

Radix et Rhizoma Glycyrrhizae



Figure 1(i) A photograph of dried root and rhizome of *Glycyrrhiza uralensis* Fisch.



Figure 1(ii) A photograph of dried root and rhizome of *Glycyrrhiza inflata* Bat.

1. NAMES

Official Name: Radix et Rhizoma Glycyrrhizae

Chinese Name: 甘草

Chinese Phonetic Name: Gancáo

2. SOURCE

Radix et Rhizoma Glycyrrhizae is the dried root and rhizome of *Glycyrrhiza uralensis* Fisch. or *Glycyrrhiza inflata* Bat. (Leguminosae). The root together with the rhizome is collected in the spring and autumn. After removal of the rootlets, the root and rhizome are dried under the sun to obtain Radix et Rhizoma Glycyrrhizae.

3. DESCRIPTION

***Glycyrrhiza uralensis* Fisch. :** Roots cylindrical, 12-56 cm long, 5-36 mm in diameter, some branched. The outer bark loose or tight. Externally reddish-brown or greyish-brown, prominently longitudinally wrinkled, lenticellate, and with sparse rootlet scars. Texture compact, fracture slightly fibrous, yellowish-white to yellow, starchy, cambium ring distinct, rays radial, some with clefts. Rhizome cylindrical, externally with bud scars, pith present in the centre of fracture. Odour slight; taste sweet and characteristic [Fig. 1(i)].

***Glycyrrhiza inflata* Bat. :** Roots and rhizomes woody and stout, 6-60 cm long, and 6-30 mm in diameter. The outer bark rough, mostly greyish-brown. Texture compact, pale yellow to yellowish-brown, with many lignified fibres, and not too starchy. The rhizome has many large adventitious buds [Fig. 1(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

The cork consists of several or more than ten layers of brown cells. The cortex relatively narrow. Phloem rays broad, mostly curved outside, and frequently with clefts; most phloem fibres in

bundles, unligified or slightly lignified, and surrounded by parenchyma cells containing prisms of calcium oxalate; sieve tube tissues often being distorted by compression. Fascicular cambium distinct. Xylem rays 2-5 cells wide, vessels frequent, up to 170 μm in diameter, xylem fibres in bundles, surrounded by parenchyma cells containing prisms of calcium oxalate. Root without, but rhizome with a pith at the centre [Fig. 2(i) and (ii)].

Powder

Colour brownish-yellow. Fibres in bundles, 5-25 μm in diameter, thick-walled, slightly lignified, surrounded by parenchyma cells containing prisms of calcium oxalate, forming crystal fibres. Crystals of calcium oxalate double-conical, rectangular or cubical, 4-18 μm in diameter, up to 40 μm in length. Vessels mostly bordered-pitted, up to 170 μm in diameter, rarely reticulated, both lignified. Starch grains frequent, single grains subspheroidal, ellipsoidal or elongated-ovoid, hilum pointed or slit-shaped, 2-23 μm in diameter; compound grains rare. Cork cells reddish-brown, polygonal in top view, slightly lignified. Pigmented masses rare, yellowish-brown or reddish-brown [Fig. 3(i) and (ii)].

4.2 Physicochemical Identification

Procedure

Weigh 1.0 g of the powdered sample and put into a 25-mL conical flask, then add 10 mL of ethanol (95%). Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a test tube. Evaporate to dryness on a water bath at about 70°C. Cool to room temperature. Dissolve the residue in 3 mL of methanol. Transfer 5 μL of the solution to a test tube, add 2 mL of dichloromethane with slight shaking. Cautiously add 1 mL of sulphuric acid along the inner wall of the test tube. Allow to stand for about 30 min. A yellow to orange-red ring is observed at the interface of the two solvent layers.

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

Standard solutions

Glycyrrhizic acid standard solution

Weigh 1.0 mg of glycyrrhizic acid CRS (Fig. 4) and dissolve in 0.5 mL of methanol.

Liquiritin standard solution

Weigh 1.0 mg of liquiritin CRS (Fig. 4) and dissolve in 0.5 mL of methanol.

Developing solvent system

Prepare a mixture of 1-butanol, glacial acetic acid and water (7:1:12, v/v) in a separating funnel. Shake well and allow to stand for 30 min. Use the upper layer.

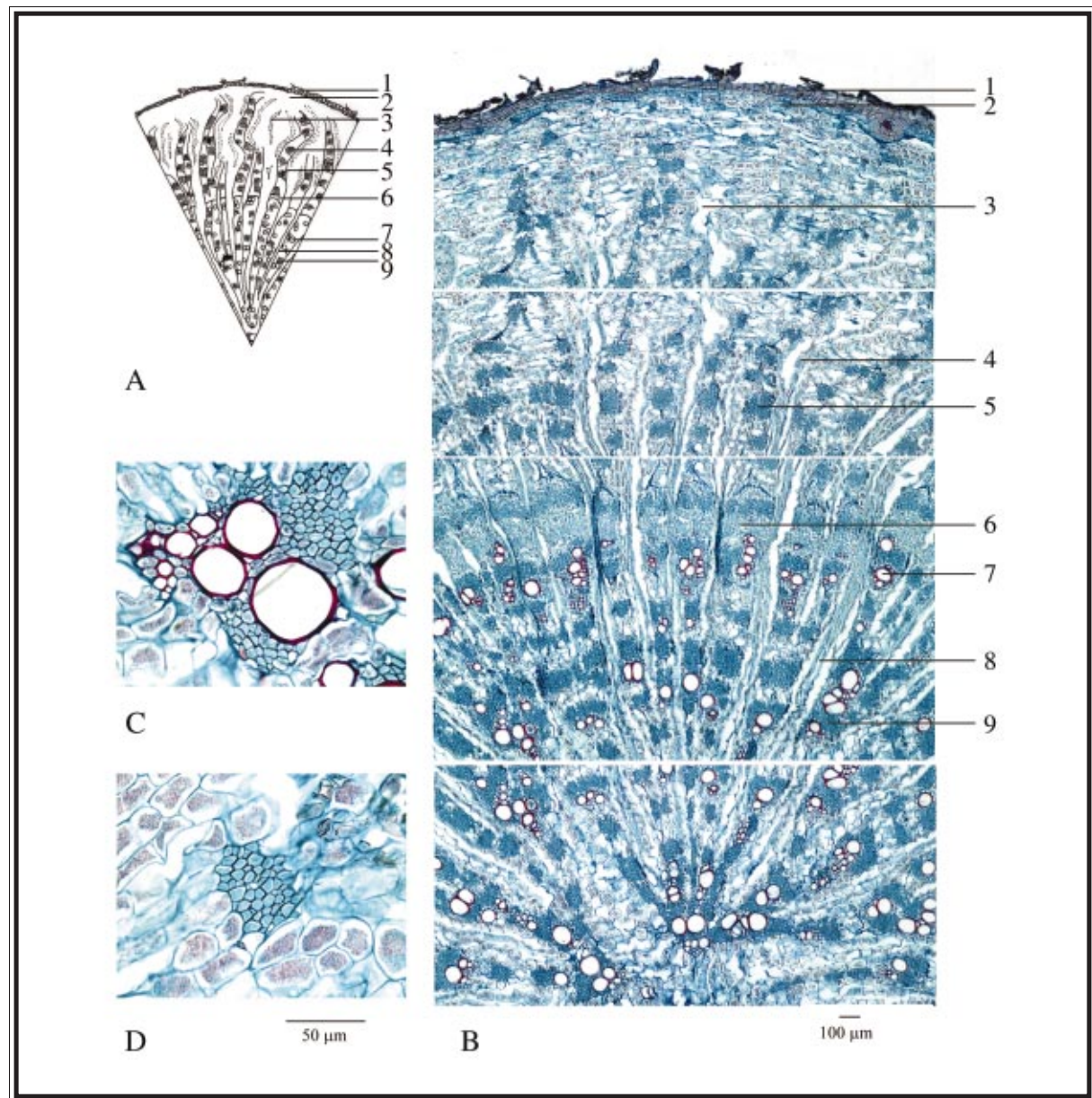


Figure 2(i) Microscopic features of transverse section of dried root and rhizome of *Glycyrrhiza uralensis* Fisch.

A. Sketch B. Section illustration C. Vessels D. Fibres

1. Cork 2. Cortex 3. Clefts 4. Phloem rays 5. Phloem fibres 6. Cambium 7. Vessels 8. Xylem rays 9. Xylem fibres

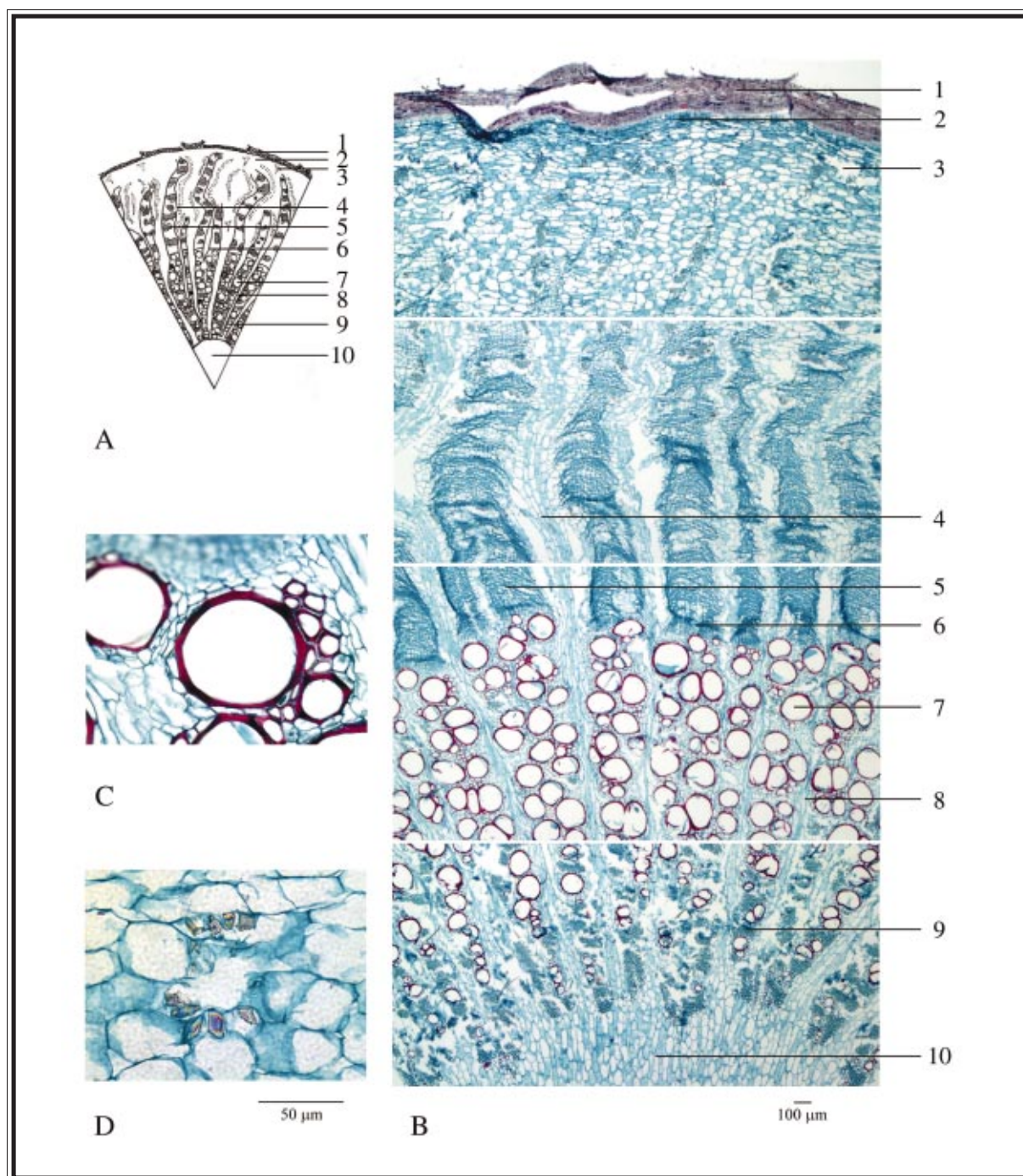


Figure 2(ii) Microscopic features of transverse section of dried root and rhizome of *Glycyrrhiza inflata* Bat.

A. Sketch B. Section illustration C. Vessels D. Prisms of calcium oxalate

1. Cork 2. Cortex 3. Clefts 4. Phloem rays 5. Phloem fibres 6. Cambium 7. Vessels 8. Xylem rays
9. Xylem fibres 10. Pith

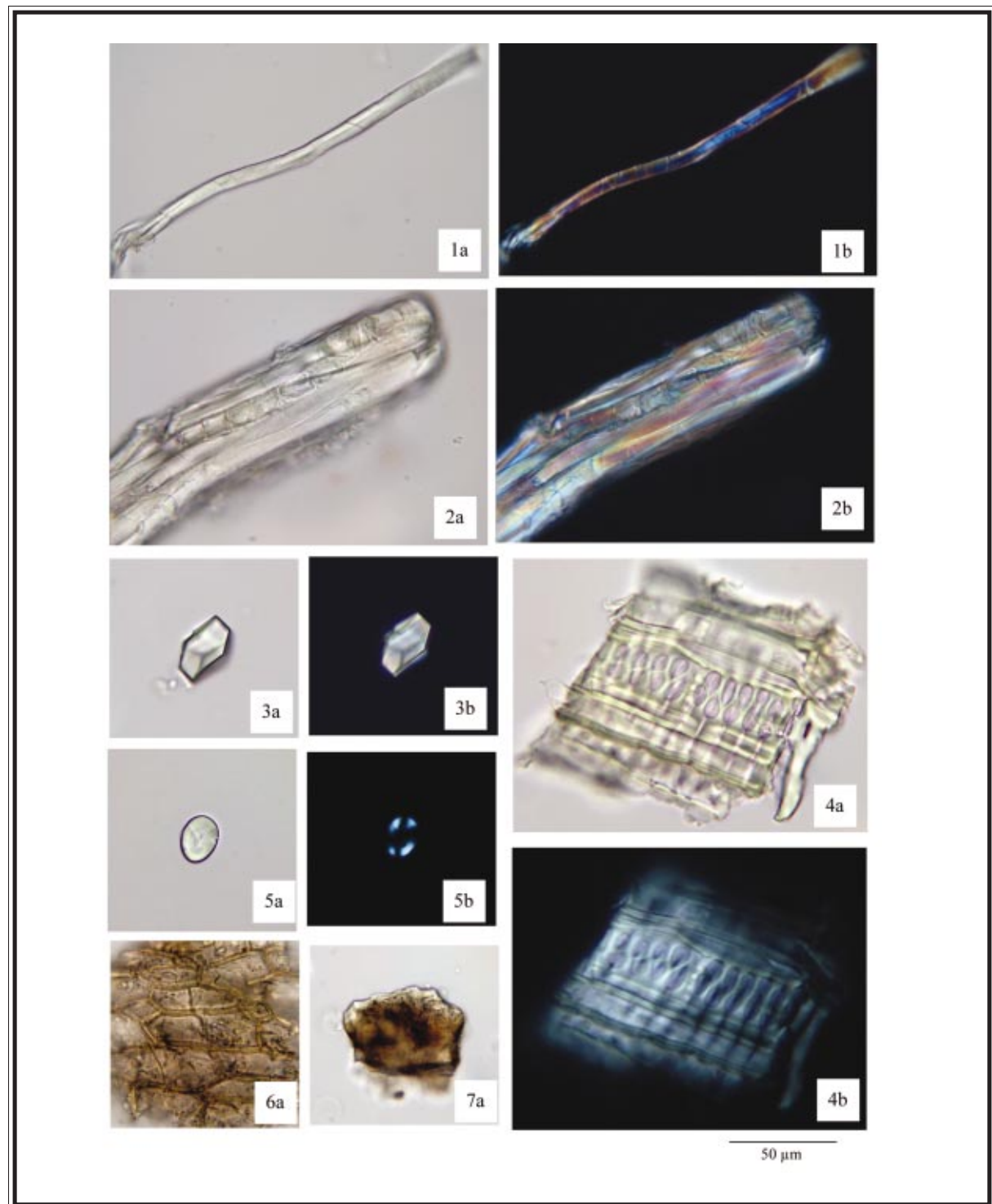


Figure 3(i) Microscopic features of powder of dried root and rhizome of *Glycyrrhiza uralensis* Fisch.

1. Single fibre
2. Crystal fibre bundle
3. Prisms of calcium oxalate
4. Bordered-pitted vessels
5. Starch granule
6. Cork cells
7. Pigmented mass

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

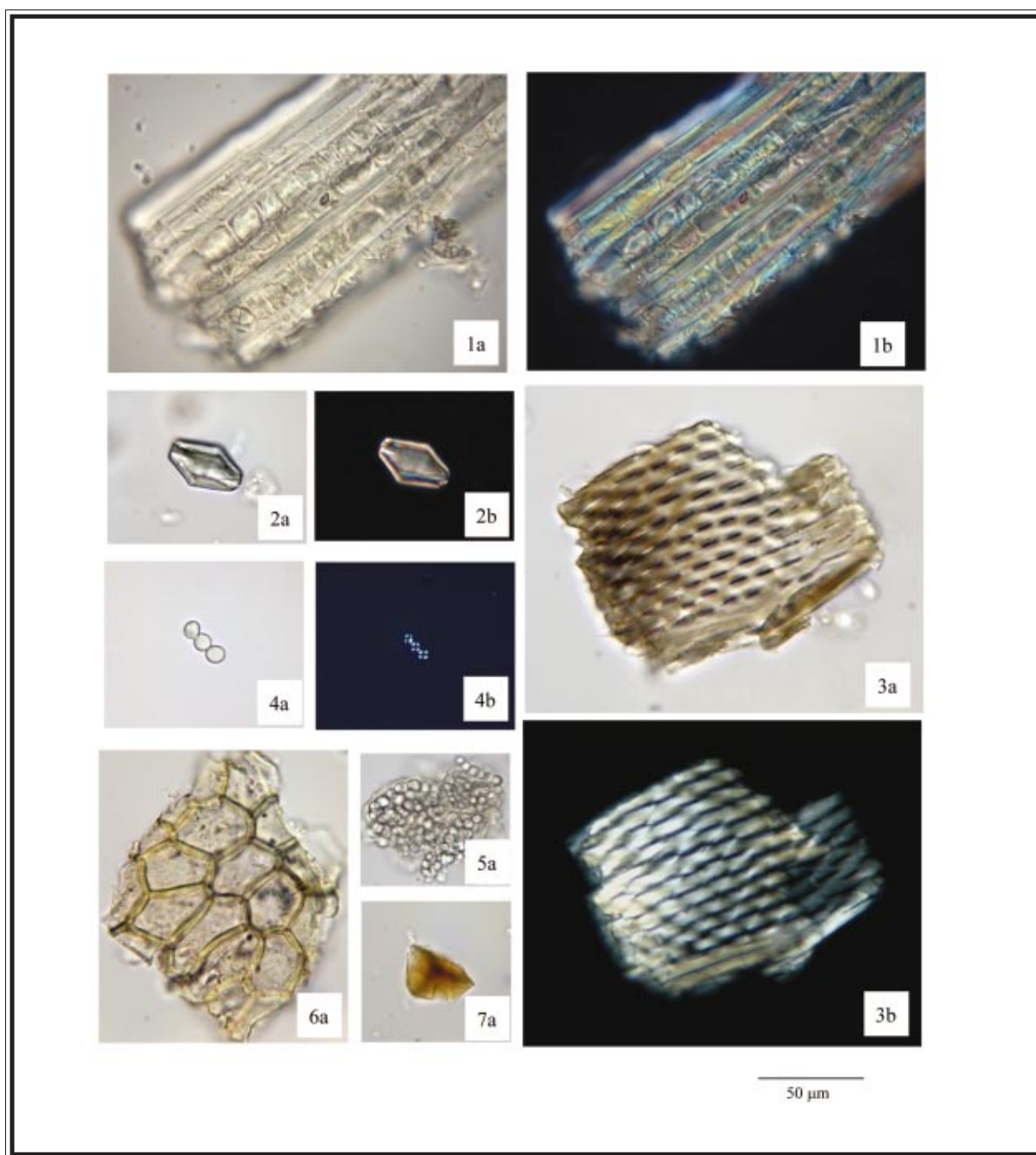


Figure 3(ii) Microscopic features of powder of dried root and rhizome of *Glycyrrhiza inflata* Bat.

1. Crystal fibres of bundle 2. Prisms of calcium oxalate 3. Bordered-pitted vessel 4. Starch granules
 5. Starch granules in parenchyma cells 6. Cork cells 7. Pigmented mass
- a. Features under the light microscope b. Features under the polarized microscope

Spray reagent

Add slowly 20 mL of sulphuric acid to 80 mL of ethanol.

Test solution

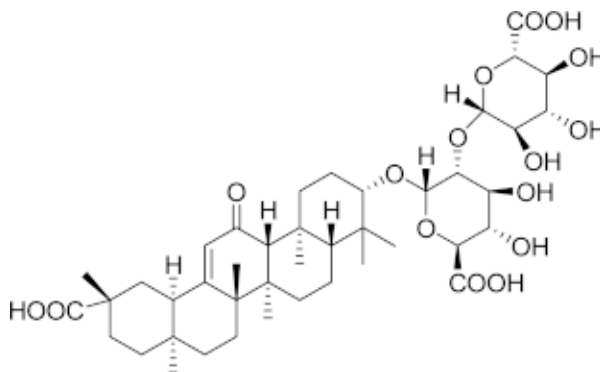
Weigh 1.0 g of the powdered sample and put into a 250-mL round-bottomed flask, then add 40 mL of diethyl ether. Reflux the mixture for 1 h. Cool to room temperature. Filter and discard the filtrate. Transfer the residue to a 250-mL round-bottomed flask, add 30 mL of methanol. Reflux the mixture for 1 h. Cool to room temperature and filter. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 40 mL of water. Extract thrice each with 20 mL of 1-butanol. Combine the 1-butanol extracts and wash thrice each with 20 mL of water. Evaporate the 1-butanol extracts 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 glycyrrhizic acid standard solution, liquiritin standard solution (1 μ L each) and the test solution (2 μ L) to the plate. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm), and then spray the plate evenly with the spray reagent. Heat the plate at about 110°C until the spots or bands become visible (about 5 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 glycyrrhizic acid and liquiritin.

(i)



Radix et Rhizoma Glycyrrhizae

(ii)

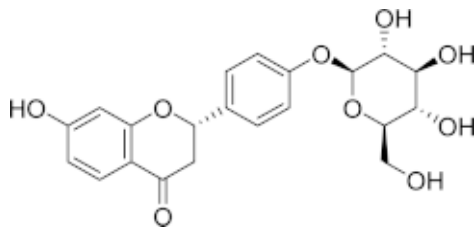


Figure 4 Chemical structures of (i) glycyrrhizic acid and (ii) liquiritin

4.4 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Glycyrrhizic acid standard stock solution, Std-Stock (1000 mg/L)

Weigh 5.0 mg of glycyrrhizic acid CRS and dissolve in 5 mL of methanol (70%).

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

Pipette 1.0 mL of glycyrrhizic acid Std-Stock into a 10-mL volumetric flask and make up to the mark with methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of methanol (70%). Sonicate (220 W) the mixture for 30 min. Centrifuge at about 3200 × g for 10 min. Filter and transfer the filtrate to a 50-mL volumetric flask. 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 detector (230 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 –

Time (min)	Acetonitrile (%, v/v)	0.03% Phosphoric acid (%, v/v)	Elution
0 – 10	5 ➔ 25	95 ➔ 75	linear gradient
10 – 20	25	75	isocratic
20 – 36	25 ➔ 50	75 ➔ 50	linear gradient
36 – 60	50 ➔ 95	50 ➔ 5	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of glycyrrhizic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of glycyrrhizic acid should not be more than 5.0%; the RSD of the retention time of glycyrrhizic acid peak should not be more than 5.0%; the column efficiency determined from glycyrrhizic acid peak should not be less than 50000 theoretical plates.

The *R* value between peak 2 and peak 3 in the chromatogram of the test solution should not be less than 1.5 [Fig. 5(i) or (ii)].

Procedure

Separately inject glycyrrhizic acid Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of glycyrrhizic acid peak in the chromatogram of the glycyrrhizic acid Std-FP and the retention times of the four characteristic peaks [Fig. 5(i) or (ii)] in the chromatogram of the test solution. Under the same HPLC conditions, identify glycyrrhizic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of glycyrrhizic acid Std-FP. The retention times of glycyrrhizic acid 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 four characteristic peaks of Radix et Rhizoma Glycyrrhizae extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Radix et Rhizoma Glycyrrhizae extract

Peak No.	RRT	Acceptable Range
1	0.29	±0.03
2	0.38	±0.03
3 (liquiritin)	0.39	±0.03
4 (marker, glycyrrhizic acid)	1.00	-

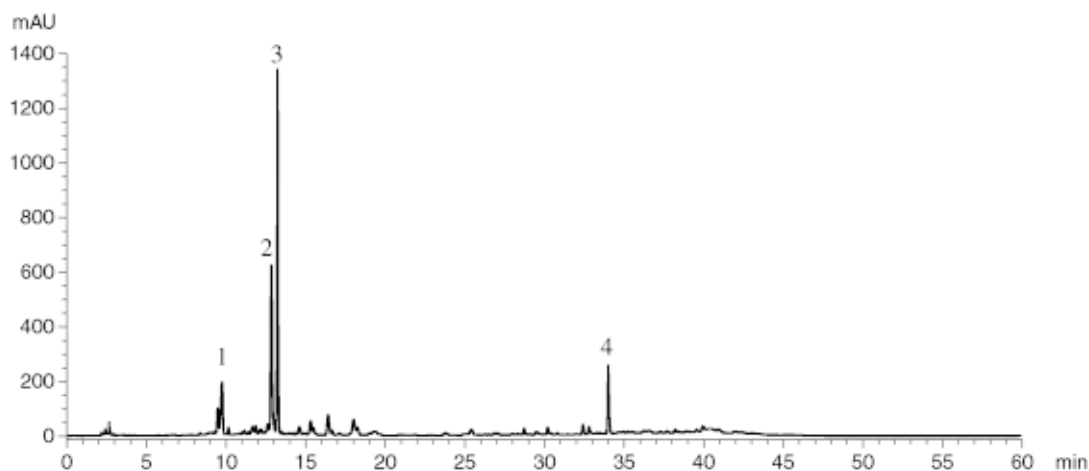


Figure 5(i) A reference fingerprint chromatogram of dried root and rhizome of *Glycyrrhiza uralensis* Fisch. extract

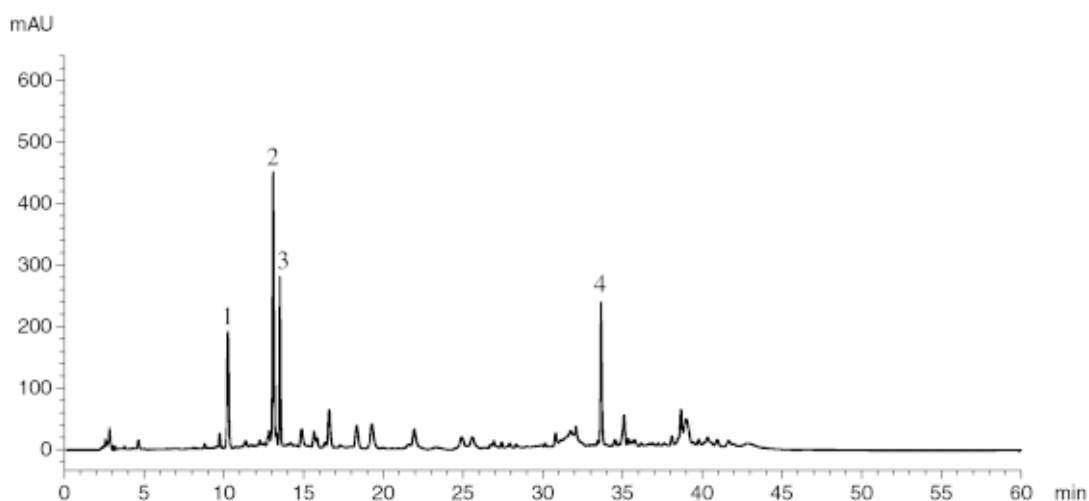


Figure 5(ii) A reference fingerprint chromatogram of dried root and rhizome of *Glycyrrhiza inflata* Bat. extract

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 respective reference fingerprint chromatograms [Fig. 5(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 – Aflatoxins (*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 1.0%.

5.7 Water Content (*Appendix X*): not more than 10.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 17.0%.

Ethanol-soluble extractives (cold extraction method): not less than 18.0%.

7. ASSAY

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

Standard solution

Mixed glycyrrhizic acid and liquiritin standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 5.0 mg of glycyrrhizic acid CRS and 5.0 mg of liquiritin CRS, and dissolve in 25 mL of methanol (70%).

Mixed glycyrrhizic acid and liquiritin standard solution for assay, Std-AS

Measure accurately the volume of the mixed glycyrrhizic acid and liquiritin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 5, 50, 100, 150, 200 mg/L for both glycyrrhizic acid and liquiritin.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of methanol (70%). Sonicate (220 W) the mixture for 30 min. Centrifuge at about $3200 \times g$ for 10 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction twice each with 10 mL

of methanol (70%) under sonication (220 W) for 10 min. Combine the filtrate 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 detector (liquiritin and glycyrrhizic acid are detected at 230 nm and 254 nm respectively) 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 –

Time (min)	Acetonitrile (%, v/v)	0.03% Phosphoric acid (%, v/v)	Elution
0 – 10	5 ➔ 25	95 ➔ 75	linear gradient
10 – 20	25	75	isocratic
20 – 36	25 ➔ 50	75 ➔ 50	linear gradient
36 – 40	50 ➔ 45	50 ➔ 55	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 μL of the mixed glycyrrhizic acid and liquiritin Std-AS (100 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of glycyrrhizic acid and liquiritin should not be more than 3.0%; the RSD of the retention times of glycyrrhizic acid peak and liquiritin peak should not be more than 3.0%; the column efficiencies determined from glycyrrhizic acid peak and liquiritin peak should not be less than 50000 theoretical plates.

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

Procedure

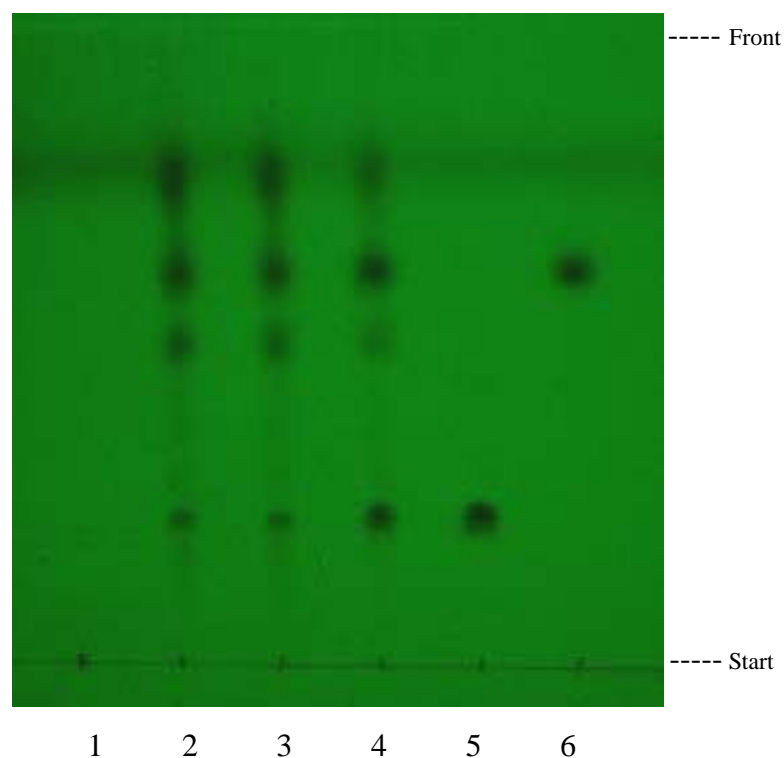
Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify glycyrrhizic acid peak and liquiritin peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed glycyrrhizic acid and liquiritin Std-AS. The retention times



of glycyrrhizic acid peaks and liquiritin peaks in both chromatograms should not differ from their counterparts by more than 3.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of glycyrrhizic acid and liquiritin in the test solution, and calculate the percentage contents of glycyrrhizic acid and liquiritin in the sample by using the equations indicated in Appendix IV(B).

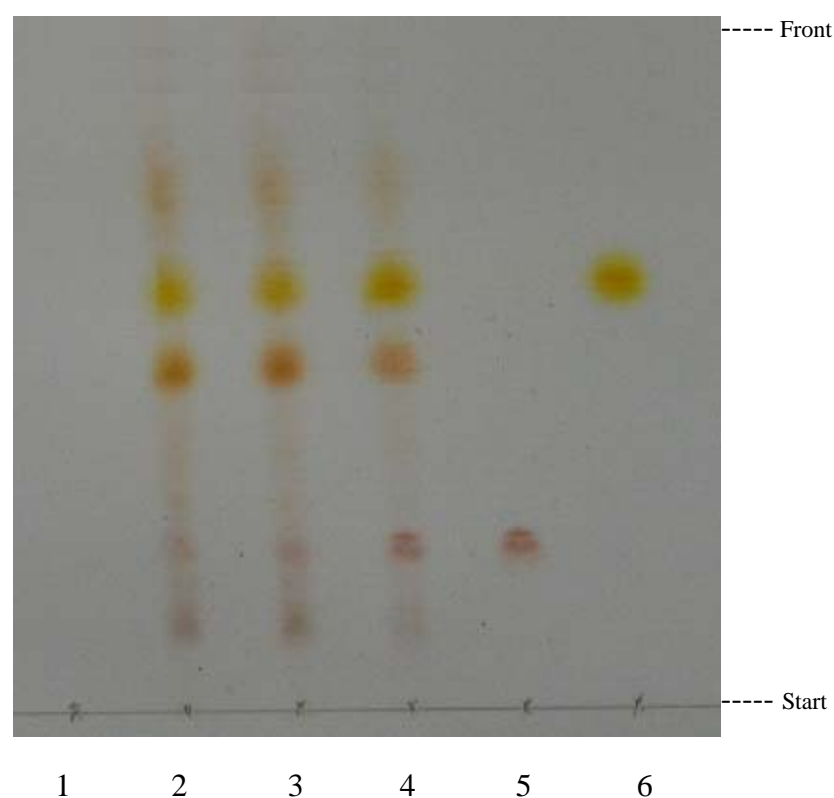
Limits

The sample contains not less than 2.0% of glycyrrhizic acid (C₄₂H₆₂O₁₆) and not less than 1.0% of liquiritin (C₂₁H₂₂O₉), calculated with reference to the dried substance.



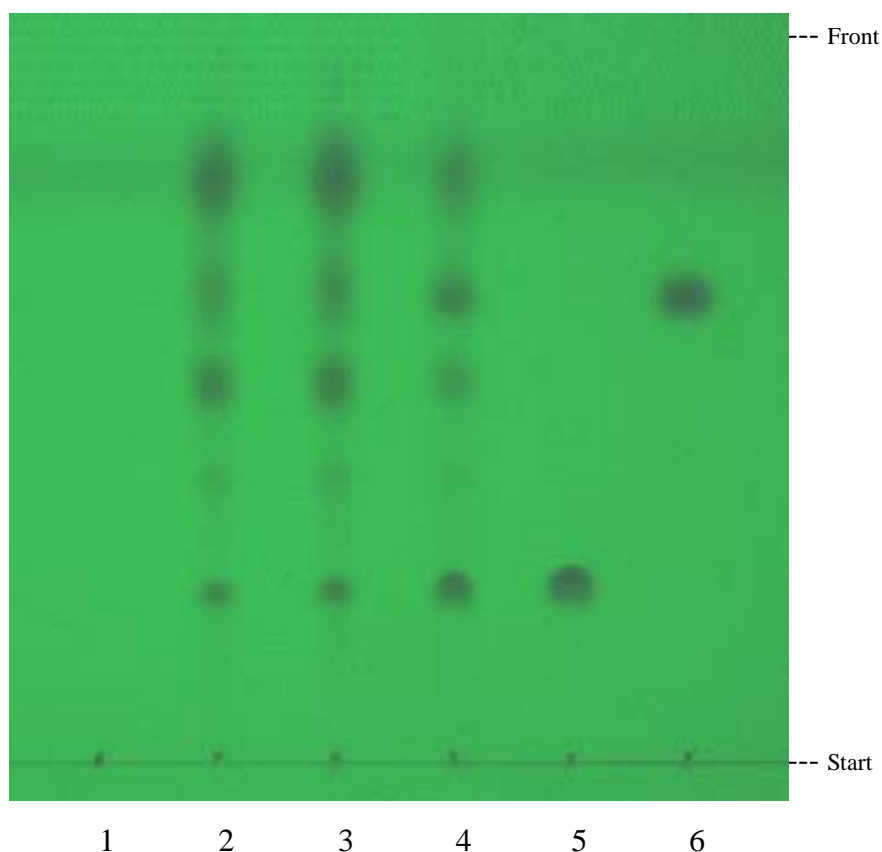
Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (<i>Glycyrrhiza uralensis</i> Fisch.)	Liquiritin and glycyrrhizic acid positive
3	Sample duplicate (<i>Glycyrrhiza uralensis</i> Fisch.)	Liquiritin and glycyrrhizic acid positive
4	Spiked sample (Sample plus liquiritin and glycyrrhizic acid)	Liquiritin and glycyrrhizic acid positive
5	Standard (Glycyrrhizic acid)	Glycyrrhizic acid positive
6	Standard (Liquiritin)	Liquiritin positive

Figure 1 TLC results of dried root and rhizome of *Glycyrrhiza uralensis* Fisch. extract observed under UV light (254 nm)



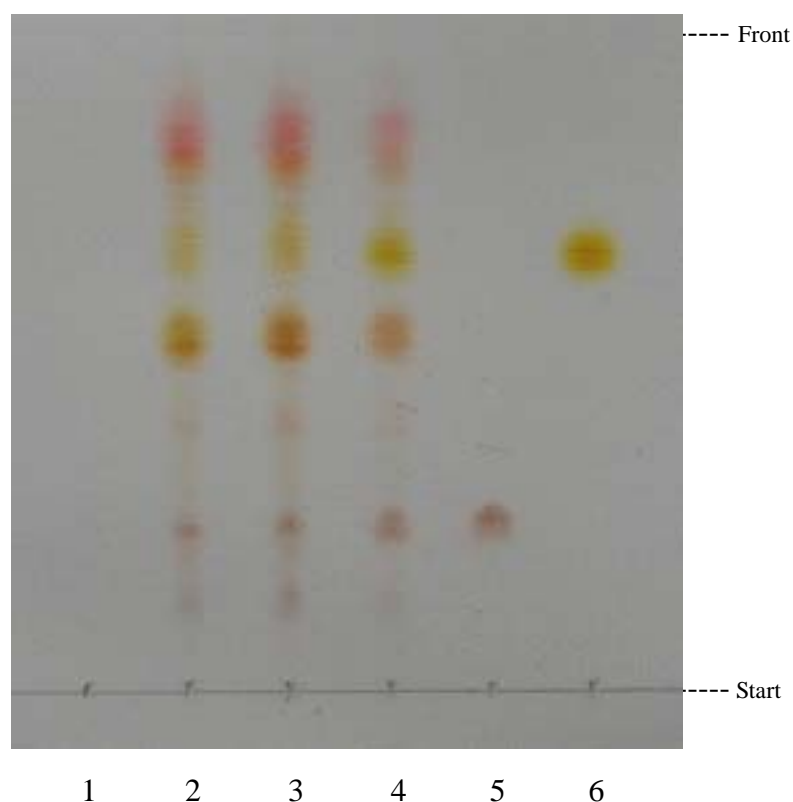
Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (<i>Glycyrrhiza uralensis</i> Fisch.)	Liquiritin and glycyrrhizic acid positive
3	Sample duplicate (<i>Glycyrrhiza uralensis</i> Fisch.)	Liquiritin and glycyrrhizic acid positive
4	Spiked sample (Sample plus liquiritin and glycyrrhizic acid)	Liquiritin and glycyrrhizic acid positive
5	Standard (Glycyrrhizic acid)	Glycyrrhizic acid positive
6	Standard (Liquiritin)	Liquiritin positive

Figure 2 TLC results of dried root and rhizome of *Glycyrrhiza uralensis* Fisch. extract observed under visible light after staining



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (<i>Glycyrrhiza inflata</i> Bat.)	Liquiritin and glycyrrhizic acid positive
3	Sample duplicate (<i>Glycyrrhiza inflata</i> Bat.)	Liquiritin and glycyrrhizic acid positive
4	Spiked sample (Sample plus liquiritin and glycyrrhizic acid)	Liquiritin and glycyrrhizic acid positive
5	Standard (Glycyrrhizic acid)	Glycyrrhizic acid positive
6	Standard (Liquiritin)	Liquiritin positive

Figure 3 TLC results of dried root and rhizome of *Glycyrrhiza inflata* Bat. extract observed under UV light (254 nm)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (<i>Glycyrrhiza inflata</i> Bat.)	Liquiritin and glycyrrhizic acid positive
3	Sample duplicate (<i>Glycyrrhiza inflata</i> Bat.)	Liquiritin and glycyrrhizic acid positive
4	Spiked sample (Sample plus liquiritin and glycyrrhizic acid)	Liquiritin and glycyrrhizic acid positive
5	Standard (Glycyrrhizic acid)	Glycyrrhizic acid positive
6	Standard (Liquiritin)	Liquiritin positive

Figure 4 TLC results of dried root and rhizome of *Glycyrrhiza inflata* Bat. extract observed under visible light after staining