Radix Rehmanniae

Figure 1　A photograph of Radix Rehmanniae

1. NAMES

Official Name: Radix Rehmanniae

Chinese Name: 地黃

Chinese Phonetic Name: Dihuang

2. SOURCE

Radix Rehmanniae is the dried root tuber of *Rehmannia glutinosa* Libosch. (Scrophulariaceae). The tuber is collected in autumn, root stocks, rootlets, soil and sand are removed, then slowly baked or dried under the sun until the inside turns black and almost dry to obtain Radix Rehmanniae, which is sometimes compressed into a mass, commonly known as "Sheng Dihuang".

3. DESCRIPTION

Mostly in irregular masses or oblong, swollen in the centre, slightly tapering at both ends. Some small pieces, slat-shaped, slightly compressed or twisted, 4.2 - 15.0 cm long, 17-65 mm in diameter. Externally brownish-black or greyish-brown, heavily shrunken, with irregular transverse wavy lines. Texture heavy, soft and pliable, broken uneasily. Fracture brownish-black or jet-black, lustrous, viscous. Odourless; taste slightly sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of elongated tangential cells. In the cortex, parenchyma cells are loosely arranged; many scattered secretory cells are found, containing oily masses of orange to orange-red colour when unstained, but dark in colour when stained. Phloem relatively broad, containing much less number of secretory cells. Cambium in a ring. Xylem rays broad, vessels arranged radially and distantly spaced (Fig. 2).

Powder

Colour dark brown. Parenchyma cells suborbicular or irregularly shaped, containing suborbicular nucleiform contents. Secretory cells mostly similar to ordinary parenchyma cells in shape, containing orange to orange-red oil droplets or oily masses. Vessels bordered-pitted and reticulate, up to 97 μm in diameter; bordered pits fairly dense. Cork cells yellowish-brown; in surface view subsquare, in transverse-section view subrectangular; arranged orderly; wall slightly curved (Fig. 3).

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

Standard solution

Catalpol standard solution

Weigh 0.5 mg of catalpol CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane and methanol (26:14, v/v).

Spray reagent

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

Test solution

Weigh 5.0 g of the powdered sample and place it in a 250-mL beaker, then add 100 mL of methanol. Disperse the sample with a mechanical mixer for 5 min. Shake the mixture for 30 min at room temperature. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of water. Transfer the aqueous solution to a separatory funnel. Extract for three times each with 20 mL of ethyl acetate. Combine the ethyl acetate extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol and then filter.

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 catalpol standard solution (2.5 μL) and the test solution (2 μL) 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 dry in air. Spray the plate evenly with the spray reagent and heat at about 120ºC until the spots or bands become visible (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of catalpol.

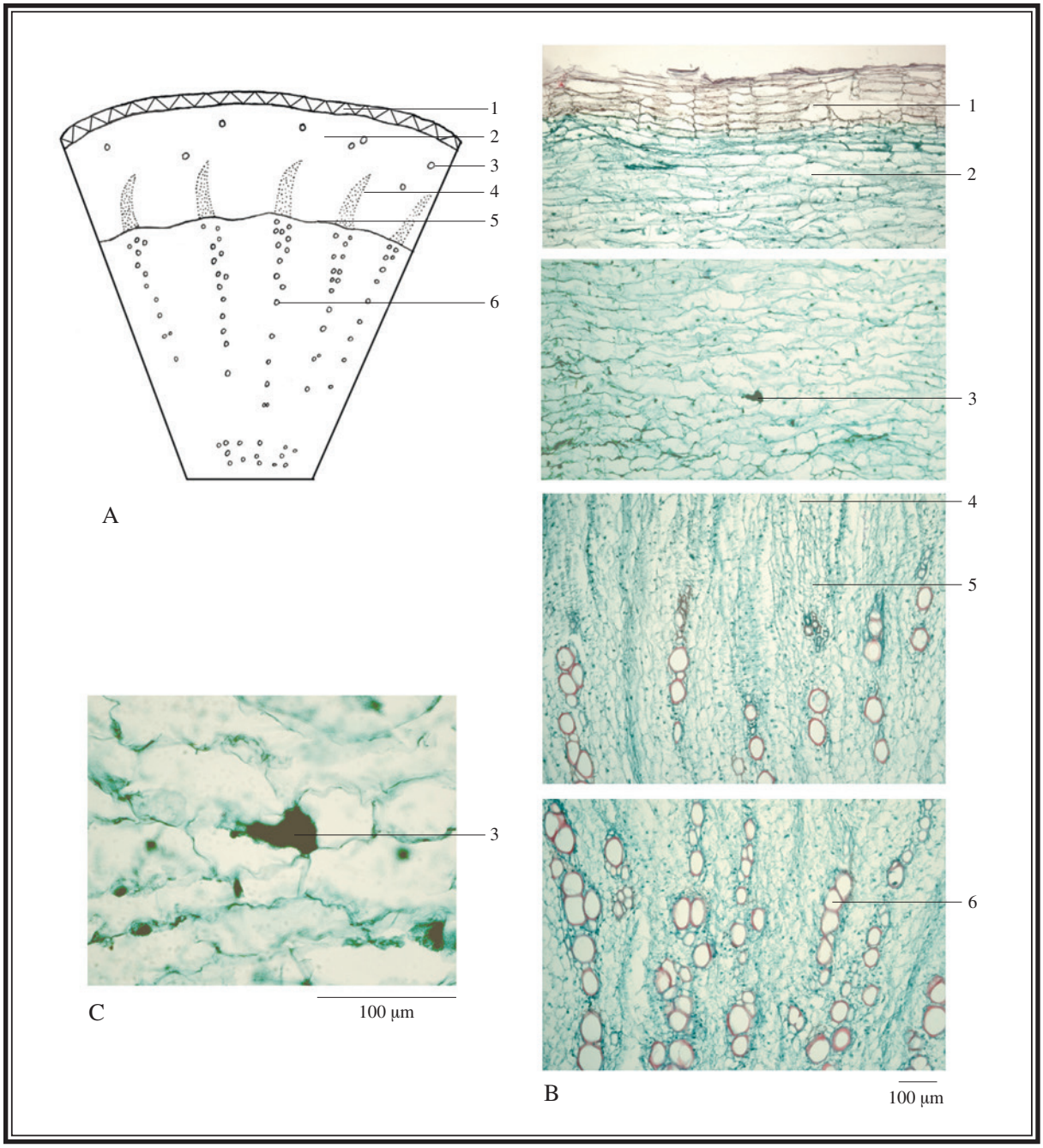


Figure 2 Microscopic features of transverse section of Radix Rehmanniae

A. Sketch B. Section illustration C. Parenchyma cells with secretory cell

1. Cork 2. Cortex 3. Secretory cell 4. Phloem 5. Cambium 6. Xylem

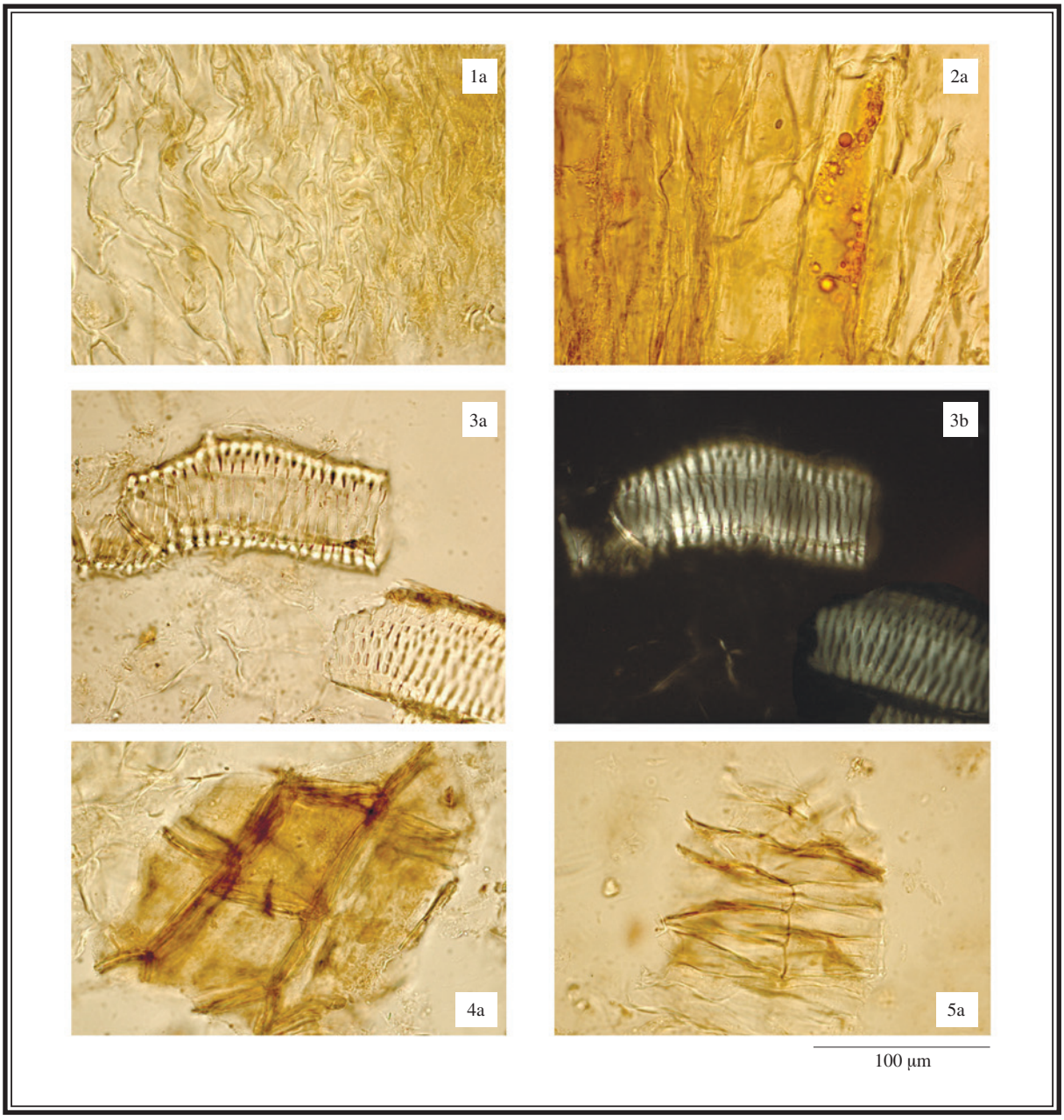


Figure 3 Microscopic features of powder of Radix Rehmanniae

1. Parenchyma cells 2. Secretory cells 3. Vessels
4. Cork cells (surface view) 5. Cork cells (transverse section view)

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

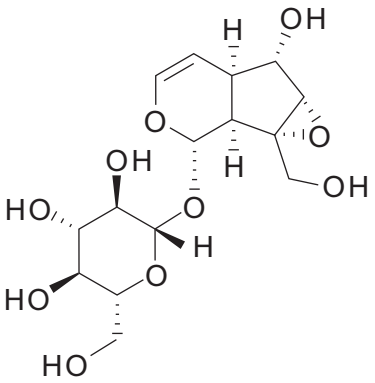


Figure 4 Chemical structure of catalpol

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

Standard solution

Catalpol standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of catalpol CRS and dissolve in 10 mL of methanol (20%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (20%). Sonicate (270 W) the mixture for 60 min and avoid overheating. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Make up to the mark with methanol (20%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0–10	99	1	isocratic
10–30	99→95.5	1→4.5	linear gradient
30–60	95.5→90	4.5→10	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of catalpol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of catalpol should not be more than 3.0%; the RSD of the retention time of catalpol peak should not be more than 2.0%; the column efficiency determined from catalpol peak should not be less than 20000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.60	±0.03
2	0.85	±0.03
3	0.91	±0.04
4 (marker, catalpol)	1.00	-
5	1.07	±0.05
6	1.88	±0.03

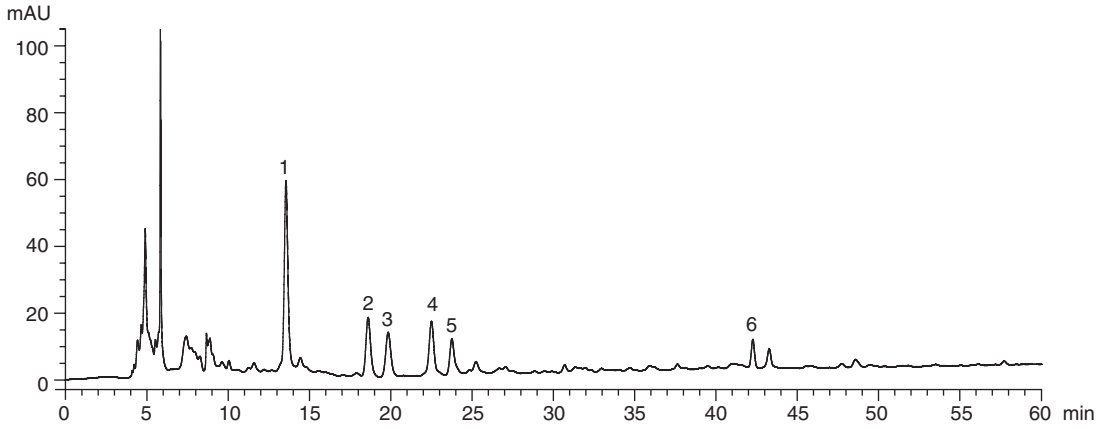


Figure 5 A reference fingerprint chromatogram of Radix Rehmanniae extract

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 – Aflatoxins *(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 6.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*: not more than 17.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 65.0%.
Ethanol-soluble extractives (cold extraction method): not less than 48.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 10.0 mg of catalpol CRS and dissolve in 10 mL of methanol (20%).

Catalpol standard solution for assay, Std-AS

Measure accurately the volume of the catalpol Std-Stock, dilute with methanol (20%) to produce a series of solutions of 20, 50, 100, 150, 200 mg/L for catalpol.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (20%). 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 3 mL of methanol (20%). Centrifuge at about 1800 × *g* for 10 min. Combine the extracts and make up to the mark with methanol (20%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 150 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	99	1	isocratic
10 – 30	99 → 95.5	1 → 4.5	linear gradient
30 – 60	95.5 → 90	4.5 → 10	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of catalpol Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of catalpol should not be more than 3.0%; the RSD of the retention time of catalpol peak should not be more than 2.0%; the column efficiency determined from catalpol peak should not be less than 5000 theoretical plates.

The *R* value between catalpol 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 catalpol Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of catalpol against the corresponding concentrations of catalpol Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

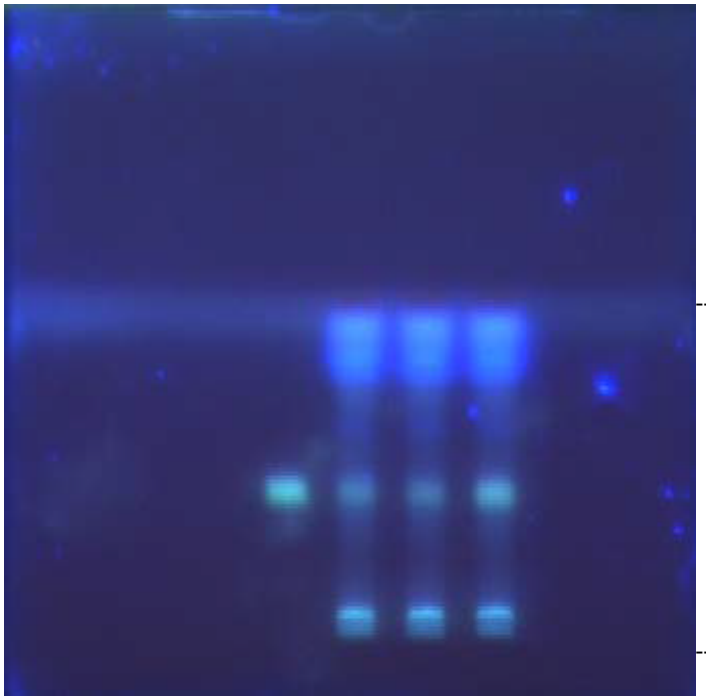
Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify catalpol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of catalpol Std-AS. The retention times of catalpol 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 catalpol in the test solution, and calculate the percentage content of catalpol in the sample by using the equations indicated in Appendix IV (B).

Limits

The sample contains not less than 0.20% of catalpol ($C_{15}H_{22}O_{10}$), calculated with reference to the dried substance.

Radix Rehmanniae (地黃)



1 2 3 4 5

Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Catalpol)	Catalpol positive
3	Sample (Radix Rehmanniae)	Catalpol positive
4	Sample duplicate (Radix Rehmanniae)	Catalpol positive
5	Spiked sample (Sample plus catalpol)	Catalpol positive

Figure 1 TLC results of Radix Rehmanniae extract observed under UV light (366 nm) after staining