

Sophorae Flavescientis Radix



Figure 1 A photograph of Sophorae Flavescientis Radix

1. NAMES

Official Name: Sophorae Flavescentis Radix

Chinese Name: 苦參

Chinese Phonetic Name: Kushen

2. SOURCE

Sophorae Flavescentis Radix is the dried root of *Sophora flavescens* Ait. (Fabaceae). The root is collected in spring or autumn, removed the top parts, rootlets and soil, washed with water, then dried to yield the intact form. Alternatively, the root is sliced while fresh, and dried immediately to obtain the sliced form of Sophorae Flavescentis Radix.

3. DESCRIPTION

Cylindrical, usually branched on the lower part, 10-60 cm long, 5-25 mm in diameter. Externally greyish-brown to brown, with distinct longitudinal wrinkles and laterally extended lenticels; bark thin, usually cracked, rolled outwards and fallen off. Texture hard, uneasily broken, with somewhat fibrous surface. Transversely cut slice rounded or oblique in shape, 2-10 mm thick, 10-60 mm in diameter, externally pale-yellow, with radial striations and clefts; sometimes hetero-vascular bundle outline appears as a concentric annular ring or irregularly scattered. Odour slight; taste very bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Cork occasionally removed, consisting of several layers of cells. Cortex narrow. Fibre bundles scattered in phloem, surrounded by parenchymatous cells with prisms of calcium oxalate, appearing as crystal fibres. Phloem rays broad. Cambium distinct, arranged in an interrupted ring. Xylem branches into 2-4 lines outwards; vessels arranged radially; crystal fibres in bundles. Xylem ray distinct and broad. Parenchymatous cells contain numerous starch granules and prisms of calcium oxalate (Fig. 2).

Powder

Colour pale brown. Simple starch granules subglobular or ellipsoid, 0.5-35 μm in diameter, with cleft hilum; compound granules mostly composed of 2-5 units; black and cruciate in shape under the polarized microscope. Fibres numerous, mostly in bundles, with thickened and slightly lignified wall, surrounded by parenchymatous cells contain prisms of calcium oxalate, appearing as crystal fibres. Prisms of calcium oxalate biconical, double rhombic or polyhedral; polychromatic under the polarized microscope. Vessels bordered-pitted, occasionally reticulate, 4-109 μm in diameter. Parenchymatous cells subrounded or subrectangular, sometimes lignified in uneven beaded shape; containing starch granules. Cork cells subpolygonal, with irregular slight cracks on the surface (Fig. 3).

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

Standard solutions

Matrine standard solution

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

Oxymatrine standard solution

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

Sophoridine standard solution

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

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), water, ethanol and ethyl acetate (1.5:1:3.5:15, v/v).

Spray reagent

Weigh 1.0 g of iodine and 10.0 g of potassium iodide, and dissolve in 50 mL of water. Heat and add 2 mL of glacial acetic acid. Transfer the solution to a 100-mL volumetric flask and make up to the mark with water.

Test solution

Weigh 4.0 g of the powdered sample and place it in a 50-mL conical flask, then add 15 mL of ethyl acetate and 0.3 mL of ammonium hydroxide solution (25%, v/v). Sonicate (90 W) the mixture for 30 min. Filter the mixture.

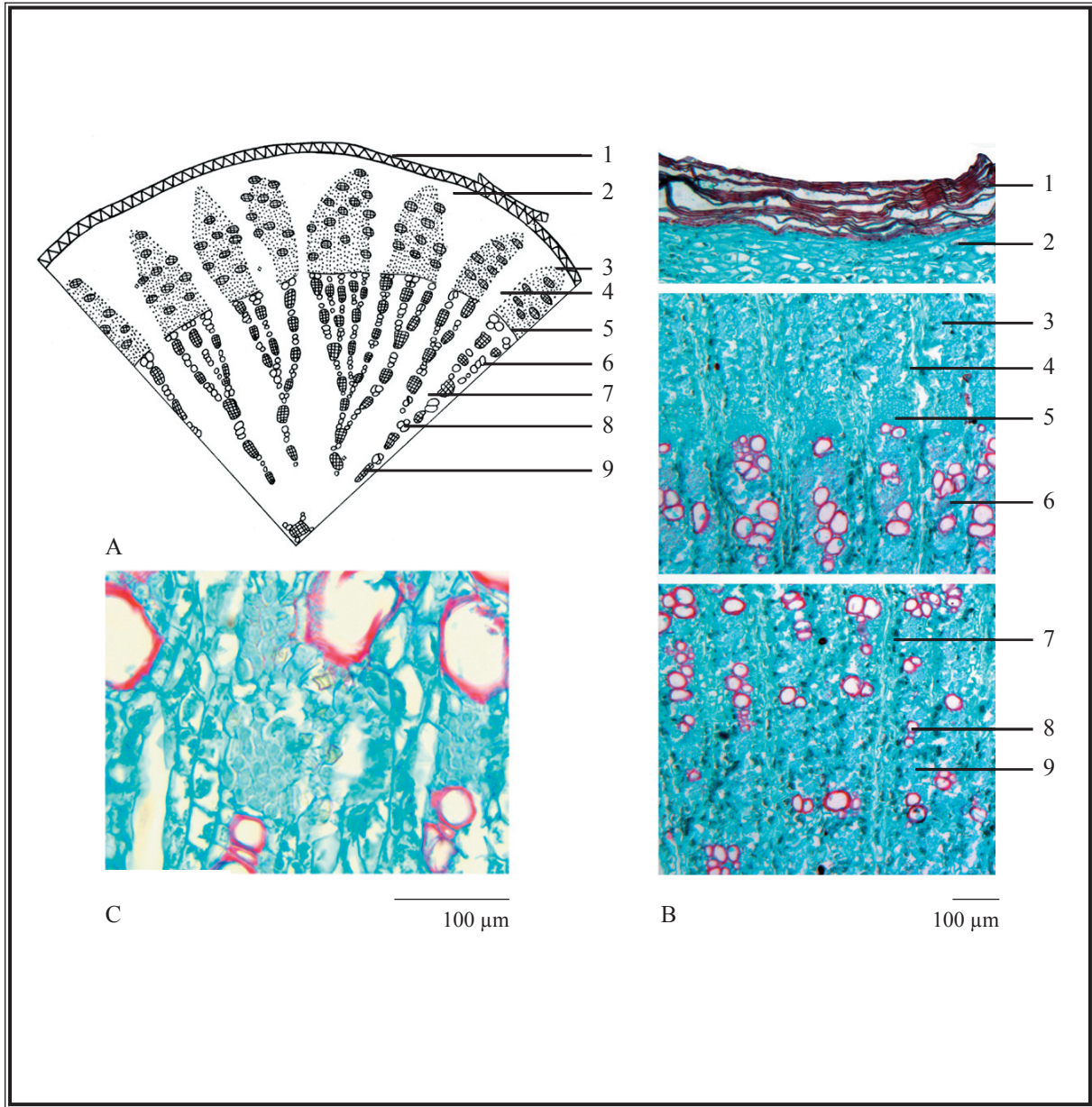


Figure 2 Microscopic features of transverse section of *Sophorae Flavescentis Radix*

A. Sketch B. Section illustration C. Crystal fibres bundle

1. Cork 2. Cortex 3. Phloem 4. Phloem ray 5. Cambium 6. Xylem 7. Xylem ray
 8. Vessels 9. Fibre bundles

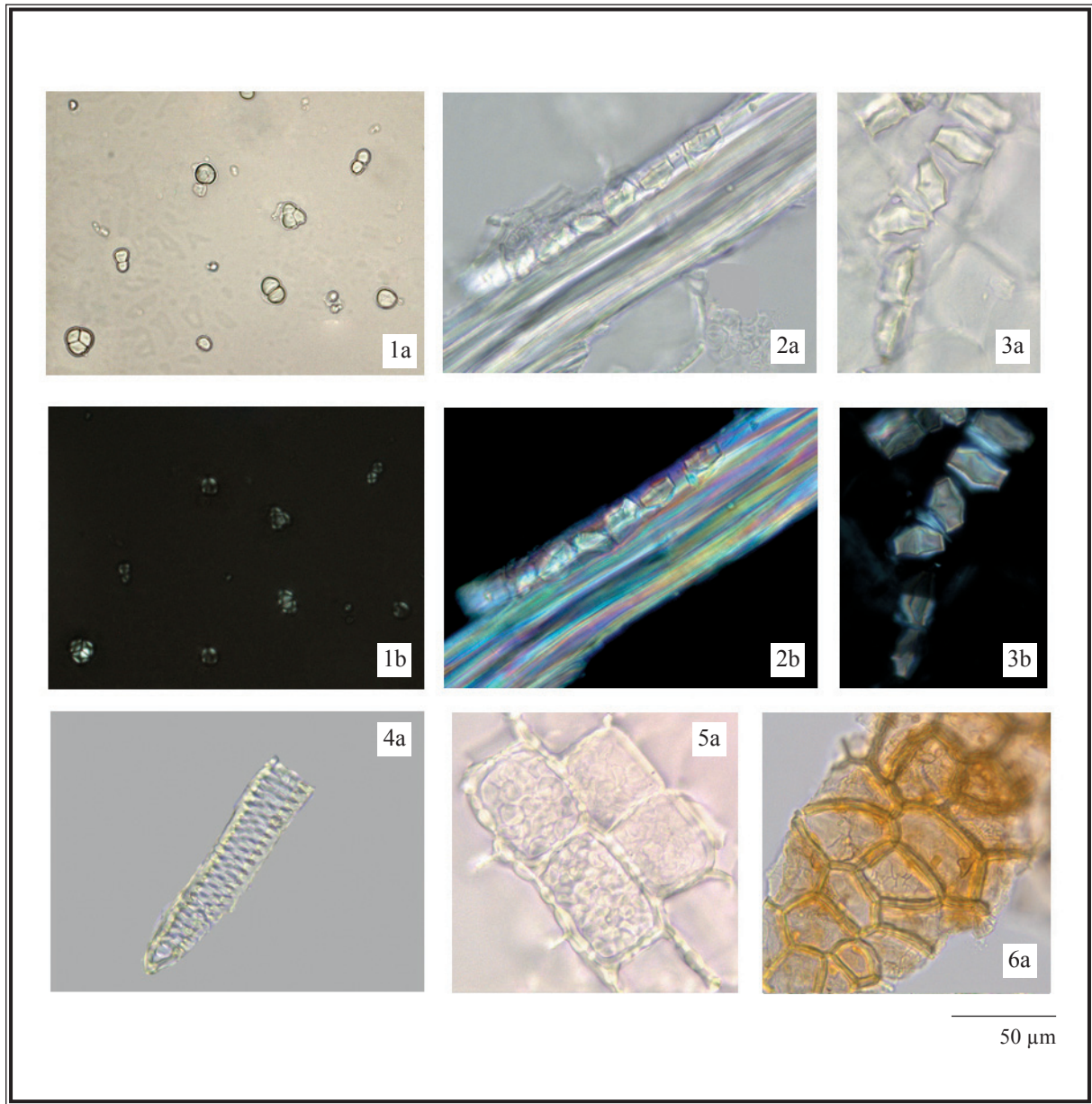


Figure 3 Microscopic features of powder of *Sophorae Flavescentis Radix*

- 1. Starch granules 2. Crystal fibres 3. Prisms of calcium oxalate 4. Bordered-pitted vessels
- 5. Lignified parenchymatous cells 6. Cork cells

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

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solutions

Matrine standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of matrine CRS and dissolve in 10 mL of ethanol (30%).

Oxymatrine standard solution for fingerprinting, Std-FP (800 mg/L)

Weigh 8.0 mg of oxymatrine CRS and dissolve in 10 mL of ethanol (30%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (30%) and 0.5 mL of ammonium hydroxide solution (25%, v/v). Sonicate (150 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (30%). Combine the solutions and make up to the mark with ethanol (30%). Filter through a 0.45- μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (4.6 \times 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 0.3% phosphoric acid with 0.3% triethylamine (4:96, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 5 μL of matrine Std-FP and oxymatrine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of matrine and oxymatrine should not be more than 5.0%; the RSD of the retention times of matrine and oxymatrine peaks should not be more than 2.0%; the column efficiencies determined from matrine and oxymatrine peaks should not be less than 6500 theoretical plates.

The *R* value between peak 1 and the closest peak; and 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. 5).

Procedure

Separately inject matrine Std-FP, oxymatrine Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of matrine and oxymatrine peaks in the chromatograms of matrine Std-FP, oxymatrine Std-FP and the retention times of the three characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify matrine and oxymatrine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of matrine Std-FP and oxymatrine Std-FP.

The retention times of matrine and oxymatrine 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 three characteristic peaks of Sophorae Flavescens Radix extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the three characteristic peaks of Sophorae Flavescens Radix extract

Peak No.	RRT	Acceptable Range
1 (matrine)	0.57	± 0.03
2 (oxysophoridine)	0.94	± 0.03
3 (marker, oxymatrine)	1.00	-

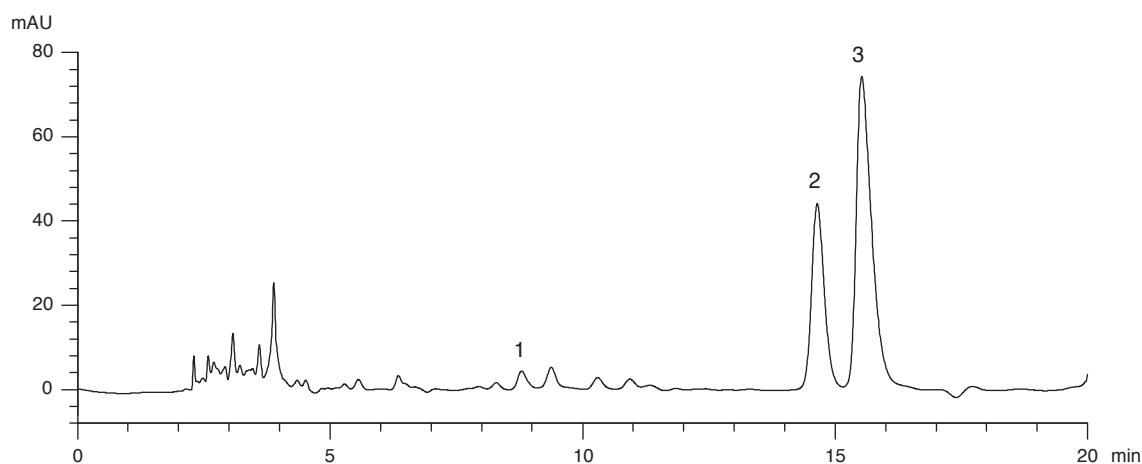


Figure 5 A reference fingerprint chromatogram of Sophorae Flavescens Radix extract

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. 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 XVIII*): meet the requirements.

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

5.6 Ash (*Appendix IX*)

Total ash: not more than 4.5%.

Acid-insoluble ash: not more than 0.5%.

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 24.0%.

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

7. ASSAY

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

Standard solution

Mixed matrine, oxymatrine and sophoridine standard solution, Std-Stock (40 mg/L for matrine, 800 mg/L for oxymatrine and 80 mg/L for sophoridine)

Weigh accurately 0.2 mg of matrine CRS, 4.0 mg of oxymatrine CRS and 0.4 mg of sophoridine CRS, and dissolve in 5 mL of ethanol (30%).

Mixed matrine, oxymatrine and sophoridine standard solution for assay, Std-AS

Measure accurately the volume of the mixed matrine, oxymatrine and sophoridine Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 12, 16, 20, 24, 32 mg/L for matrine, 240, 320, 400, 480, 640 mg/L for oxymatrine and 3.2, 8, 16, 24, 32 mg/L for sophoridine.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (30%) and 0.5 mL of ammonium hydroxide solution (25%, v/v). Sonicate (150 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (30%). Combine the solutions and make up to the mark with ethanol (30%). Filter through a 0.45- μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 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 0.3% phosphoric acid with 0.3% triethylamine (4:96, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed matrine, oxymatrine and sophoridine Std-AS (20 mg/L for matrine, 400 mg/L for oxymatrine and 16 mg/L for sophoridine). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of matrine, oxymatrine and sophoridine should not be more than 5.0%; the RSD of the retention times of matrine, oxymatrine and sophoridine peaks should not be more than 2.0%; the column efficiencies determined from matrine, oxymatrine and sophoridine peaks should not be less than 6500 theoretical plates.

The *R* value between matrine peak and the closest peak; the *R* value between oxymatrine peak and the closest peak; and the *R* value between sophoridine peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed matrine, oxymatrine and sophoridine Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of matrine, oxymatrine and sophoridine against the corresponding concentrations of the mixed matrine, oxymatrine and sophoridine 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 matrine, oxymatrine and sophoridine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed matrine, oxymatrine and sophoridine Std-AS. The retention times of matrine, oxymatrine and sophoridine 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 matrine, oxymatrine and sophoridine in the test solution, and calculate the percentage contents of matrine, oxymatrine and sophoridine in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 1.9% of the total content of matrine (C₁₅H₂₄N₂O), oxymatrine (C₁₅H₂₄N₂O₂) and sophoridine (C₁₅H₂₄N₂O), calculated with reference to the dried substance.