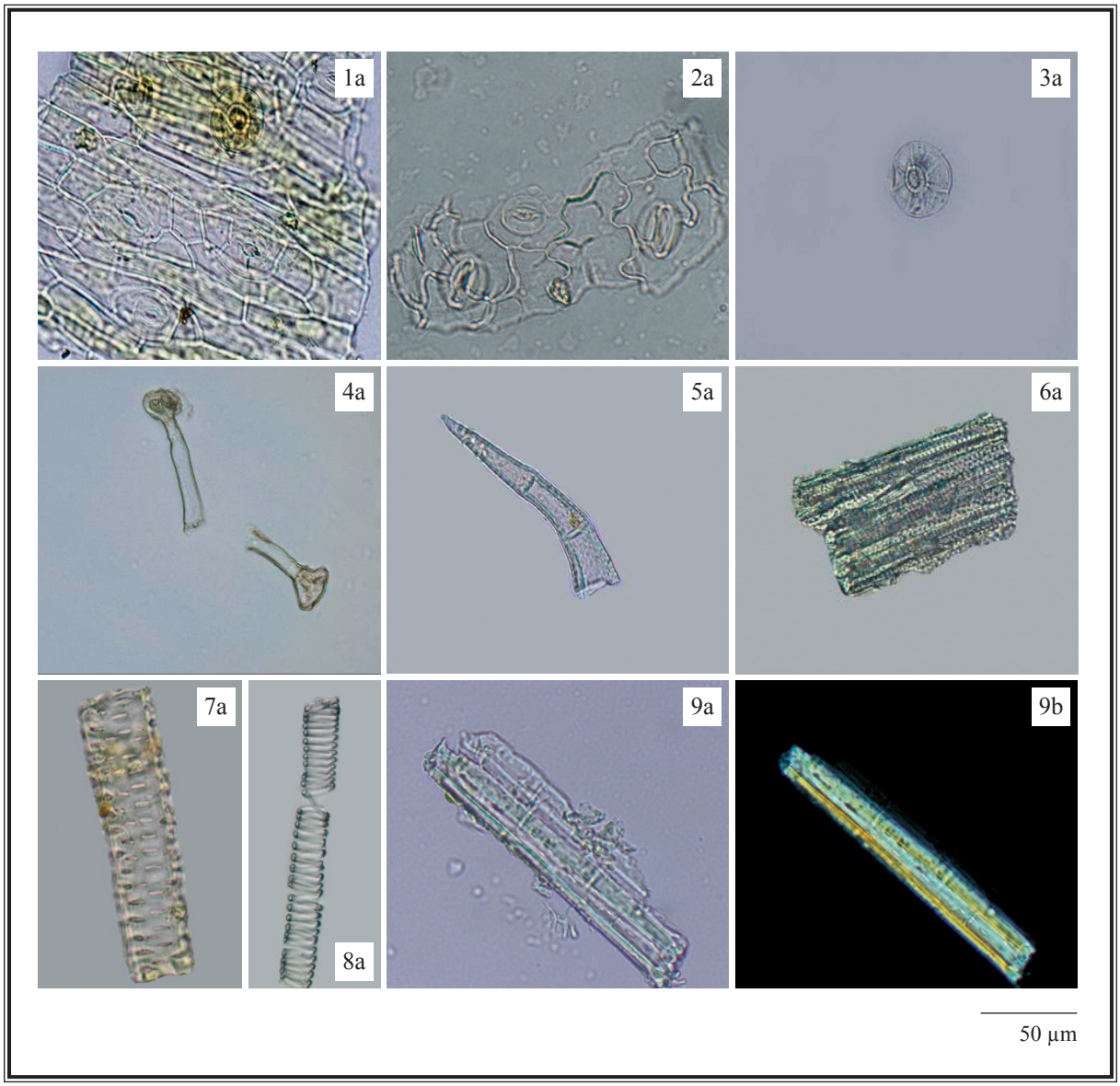


Figure 4 Microscopic features of powder of Scutellariae Barbatae Herba

1. Epidermal cells of stem 2. Epidermal cells of leaf 3. Glandular scale 4. Glandular hairs
5. Non-glandular hair 6. Reticulate vessel 7. Bordered-pitted vessel 8. Spiral vessel 9. Fibres

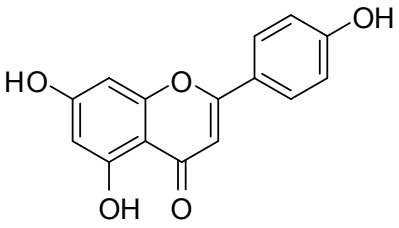
a. Features under the light microscope b. Features under the polarized microscope

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 apigenin standard solution (1 µL) and the test solution (10 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 10 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 8.5 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 105°C until the spots or bands become visible (about 3 min). 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 apigenin.

(i)



(ii)

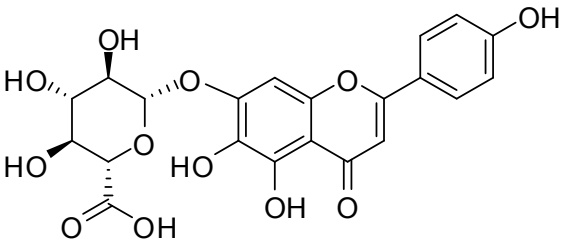


Figure 5 Chemical structures of (i) apigenin and (ii) scutellarin

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

Standard solution

Scutellarin standard solution for fingerprinting, Std-FP (24 mg/L)

Weigh 0.24 mg of scutellarin CRS (Fig. 5) and dissolve in 10 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 25 mL of ethanol (70%). Immediately reflux the mixture for 15 min. Cool down to room temperature. 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 ethanol (70%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 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)	1% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	83 → 75	17 → 25	linear gradient
15 – 30	75	25	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of scutellarin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of scutellarin should not be more than 5.0%; the RSD of the retention time of scutellarin peak should not be more than 2.0%; the column efficiency determined from scutellarin peak should not be less than 10000 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. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Scutellariae Barbatae Herba extract

Peak No.	RRT	Acceptable Range
1	0.93	± 0.04
2 (marker, scutellarin)	1.00	-
3	1.67	± 0.08

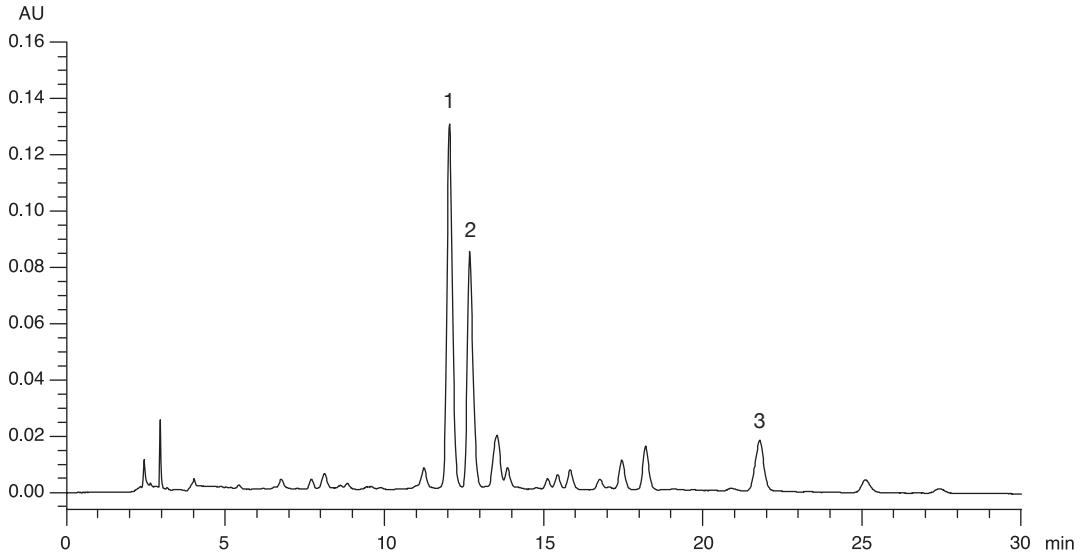


Figure 6 A reference fingerprint chromatogram of Scutellariae Barbatae Herba extract

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. 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 XVIII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 10.5%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 20.0%.
Ethanol-soluble extractives (hot extraction method): not less than 26.0%.

7. ASSAY

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

Standard solution

Scutellarin standard stock solution, Std-Stock (240 mg/L)

Weigh accurately 1.2 mg of scutellarin CRS and dissolve in 5 mL of methanol.

Scutellarin standard solution for assay, Std-AS

Measure accurately the volume of the scutellarin Std-Stock, dilute with methanol to produce a series of solutions of 6, 12, 24, 36, 60 mg/L for scutellarin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 25 mL of ethanol (70%). Immediately reflux the mixture for 15 min. Cool down to room temperature. 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 ethanol (70%). Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (335 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)	1% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	83 → 75	17 → 25	linear gradient
15 – 30	75	25	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of scutellarin Std-AS (24 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of scutellarin should not be more than 5.0%; the RSD of the retention time of scutellarin peak should not be more than 2.0%; the column efficiency determined from scutellarin peak should not be less than 10000 theoretical plates.

The *R* value between scutellarin 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 scutellarin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of scutellarin against the corresponding concentrations of scutellarin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

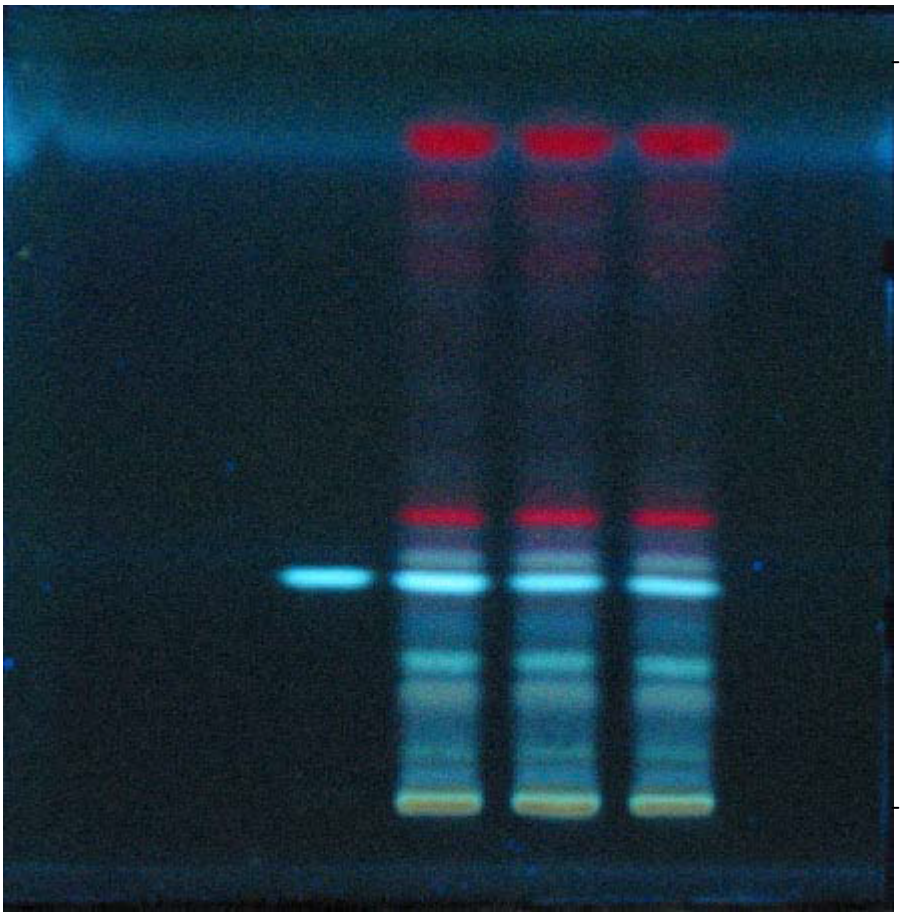
Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify scutellarin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of scutellarin Std-AS. The retention times of scutellarin 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 scutellarin in the test solution, and calculate the percentage content of scutellarin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.52% of scutellarin ($C_{21}H_{18}O_{12}$), calculated with reference to the dried substance.

Scutellariae Barbatae Herba　(半枝蓮)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Apigenin)	Apigenin positive
3	Spiked sample (Sample plus apigenin)	Apigenin positive
4	Sample (Scutellariae Barbatae Herba)	Apigenin positive
5	Sample duplicate (Scutellariae Barbatae Herba)	Apigenin positive

Figure 1　TLC results of Scutellariae Barbatae Herba extract observed under UV light (366 nm) after staining