Sanguisorbae Radix

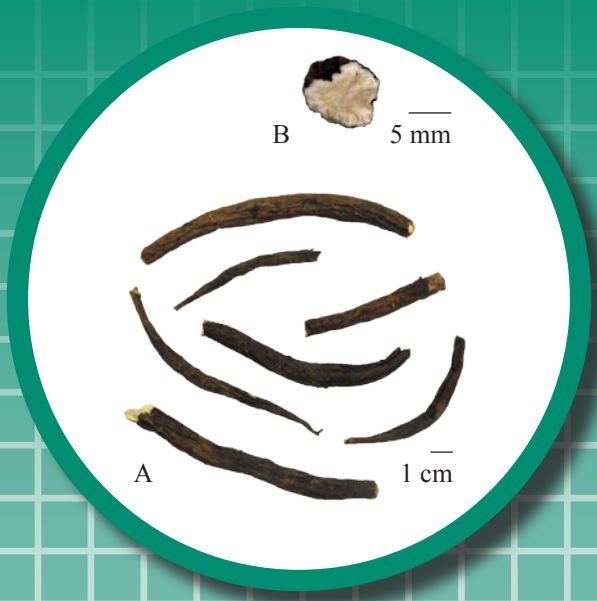


Figure 1 (i) A photograph of dried root of *Sanguisorba officinalis* L.

A. Root B. Magnified transverse section of root

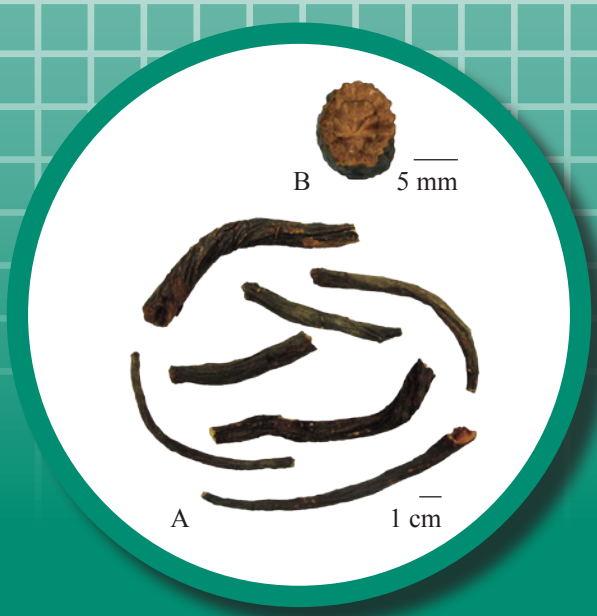


Figure 1 (ii) A photograph of dried root of *Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li

A. Root B. Magnified transverse section of root

1. NAMES

Official Name: Sanguisorbae Radix

Chinese Name: 地榆

Chinese Phonetic Name: Diyu

2. SOURCE

Sanguisorbae Radix is the dried root of *Sanguisorba officinalis* L. or *Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li (Rosaceae). The root is collected in spring before budding or in autumn after withering, rootlets removed, washed clean and dried, or cut into slices when fresh, then dried under the sun or baked to obtain Sanguisorbae Radix.

3. DESCRIPTION

***Sanguisorba officinalis* L.:** Cylindrical or fusiform, slightly curved, sometimes with rootlets, 4-22 cm long, 2-15 mm in diameter. Externally dark brown, rough, with longitudinal wrinkles. Texture hard and slightly brittle; fracture white to yellowish-white, slightly starchy, cambium ring distinct, wood radially arranged. Odour slight; taste slightly bitter and astringent [Fig. 1 (i)].

***Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li:** Cylindrical, slightly curved or twisted, sometimes with rootlets, 4-22 cm long, 2-49 mm in diameter. Texture hard and tenacious, fracture yellow to yellowish-brown, bark with numerous yellowish-brown wooly fibres, cambium ring distinct, wood radially arranged [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

***Sanguisorba officinalis* L.:** Cork consists of several layers of brown cells. Cortex consists of several layers of oblong cells. Phloem broad, with clefts; fibres rare, mostly scattered singly; phloem rays distinct. Cambium distinct, arranged in a ring. In xylem, vessels arranged radially, surrounded by fibres; xylem rays distinct. Parenchymatous cells contain abundant starch granules, scattered with clusters of calcium oxalate, prisms of calcium oxalate occasionally found [Fig. 2 (i)].

***Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li:** In phloem, fibres scattered singly or arranged in bundles. Parenchymatous cells scattered with a few clusters of calcium oxalate [Fig. 2 (ii)].

Powder

***Sanguisorba officinalis* L.:** Colour greyish-brown to yellowish-brown. Clusters of calcium oxalate abundant, with broad angles, 10-55 μm in diameter; polychromatic under the polarized microscope. Prisms of calcium oxalate subsquare or irregular, 4-23 μm in diameter; bright yellowish-white to polychromatic under the polarized microscope. Starch granules mostly simple, long ovate or oblong, 3-28 μm long, 2-20 μm in diameter, hilum indistinct, some slit-shaped, striations indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules occasionally found. Fibres relatively few, usually singly, 3-33 μm in diameter; yellowish-white to brownish-yellow under the polarized microscope. Cork cells brownish-yellow, polygonal or subsquare in surface view, some contain yellowish-brown contents. Vessels mainly bordered-pitted and scalariform, 12-80 μm in diameter [Fig. 3 (i)].

***Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li:** Colour yellowish-brown to brown. Clusters of calcium oxalate with broad angles, 10-69 μm in diameter; polychromatic under the polarized microscope. Prisms of calcium oxalate irregular, 3-21 μm in diameter; bright yellowish-white to polychromatic under the polarized microscope. Starch granules mostly simple, subrounded or ovate, 2-19 μm in diameter, hilum and striations indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules occasionally found. Fibres abundant, usually singly, 3-46 μm in diameter; bright white under the polarized microscope. Cork cells reddish-brown, subrectangular or polygonal in surface view, some contain yellowish-brown contents. Vessels mainly bordered-pitted, 10-81 μm in diameter [Fig. 3 (ii)].

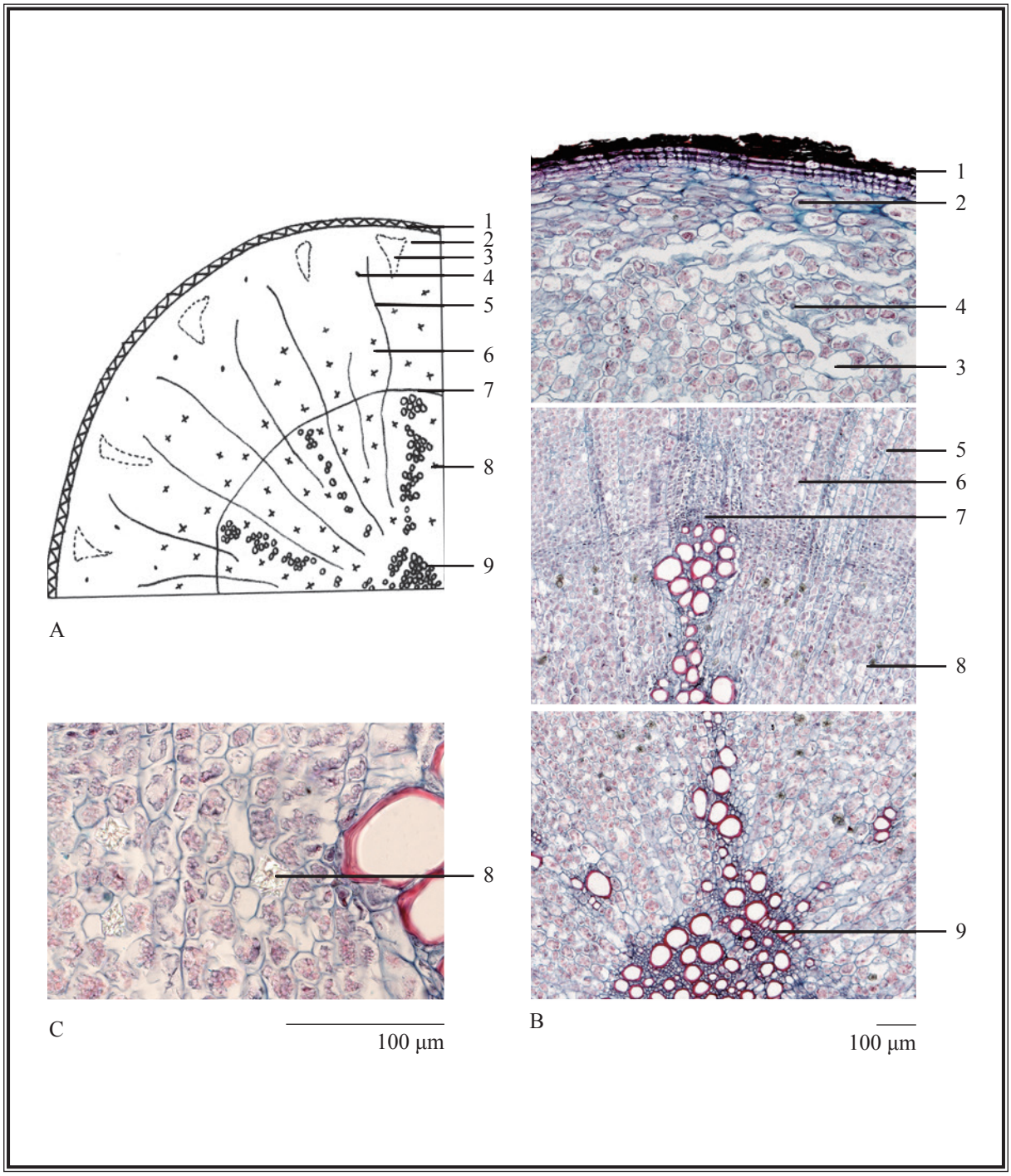


Figure 2 (i) Microscopic features of transverse section of dried root of *Sanguisorba officinalis* L.

A. Sketch B. Section illustration C. Cluster of calcium oxalate

1. Cork 2. Cortex 3. Clefts 4. Phloem fibre 5. Ray
6. Phloem 7. Cambium 8. Cluster of calcium oxalate 9. Xylem

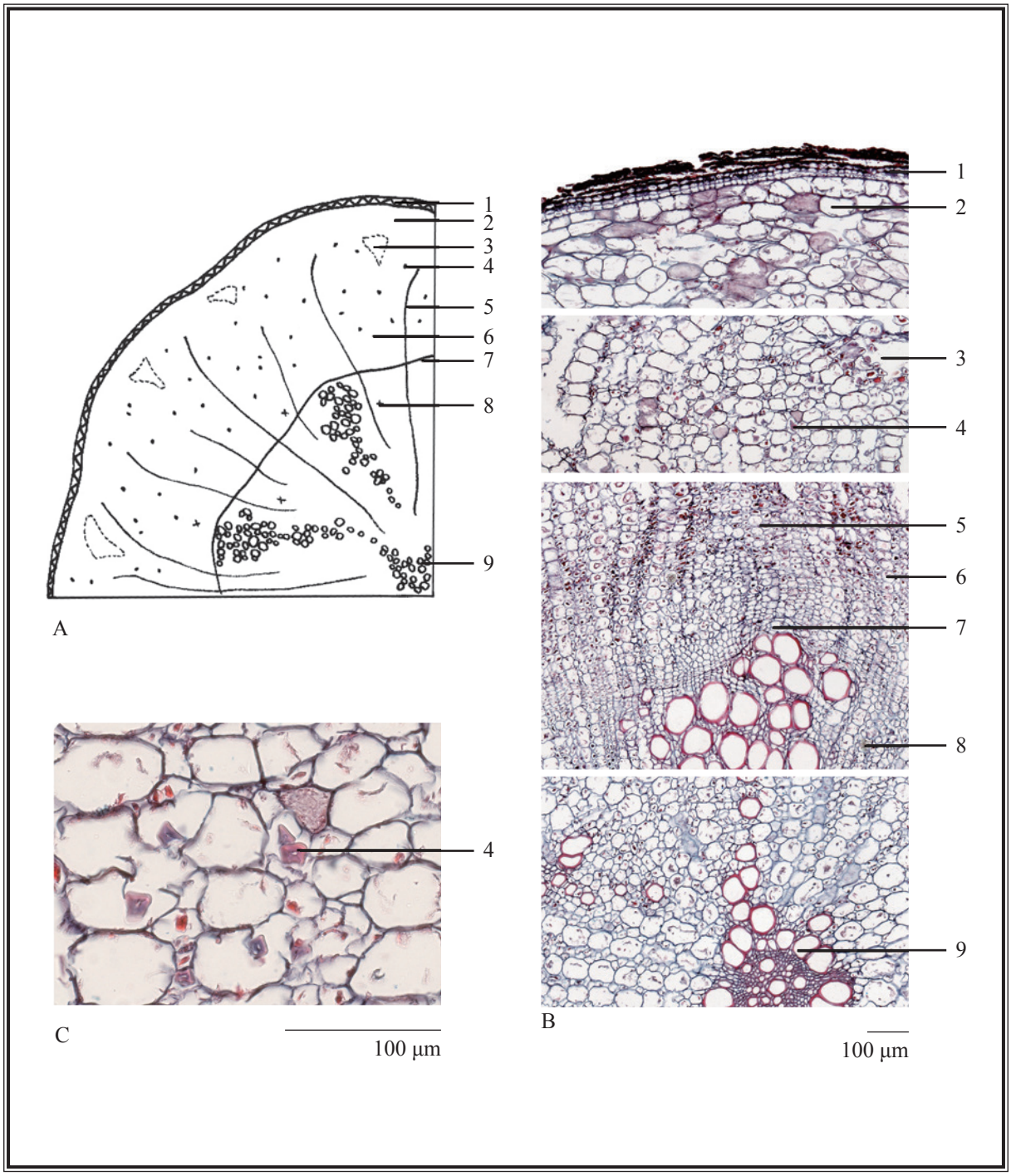


Figure 2 (ii) Microscopic features of transverse section of dried root of *Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li

A. Sketch B. Section illustration C. Phloem fibres

1. Cork 2. Cortex 3. Clefts 4. Phloem fibres 5. Ray
6. Phloem 7. Cambium 8. Cluster of calcium oxalate 9. Xylem

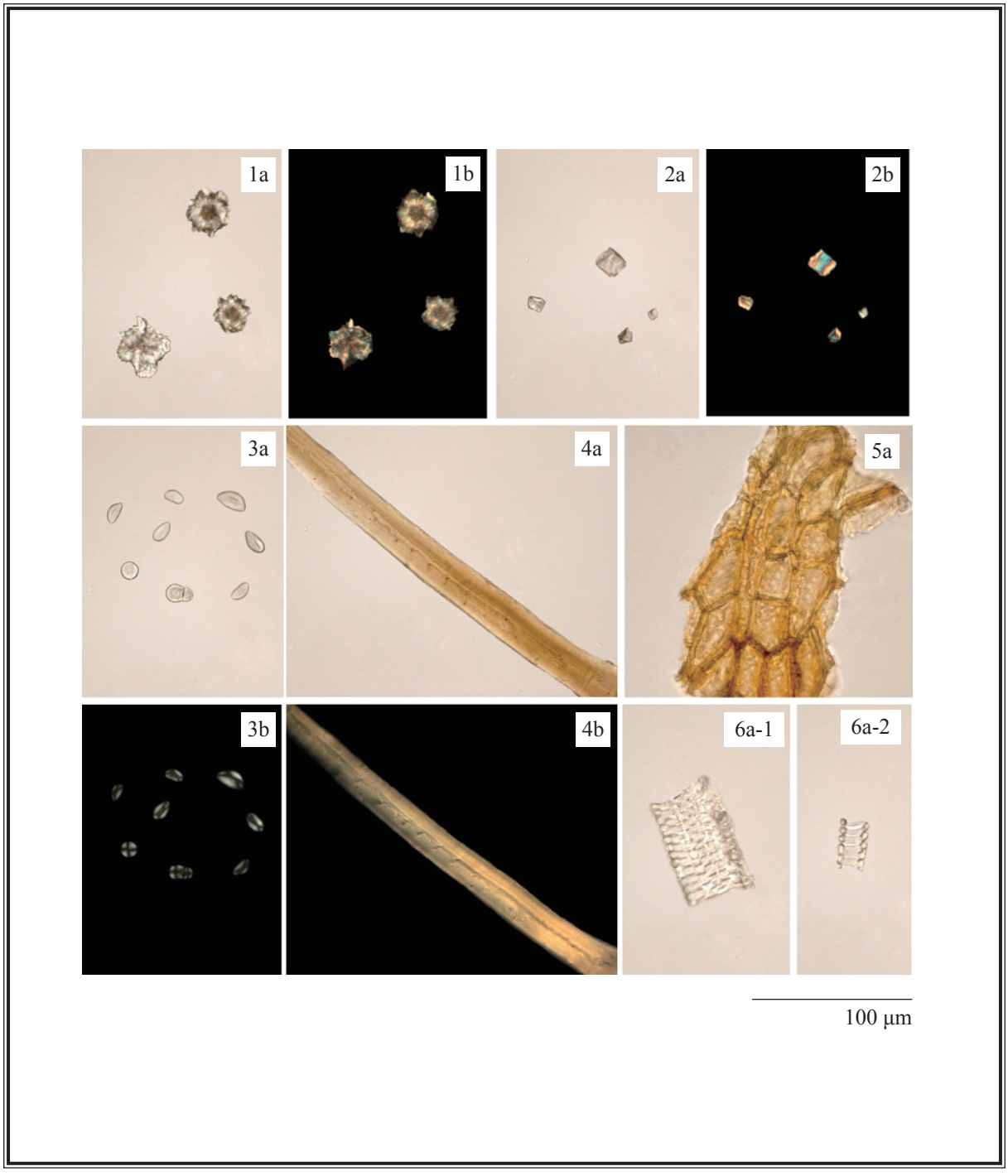


Figure 3 (i) Microscopic features of powder of dried root of *Sanguisorba officinalis* L.

1. Clusters of calcium oxalate 2. Prisms of calcium oxalate 3. Starch granules
4. Fibre 5. Cork cells 6. Vessels (6-1 bordered-pitted vessel, 6-2 scalariform vessel)

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

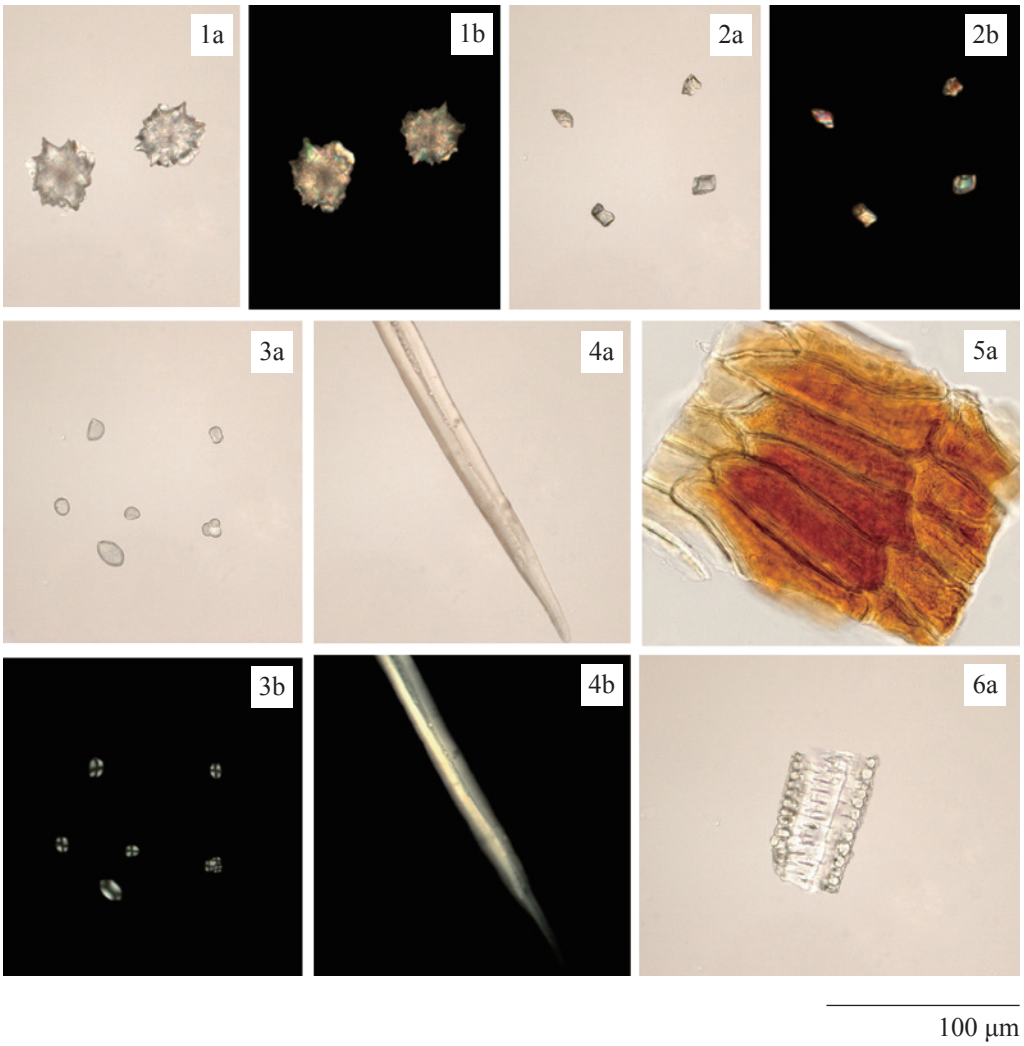


Figure 3 (ii) Microscopic features of powder of dried root of *Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li

1. Clusters of calcium oxalate 2. Prisms of calcium oxalate 3. Starch granules
4. Fibre 5. Cork cells 6. Bordered-pitted vessel

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

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

Standard solutions

Ziyu-glycoside I standard solution

Weigh 1.0 mg of ziyu-glycoside I CRS (Fig. 4) and dissolve in 1 mL of methanol.

Ziyu-glycoside II standard solution

Weigh 1.0 mg of ziyu-glycoside II CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, methanol and water (15:1:0.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

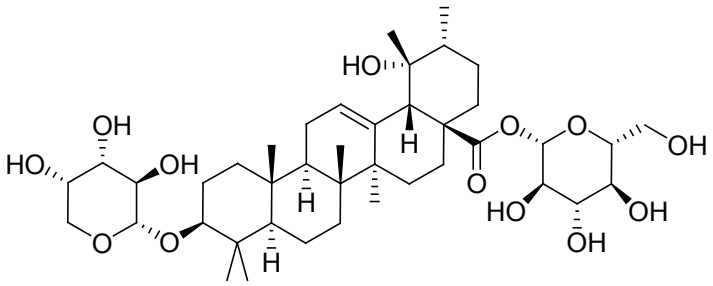
Test solution

Weigh 0.5 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

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 ziyu-glycoside I standard solution (1 μL), ziyu-glycoside II standard solution (0.5 μL) and the test solution (1 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 15 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 6 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 105ºC until the spots or bands become visible (about 3-5 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

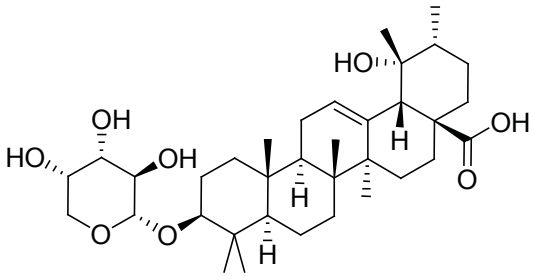


Figure 4 Chemical structures of (i) ziyu-glycoside I and (ii) ziyu-glycoside II

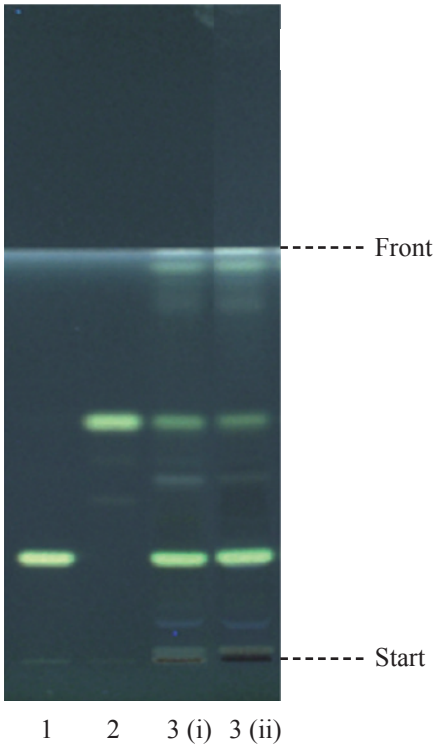


Figure 5 A reference HPTLC chromatogram of Sanguisorbae Radix extract observed under UV light (366 nm) after staining

1. Ziyu-glycoside I standard solution
2. Ziyu-glycoside II standard solution
3. Test solution of
(i) dried root of *Sanguisorba officinalis* L.
(ii) dried root of *Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li

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 ziyu-glycoside I and ziyu-glycoside II (Fig. 5).

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

Reagents

0.2 M Potassium dihydrogen phosphate solution

Weigh 27.2 g of potassium dihydrogen phosphate and dissolve in 1000 mL of water.

0.2 M Sodium hydroxide solution

Weigh 8.0 g of sodium hydroxide and dissolve in 1000 mL of water.

0.01 M Potassium dihydrogen phosphate buffer solution (pH 6.2)

Pipette 50 mL of 0.2 M potassium dihydrogen phosphate solution and 8.6 mL of 0.2 M sodium hydroxide solution to a 1000-mL volumetric flask. Make up to the mark with water.

Standard solutions

Ziyu-glycoside I standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of ziyu-glycoside I CRS and dissolve in 10 mL of methanol (70%).

Ziyu-glycoside II standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of ziyu-glycoside II CRS and dissolve in 5 mL of methanol. Pipette 1 mL of the solution to a 10-mL volumetric flask and make up to the mark with methanol (70%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and 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 (206 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.01 M potassium dihydrogen phosphate buffer solution (pH 6.2) and methanol (37:63, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of ziyu-glycoside I Std-FP and ziyu-glycoside II Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ziyu-glycoside I and ziyu-glycoside II should not be more than 5.0%; the RSD of the retention times of ziyu-glycoside I and ziyu-glycoside II peaks should not be more than 2.0%; the column efficiencies determined from ziyu-glycoside I and ziyu-glycoside II peaks should not be less than 5000 theoretical plates.

The *R* value between peak 2 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. 6 (i) or (ii)].

Procedure

Separately inject ziyu-glycoside I Std-FP, ziyu-glycoside II Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention times of ziyu-glycoside I and ziyu-glycoside II peaks in the chromatograms of ziyu-glycoside I Std-FP, ziyu-glycoside II Std-FP and the retention times of the three characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify ziyu-glycoside I and ziyu-glycoside II peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of ziyu-glycoside I Std-FP and ziyu-glycoside II Std-FP. The retention times of ziyu-glycoside I and ziyu-glycoside II 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 Sanguisorbae Radix extract are listed in Table 1.

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

Peak No.	RRT	Acceptable Range
1	0.92	± 0.03
2 (marker, ziyu-glycoside I)	1.00	-
3 (ziyu-glycoside II)	1.21	± 0.04

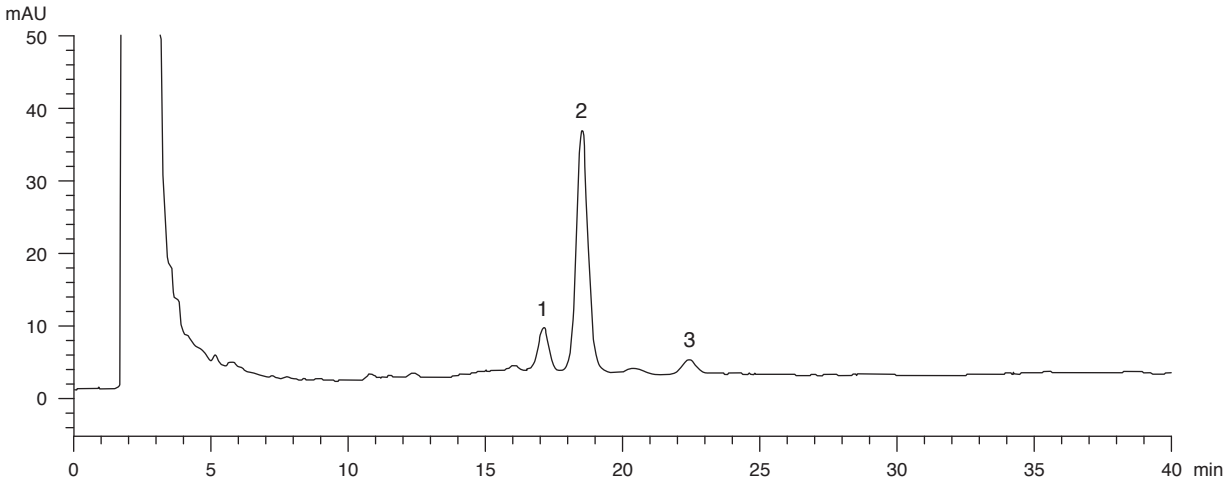


Figure 6 (i) A reference fingerprint chromatogram of dried root of *Sanguisorba officinalis* L. extract

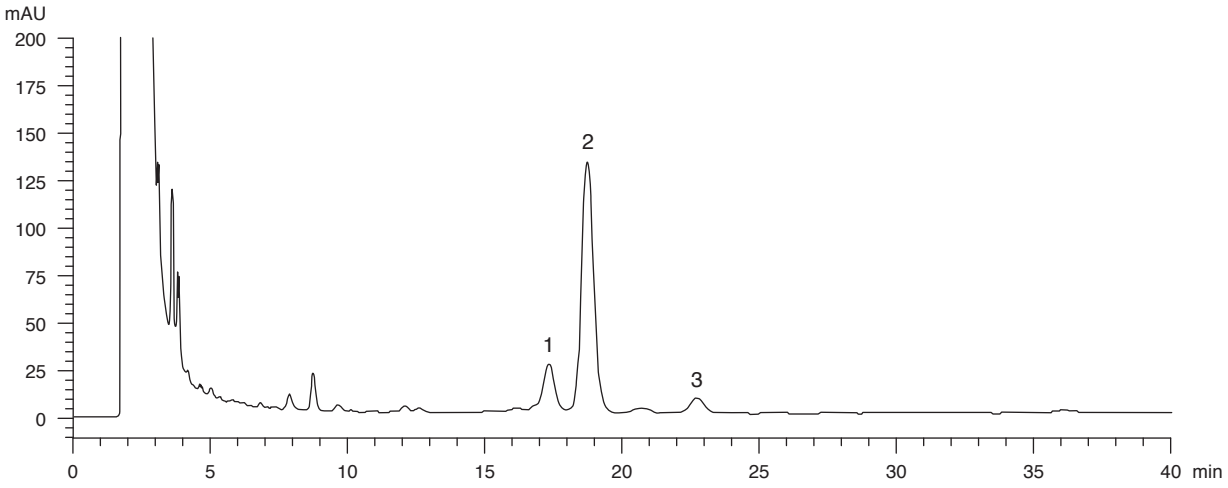


Figure 6 (ii) A reference fingerprint chromatogram of dried root of *Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yü et Li extract

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 respective reference fingerprint chromatograms [Fig. 6 (i) or (ii)].

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 XVII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 30.0%.
Ethanol-soluble extractives (cold extraction method): not less than 32.0%.

7. ASSAY

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

Reagents

0.2 M Potassium dihydrogen phosphate solution

Weigh 27.2 g of potassium dihydrogen phosphate and dissolve in 1000 mL of water.

0.2 M Sodium hydroxide solution

Weigh 8.0 g of sodium hydroxide and dissolve in 1000 mL of water.

0.01 M Potassium dihydrogen phosphate buffer solution (pH 6.2)

Pipette 50 mL of 0.2 M potassium dihydrogen phosphate solution and 8.6 mL of 0.2 M sodium hydroxide solution to a 1000-mL volumetric flask. Make up to the mark with water.

Standard solution

Ziyu-glycoside I standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of ziyu-glycoside I CRS and dissolve in 5 mL of methanol (70%).

Ziyu-glycoside II standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 0.5 mg of ziyu-glycoside II CRS and dissolve in 5 mL of methanol.

Mixed ziyu-glycoside I and ziyu-glycoside II standard solution for assay, Std-AS

Measure accurately the volume of the ziyu-glycoside I Std-Stock and ziyu-glycoside II Std-Stock, dilute with methanol (70%) to produce a series of solutions of 50, 100, 200, 500, 1000 mg/L for ziyu-glycoside I and 2, 5, 10, 20, 50 mg/L for ziyu-glycoside II.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and 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 (206 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.01 M potassium dihydrogen phosphate buffer solution (pH 6.2) and methanol (37:63, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed ziyu-glycoside I and ziyu-glycoside II Std-AS (200 mg/L for ziyu-glycoside I and 10 mg/L for ziyu-glycoside II). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of ziyu-glycoside I and ziyu-glycoside II should not be more than 5.0%; the RSD of the retention times of ziyu-glycoside I and ziyu-glycoside II peaks should not be more than 2.0%; the column efficiencies determined from ziyu-glycoside I and ziyu-glycoside II peaks should not be less than 5000 theoretical plates.

The *R* value between ziyu-glycoside I peak and the closest peak; and the *R* value between ziyu-glycoside II 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 ziyu-glycoside I and ziyu-glycoside II Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of ziyu-glycoside I and ziyu-glycoside II against the corresponding concentrations of the mixed ziyu-glycoside I and ziyu-glycoside II Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify ziyu-glycoside I and ziyu-glycoside II peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed ziyu-glycoside I and ziyu-glycoside II Std-AS. The retention times of ziyu-glycoside I and ziyu-glycoside II 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 ziyu-glycoside I and ziyu-glycoside II in the test solution, and calculate the percentage contents of ziyu-glycoside I and ziyu-glycoside II in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.5% of ziyu-glycoside I ($C_{41}H_{66}O_{13}$) and not less than 0.091% of ziyu-glycoside II ($C_{35}H_{56}O_8$), calculated with reference to the dried substance.