

Sophorae Tonkinensis Radix et Rhizoma

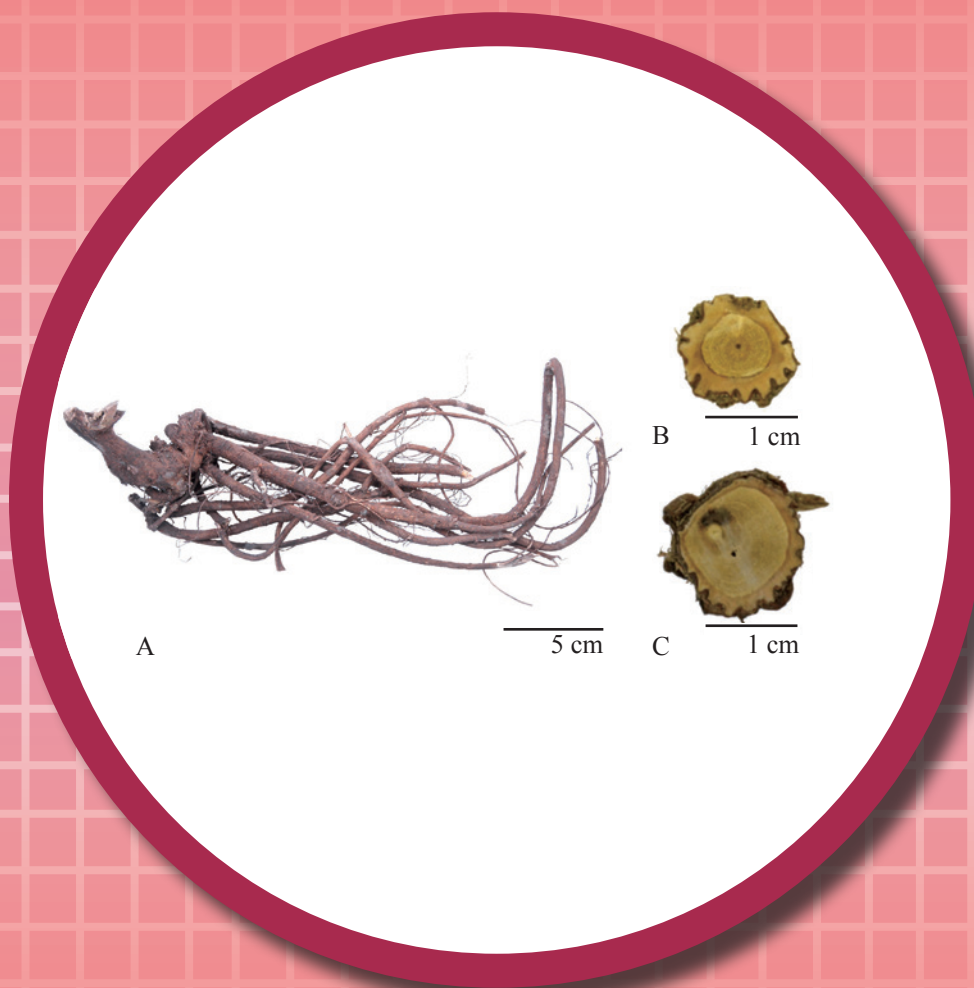


Figure 1 A photograph of Sophorae Tonkinensis Radix et Rhizoma

A. Sophorae Tonkinensis Radix et Rhizoma

B. Magnified image of transverse section of root

C. Magnified image of transverse section of rhizome

1. NAMES

Official Name: *Sophorae Tonkinensis Radix et Rhizoma*

Chinese Name: 山豆根

Chinese Phonetic Name: Shandougen

2. SOURCE

Sophorae Tonkinensis Radix et Rhizoma is the dried root and rhizome of *Sophora tonkinensis* Gagnep. (Fabaceae). The root and rhizome are collected in autumn, foreign matter removed, washed clean, then dried to obtain *Sophorae Tonkinensis Radix et Rhizoma*.

3. DESCRIPTION

Roots long-cylindrical, usually branched, varied in length, 20-80 cm long, 3-15 mm in diameter. Externally brown to dark brown, with irregular longitudinal wrinkles and transversely elongated lenticels-like protuberances. Rhizomes irregularly nodulated, bearing remnants of stems at the apex and several roots on the lower part. Texture hard, uneasily broken. Fracture pale brown in bark and pale yellow in wood. Odour soybean-like flavour (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (*Appendix III*)

Transverse section

Root: Cork consists of several to 10 or more layers of cells. Phelloderm consists of 1-4 layers of cells, the outer 1-2 rows of cells with lignified and thickened walls, forming an interrupted ring of cells containing prisms of calcium oxalate. Phloem rays a bit broad, slightly curved; fibre bundles scattered in phelloderm and phloem. Cambium in a ring. Xylem developed, scattered with fibre bundles, fibre bundles arranged alternately with vessels. Xylem rays consist of 1-8 rows of cells. Vessels subrounded, mostly singly scattered or 2 or more in groups, some containing yellowish-brown contents. Xylem fibres bundles scattered. Parenchymatous cells filled with starch granules, a few cells contain prisms of calcium oxalate [Fig. 2 (i)].

Rhizome: Phloem rays a bit broad, slightly curved; fibre bundles scattered in phelloderm and phloem. Xylem broad, developed, xylem fibres abundant, arranged alternately with vessels. Pith visible in the centre, mainly consisting of parenchymatous cells [Fig. 2 (ii)].

Powder

Colour pale yellow. Cork cells yellowish-brown to pale brown, flat rectangular in lateral view, wall slightly curved; polygonal in surface view, anticlinal wall thin or slightly thickened, some contain intermittent pits. Fibres abundant, in loose bundles or scattered singly, colourless or yellowish-brown, slender, usually twisted, endings blunt and round, 11-13 μm in diameter, a few with swollen part up to 54 μm in diameter, walls extremely thick, non-lignified, primary wall easily separated from secondary wall, with irregular longitudinal fissures on the surface; fibre bundles lining the surrounding cells contain prisms of calcium oxalate, forming crystal fibres, walls of crystal cells unevenly thickened; polychromatic under the polarized microscope. Prisms of calcium oxalate biconical, subsquare, rhombic, polyhedral or irregularly lumpy, 5-30 μm in diameter; polychromatic under the polarized microscope. Vessels mainly reticulate and bordered-pitted, pale yellow to golden yellow, reticulate vessels relatively slender, endings sharp or blunt and rounded, 10-40 μm in diameter; bordered-pitted vessels relatively large, usually fragmented, intact vessels cylindrical, 30-126 μm in diameter. Xylem parenchymatous cells long stripe-shaped or rectangular, wall slightly thickened and slightly lignified, with small pits. Starch granules slightly gelatinized; non-gelatinized simple starch granules rounded to subrounded, 4-22 μm in diameter, hilum and striation not obvious; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-8(-14) units. Stone cells occasionally found, scattered singly or in groups of 2, some connected with fibres, pale yellow, subrounded, rectangular or subelliptic, 45-70 μm in diameter (Fig. 3).

Sophorae Tonkinensis Radix et Rhizoma

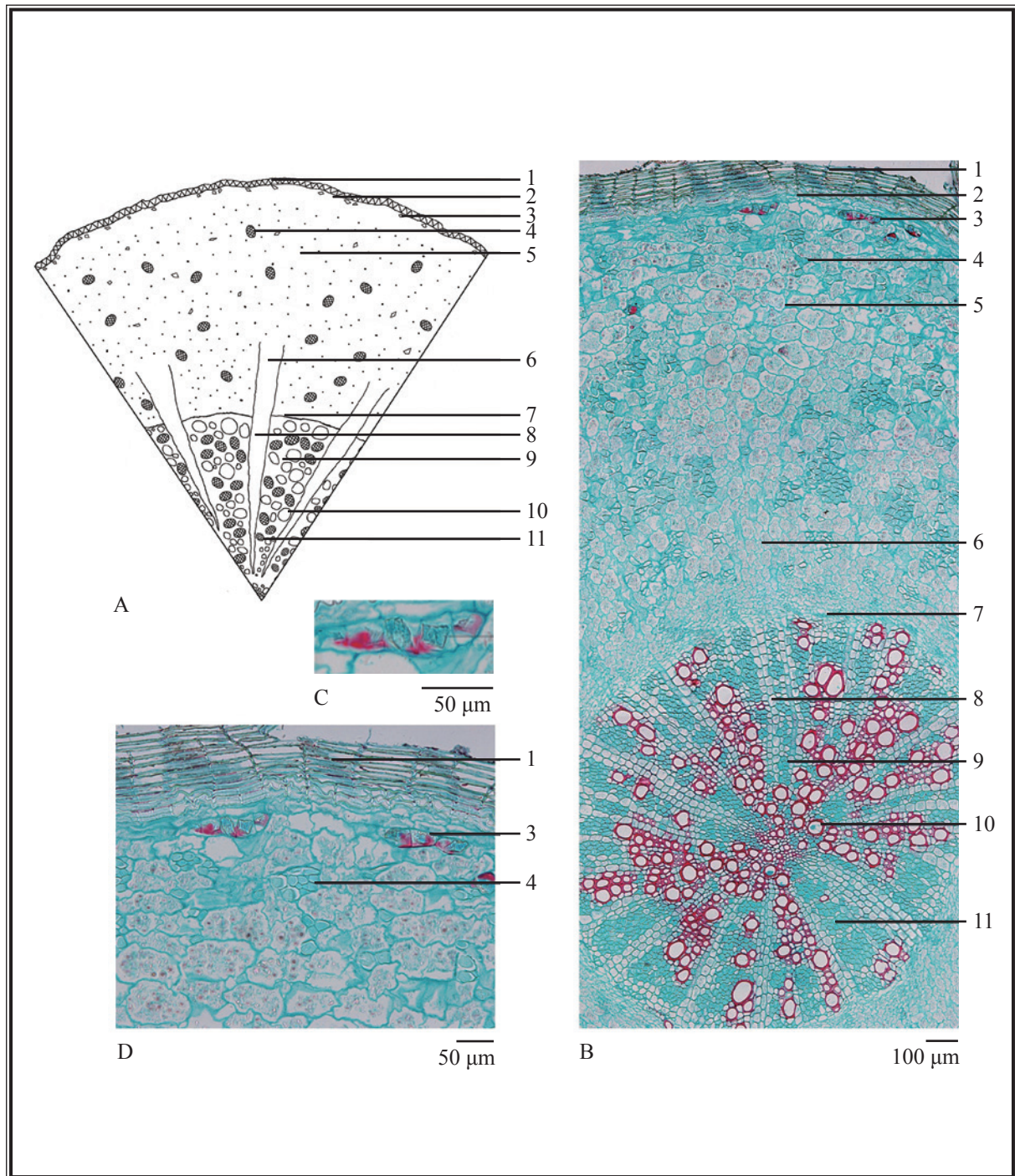


Figure 2 (i) Microscopic features of transverse section of root of *Sophorae Tonkinensis Radix et Rhizoma*

A. Sketch B. Section illustration C. Prisms of calcium oxalate D. Cork, phelloderm and phloem

1. Cork 2. Phelloderm 3. Prism of calcium oxalate 4. Phloem fibre 5. Phloem 6. Phloem ray
 7. Cambium 8. Xylem ray 9. Xylem 10. Vessel 11. Xylem fibre

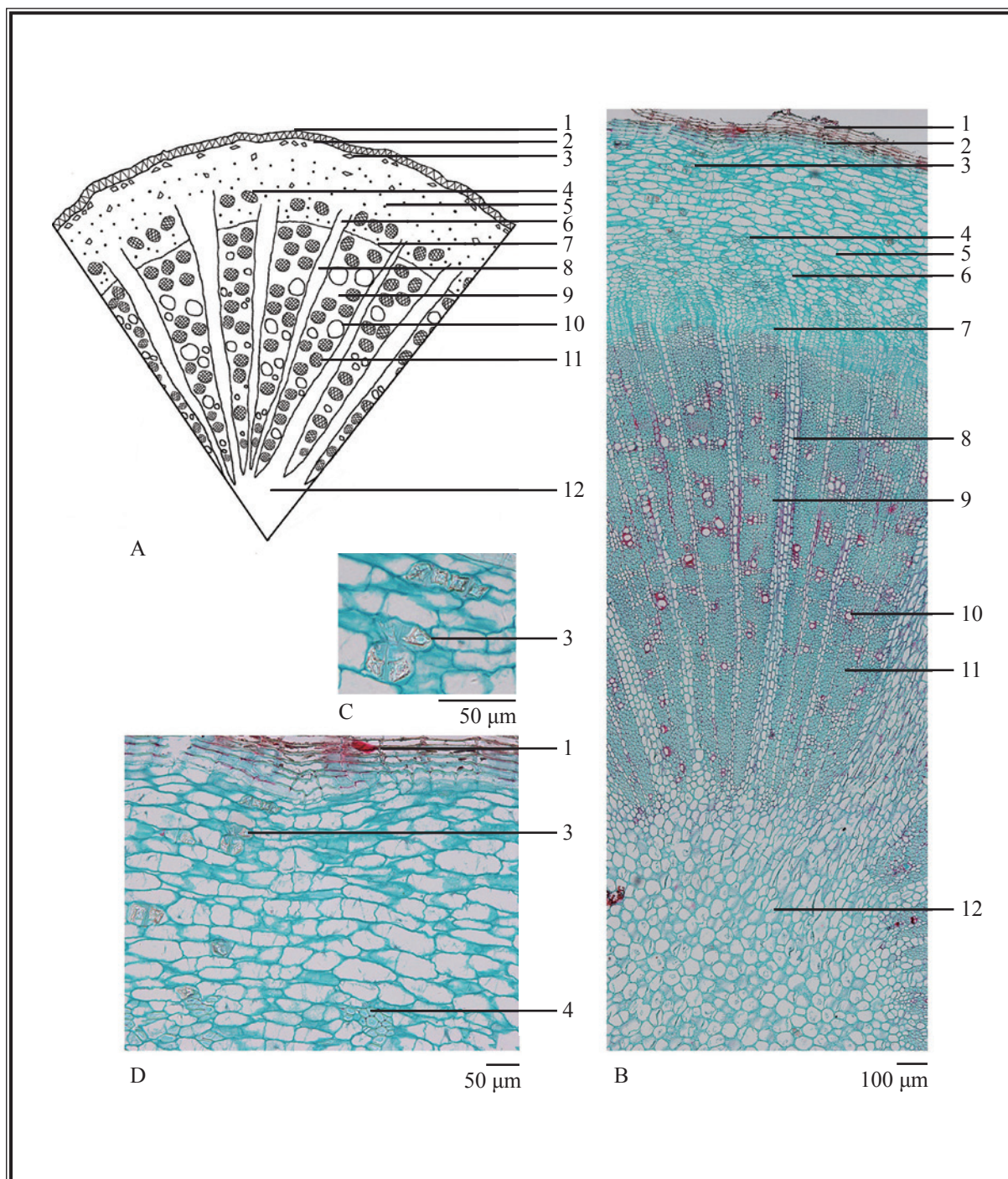


Figure 2 (ii) Microscopic features of transverse section of rhizome of *Sophorae Tonkinensis Radix et Rhizoma*

A. Sketch B. Section illustration C. Prisms of calcium oxalate D. Cork, phelloderm and fibre bundles

1. Cork 2. Phelloderm 3. Prism of calcium oxalate 4. Phloem fibre 5. Phloem 6. Phloem ray
7. Cambium 8. Xylem ray 9. Xylem 10. Vessel 11. Xylem fibre 12. Pith

Sophorae Tonkinensis Radix et Rhizoma

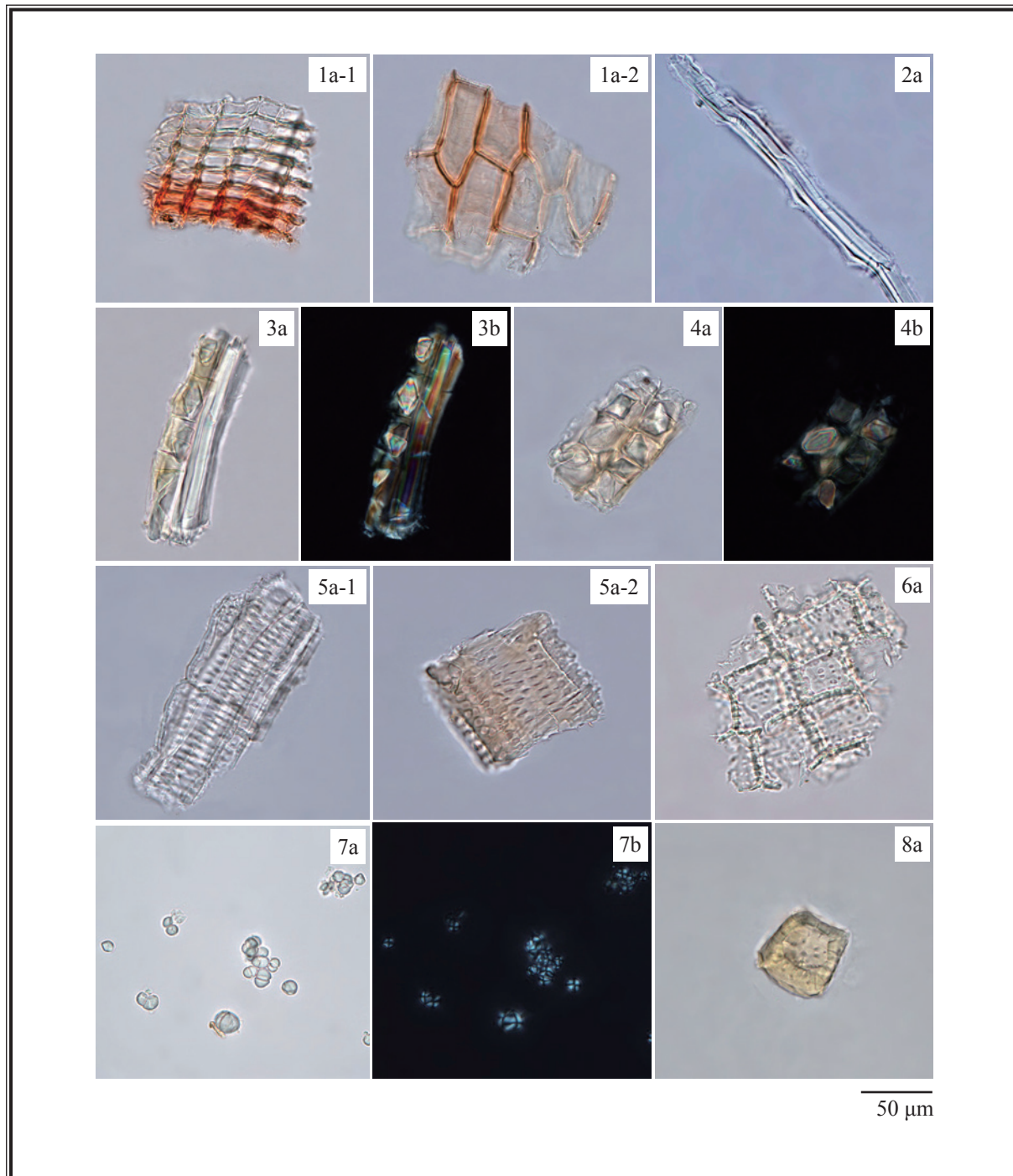


Figure 3 Microscopic features of powder of *Sophorae Tonkinensis Radix et Rhizoma*

1. Cork cells (1-1 in lateral view, 1-2 in surface view)
2. Fibre
3. Crystal fibre
4. Prisms of calcium oxalate
5. Vessels (5-1 reticulate vessel, 5-2 bordered-pitted vessel)
6. Lignified parenchymatous cells of xylem
7. Starch granules
8. Stone cell

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

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

Standard solutions

Matrine standard solution

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

Oxymatrine standard solution

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

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (9.1%, w/v) and *n*-butanol (2:3, v/v). Use the upper layer.

Spray reagent

Solution A

Weigh 0.85 g of bismuth oxynitrate and dissolve in a mixture of 10 mL of glacial acetic acid and 40 mL of water.

Solution B

Weigh 4 g of potassium iodide and dissolve in 10 mL of water.

Spray reagent

Transfer 5 mL of Solution A, 5 mL of Solution B and 20 mL of glacial acetic acid into a 100-mL volumetric flask and make up to the mark with water. Freshly prepare the reagent.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 0.2 mL of ammonium hydroxide solution (25%, v/v) and 10 mL of methanol. Oscillate (120 r/min) the mixture for 15 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate and a freshly prepared developing solvent system as described above. Apply separately matrine standard solution, oxymatrine standard solution and the test solution (4 μ L each) to the plate. Develop over a path of about 5 cm. After the development, remove the plate from the chamber, mark the solvent front and heat at about 105°C (about 1-2 min). Spray the plate evenly with the spray reagent and heat at about 105°C until the spots or bands become visible (about 1-2 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

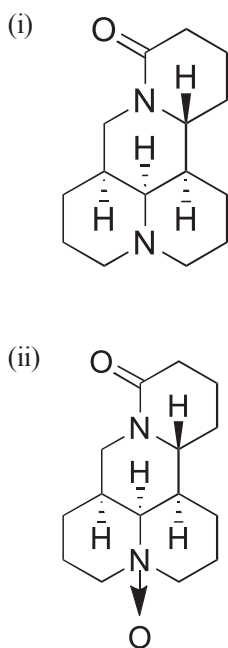


Figure 4 Chemical structures of (i) matrine and (ii) oxymatrine

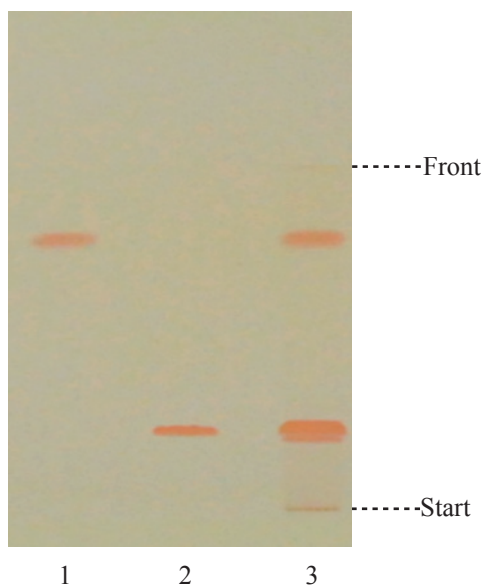


Figure 5 A reference HPTLC chromatogram of *Sophorae Tonkinensis Radix et Rhizoma* extract observed under visible light after staining

1. Matrine standard solution 2. Oxymatrine standard solution 3. Test solution

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 matrine and oxymatrine (Fig. 5).

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

Reagent

0.025 M Potassium dihydrogen phosphate solution (pH 2.0)

Weigh 3.4 g of potassium dihydrogen phosphate and dissolve in 1000 mL of water. Adjust the pH value to 2.0 with phosphoric acid.

Standard solutions

Matrine standard solution for fingerprinting, Std-FP (240 mg/L)

Weigh 2.4 mg of matrine CRS and dissolve in 10 mL of methanol. Keep at about 4°C.

Oxymatrine standard solution for fingerprinting, Std-FP (520 mg/L)

Weigh 5.2 mg of oxymatrine CRS and dissolve in 10 mL of methanol. Keep at about 4°C.

Test solution

Weigh 1.5 g of the powdered sample and place it in a 150-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture in a water bath for 1 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about $5000 \times g$ for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask 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 (220 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C 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)	Methanol (% v/v)	0.025 M Potassium dihydrogen phosphate solution (pH 2.0) (% v/v)	Elution
0 – 35	3 → 12	97 → 88	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 3 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 5000 and 10000 theoretical plates respectively.

The *R* value between peak 1 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 matrine Std-FP, oxymatrine Std-FP and the test solution (3 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 four characteristic peaks (Fig. 6) 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 four characteristic peaks of Sophorae Tonkinensis Radix et Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Sophorae Tonkinensis Radix et Rhizoma extract

Peak No.	RRT	Acceptable Range
1 (matrine)	0.61	± 0.03
2	0.81	± 0.05
3 (oxysophocarpine)	0.91	± 0.03
4 (marker, oxymatrine)	1.00	-

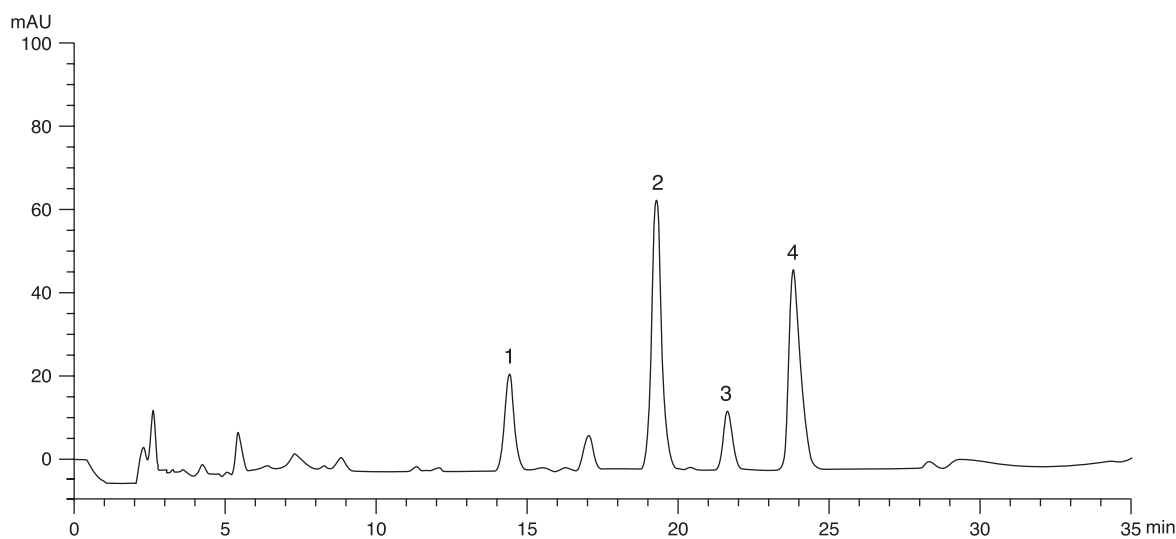


Figure 6 A reference fingerprint chromatogram of Sophorae Tonkinensis Radix et Rhizoma extract

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 (*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 6.0%.

Acid-insoluble ash: not more than 1.0%.

5.7 Water Content (Appendix X)

Oven dried method: not more than 8.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (hot extraction method): not less than 23.0%.

Ethanol-soluble extractives (hot extraction method): not less than 17.0%.

7. ASSAY

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

Reagent

0.025 M Potassium dihydrogen phosphate solution (pH 2.0)

Weigh 3.4 g of potassium dihydrogen phosphate and dissolve in 1000 mL of water. Adjust the pH value to 2.0 with phosphoric acid.

Standard solution

Mixed matrine and oxymatrine standard stock solution, Std-Stock (1200 mg/L for matrine and 2800 mg/L for oxymatrine)

Weigh accurately 12.0 mg of matrine CRS and 28.0 mg of oxymatrine CRS, and dissolve in 10 mL of methanol. Keep at about 4°C.

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

Measure accurately the volume of the mixed matrine and oxymatrine Std-Stock, dilute with methanol to produce a series of solutions of 24, 40, 120, 240, 600 mg/L for matrine and 56, 140, 560, 1400, 2800 mg/L for oxymatrine. Keep at about 4°C.

Test solution

Weigh accurately 1.5 g of the powdered sample and place it in a 150-mL round-bottomed flask, then add 50 mL of methanol. Reflux the mixture in a water bath for 1 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about $5000 \times g$ for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask 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 (220 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C 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)	Methanol (% v/v)	0.025 M Potassium dihydrogen phosphate solution (pH 2.0) (% v/v)	Elution
0 – 35	3 → 12	97 → 88	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 3 μL of the mixed matrine and oxymatrine Std-AS (120 mg/L for matrine and 560 mg/L for oxymatrine). 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 5000 and 10000 theoretical plates respectively.

The R value between matrine peak and the closest peak; and the R value between oxymatrine 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 the mixed matrine and oxymatrine Std-AS (3 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of matrine and oxymatrine against the corresponding concentrations of the mixed matrine and oxymatrine Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 3 μL of the test solution into the HPLC system and record the chromatogram. Identify matrine and oxymatrine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed matrine and oxymatrine Std-AS. The retention times of matrine and oxymatrine 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 and oxymatrine in the test solution, and calculate the percentage contents of matrine and oxymatrine in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.0% of the total content of matrine ($\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}$) and oxymatrine ($\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_2$), calculated with reference to the dried substance.

8. CAUTION

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.