Radix Scutellariae

Figure 1　A photograph of Radix Scutellariae

1. NAMES

Official Name: Radix Scutellariae

Chinese Name: 黃芩

Chinese Phonetic Name: Huangqin

2. SOURCE

Radix Scutellariae is the dried root of *Scutellaria baicalensis* Georgi (Lamiaceae). The root is collected in spring or autumn, the outer cork and rootlets removed, then dried under the sun to obtain Radix Scutellariae.

3. DESCRIPTION

Conical, the broader part at the top, tapering towards the distal end, twisted, 8-25 cm long, 5-25 mm in diameter. Externally brownish-yellow or dark yellow, marked by sparse warty traces of rootlets; the upper portion rough, with twisted longitudinal wrinkles or irregular reticula, the lower distal portion with longitudinal striations and fine wrinkles. Texture hard and fragile, easily broken. Fracture yellow, reddish-brown in the centre, dark brown to brownish-black in the old root, withered or hollowed. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells, mostly broken. Cortex narrow, scattered with stone cells. Phloem broad, scattered with single or groups of stone cells. Cambium distinct. Xylem vessels occur singly or grouped, surrounded by xylem fibres bundles, with xylem rays relatively broad. Interxylary cork tissue is found in the xylem of roots of plant older than 2-year old, with several layers of well ordered cork cells enclosing the vessels. Parenchyma cells replete with starch granules (Fig. 2).

Powder

Colour yellow. Phloem fibres fusiform, scattered singly or in bundles, 60-250 μm long, 9-33 μm in diameter, thick-walled, with fine pit-canals. Stone cells suborbicular, subsquare or rectangular, relatively thick-walled or heavily thick-walled. Xylem fibres mostly broken,

with sparse oblique pits. Cork cells brownish-yellow, polygonal in surface view. Vessels mainly reticulated, 24-72 μm in diameter. Starch granules abundant, 2-10 μm, simple granules spheroidal, hilum distinct, compound granules composed of 2-3 units (Fig. 3).

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

Standard solutions

Baicalein standard solution

Weigh 2.0 mg of baicalein CRS (Fig. 4) and dissolve in 1 mL of methanol.

Baicalin standard solution

Weigh 2.0 mg of baicalin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Wogonin standard solution

Weigh 2.0 mg of wogonin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of toluene, ethyl acetate, methanol and formic acid (10:3:1:2, v/v).

Spray reagent

Weigh 1 g of iron(III) chloride hexahydrate and dissolve in 100 mL of absolute ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 30 mL of a mixture of ethyl acetate and methanol (3:1, v/v). Reflux the mixture for 30 min. Cool to room temperature. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately baicalein standard solution, baicalin standard solution, wogonin standard solution and the test solution (5 μL each) to the plate. 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, then heat at 105°C until the spots or 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).

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 baicalein, baicalin and wogonin.

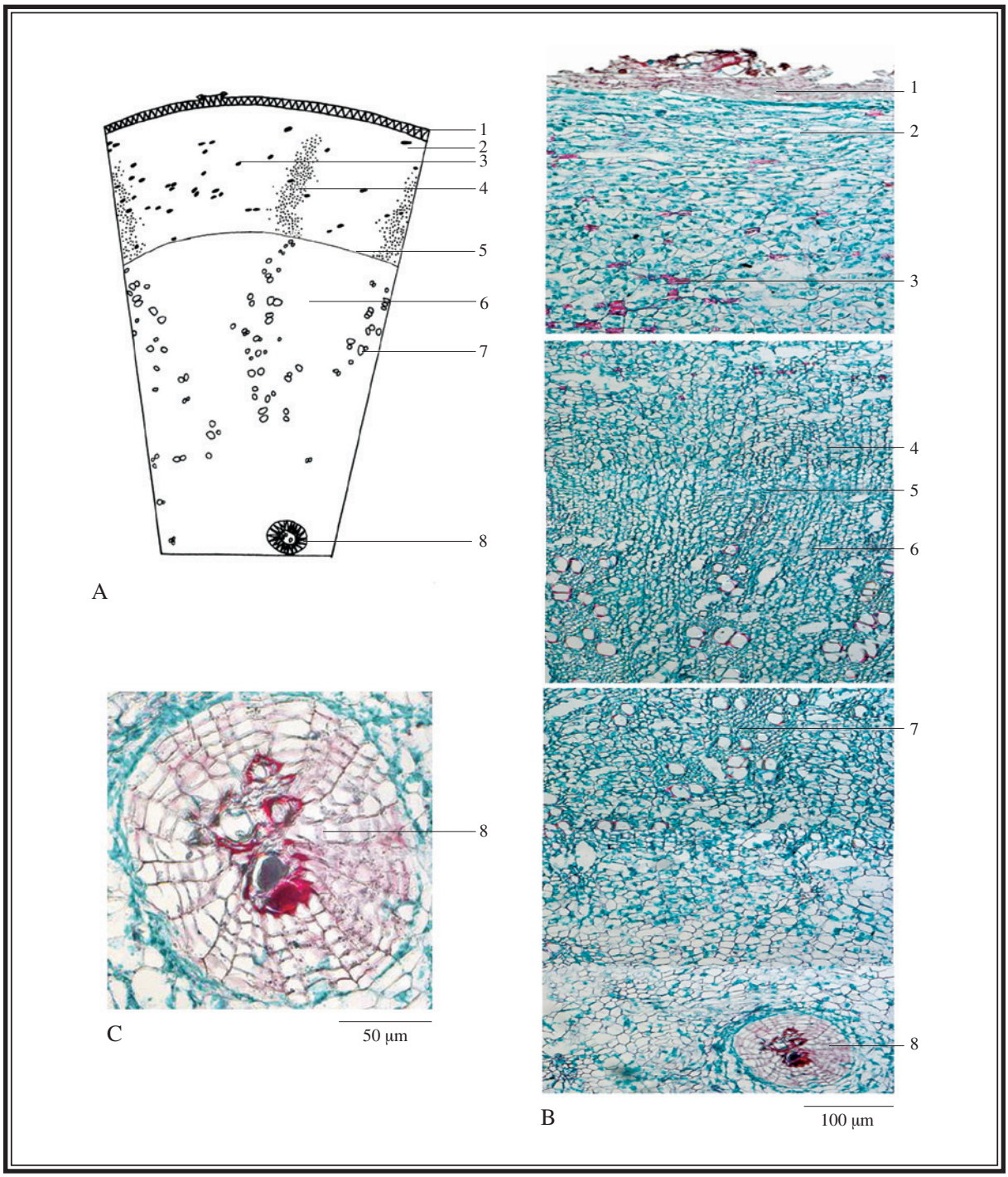


Figure 2 Microscopic features of transverse section of Radix Scutellariae

A. Sketch B. Section illustration C. Interxylary cork tissue exists in xylem

1. Cork 2. Cortex 3. Stone cells 4. Phloem 5. Cambium
6. Xylem rays 7. Xylem 8. Interxylary cork tissue

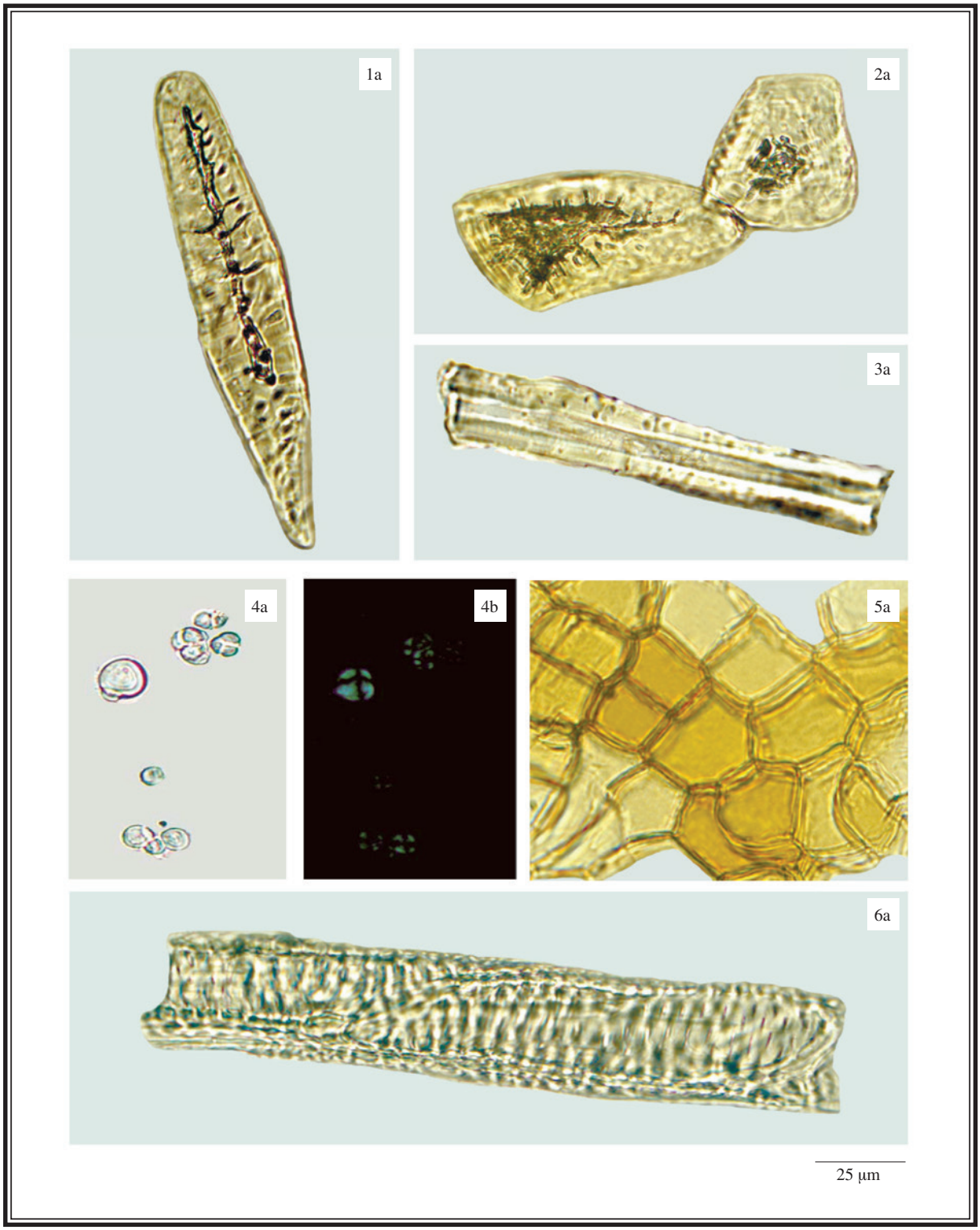
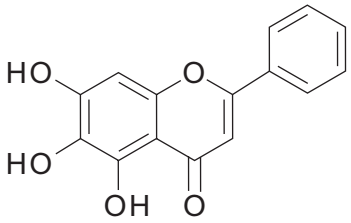


Figure 3 Microscopic features of powder of Radix Scutellariae

1. Phloem fibres 2. Stone cells 3. Xylem fibres 4. Starch granules 5. Cork cells
6. Reticulate vessels

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

(i)



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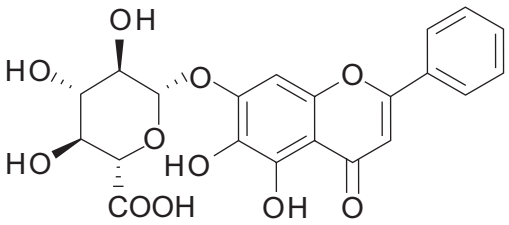

(iii)

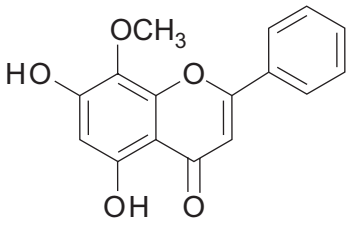

Figure 4 Chemical structures of (i) baicalein (ii) baicalin and (iii) wogonin

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

Standard solution

Baicalein standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of baicalein CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 40 mL of methanol (70%). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL volumetric flask. 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 (276 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)	Methanol (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0–25	40	60	isocratic
25–45	40→60	60→40	linear gradient
45–60	60	40	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of baicalein Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of baicalein should not be more than 3.0%; the RSD of the retention time of baicalein peak should not be more than 2.0%; the column efficiency determined from baicalein peak should not be less than 150000 theoretical plates.

The *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Radix Scutellariae extract

Peak No.	RRT	Acceptable Range
1 (baicalin)	0.54	±0.04
2	0.74	±0.04
3	0.81	±0.03
4	0.85	±0.03
5 (marker, baicalein)	1.00	-
6 (wogonin)	1.14	±0.03

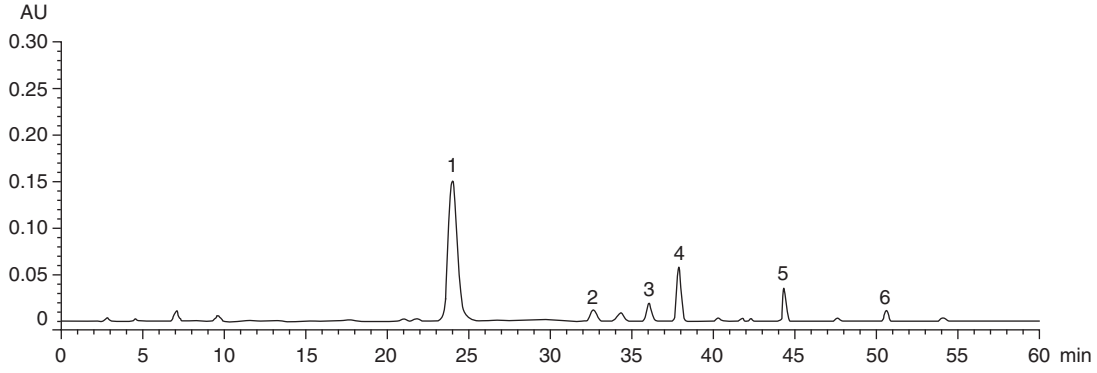

Figure 5 A reference fingerprint chromatogram of Radix Scutellariae extract

For positive identification, the sample must give the above six 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 XV)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.

Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 30.0%.

Ethanol-soluble extractives (hot extraction method): not less than 46.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 2.0 mg of baicalin CRS and dissolve in 10 mL of methanol (70%).

Baicalin standard solution for assay, Std-AS

Measure accurately the volume of the baicalin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 20, 60, 100, 160, 200 mg/L for baicalin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (70%). Sonicate (220 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 4 min. Filter and transfer the filtrate to a 250-mL volumetric flask. Repeat the extraction for four more times. Combine the filtrate. Make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (276 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 acetonitrile and 0.1% phosphoric acid (20:80, v/v). The elution time is about 35 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of baicalin Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of baicalin should not be more than 5.0%; the RSD of the retention time of baicalin peak should not be more than 2.0%; the column efficiency determined from baicalin peak should not be less than 12000 theoretical plates.

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

Limits

The sample contains not less than 12% of baicalin ($C_{21}H_{18}O_{11}$), calculated with reference to the dried substance.