Radix Bupleuri

Figure 1 A photograph of Radix Bupleuri

1. NAMES

Official Name: Radix Bupleuri

Chinese Name: 柴胡

Chinese Phonetic Name: Chaihu

2. SOURCE

Radix Bupleuri is the dried root of *Bupleurum chinense* DC. (Apiaceae/Umbelliferae). The root is collected in the spring or autumn. After removal of the aerial part and the soil, the root is dried to obtain Radix Bupleuri.

3. DESCRIPTION

Cylindrical, or long and conical; 3.5-16 cm long and 3-12 mm in diameter. The top part bulgy; the apex shows 3-15 stem-bases or short fibrous leaf-bases, and branched at the lower part. Outer surface dull blackish-brown or light brown, marked with longitudinal wrinkles, rootlet scars and lenticels. Texture hard and tenacious, not easy to break; fracture laminated-fibrous, bark light brown, wood yellowish-white. Odour slightly fragrant; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consisting of several layers of flat cells. Cortex narrow, containing a few oil canals. Phloem shows scattered oil canals and, often with clefts; oil canals surrounded by secretory cells and contain yellowish-brown or greenish-yellow secretions or oily drops in canals. Cambium in a ring. Xylem broad, occupying more than half of the radius of the root; vessels single or grouped; xylem rays visible. Xylem fibres well developed and arranged with xylem parenchyma cells in several discontinuous rings (Fig. 2).

Powder

Colour greyish-brown. Oil canals mostly fractured, containing yellowish-brown or greenish-yellow strip-shaped secretions, 10-50 µm in diameter. Xylem fibres frequent, single or in bundles, colourless or slightly yellow, long fusiform, 7-25 µm in diameter, 2-8 µm in wall thickness, lignified. Reticulated and spiral vessels predominant, 5-80 µm in diameter. Cork cells yellowish-brown, polygonal (Fig. 3).

4.2 Physicochemical Identification

Procedure

Weigh 1.0 g of the powdered sample and put into a 50-mL conical flask, then add 20 mL of methanol. Shake the mixture for 2 h. Allow to stand for about 30 min. Filter and transfer 100 µL of filtrate to a test tube, add 3 mL of dichloromethane with slight shaking. Cautiously add 1.5 mL of sulphuric acid along the inner wall of the test tube. A red to reddish-brown ring is observed at the interface of the two solvent layers.

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

Standard solutions

Saikosaponin A standard solution

Weigh 1.0 mg of saikosaponin A CRS (Fig. 4) and dissolve in 2 mL of methanol.

Saikosaponin C standard solution

Weigh 1.0 mg of saikosaponin C CRS (Fig. 4) and dissolve in 2 mL of methanol.

Saikosaponin D standard solution

Weigh 1.0 mg of saikosaponin D CRS (Fig. 4) and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol and water (8:2:1, v/v).

Spray reagent

Dissolve 2.0 g of 4-(dimethylamino)benzaldehyde in 100 mL of dilute sulphuric acid (40%, v/v). Freshly prepare the reagent.

Test solution

Weigh 0.5 g of the powdered sample and put into a 50-mL round-bottomed flask, then add about 20 mL of methanol. Reflux the mixture for 1 h. Cool to room temperature. Filter and transfer the filtrate to a 100-mL conical flask. Concentrate the filtrate to about 5 mL on a water bath, and filter.

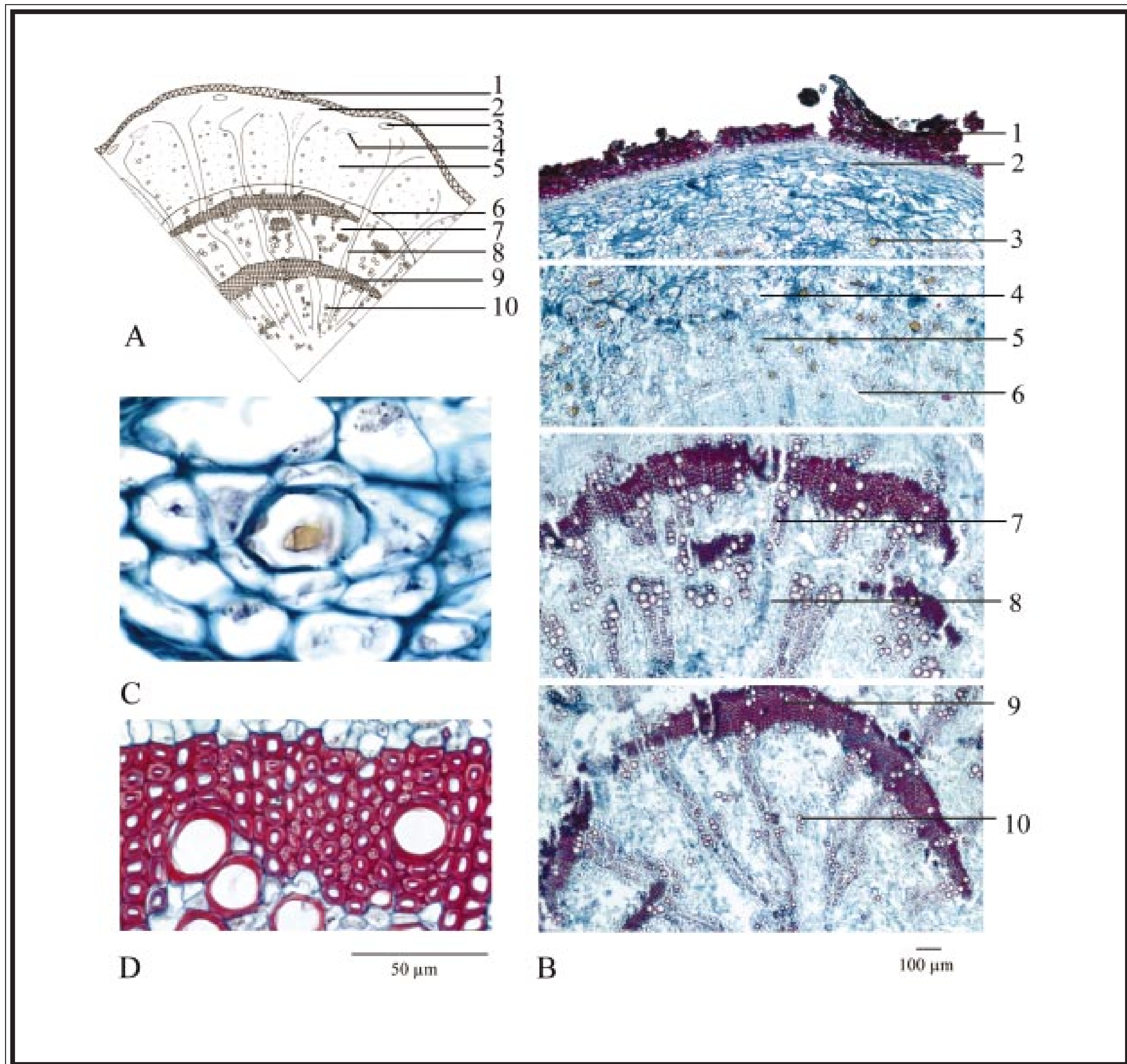


Figure 2 Microscopic features of transverse section of Radix Bupleuri

A. Sketch B. Section illustration C. Oil canal D. Xylem fibres

1. Cork 2. Cortex 3. Oil canal 4. Cleft 5. Phloem 6. Cambium 7. Xylem 8. Xylem ray 9. Xylem fibres 10. Vessels

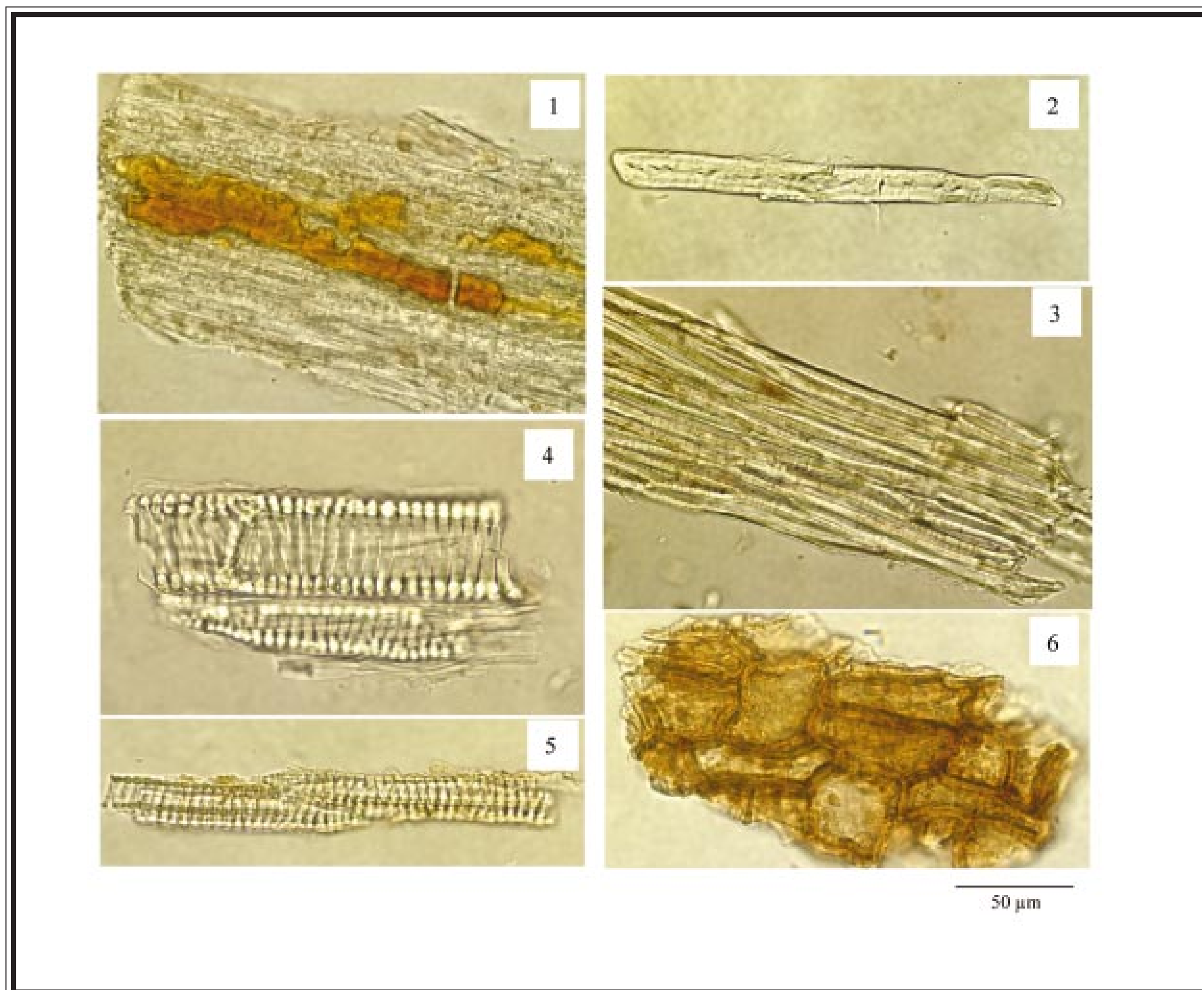


Figure 3 Microscopic features of powder of Radix Bupleuri (under the light microscope)

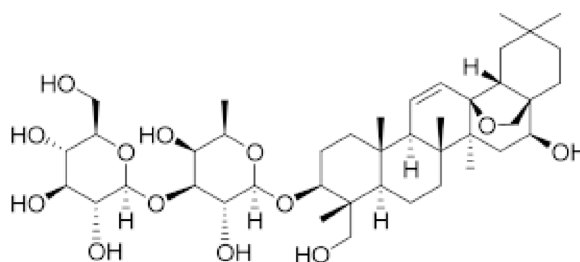
1. Oil canal 2. Xylem fibre 3. Xylem fibres in a bundle 4. Reticulated vessels 5. Spiral vessels 6. Cork cells

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 saikosaponin A standard solution, saikosaponin C standard solution, saikosaponin D standard solution and the test solution (5 µL each) 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. Spray the plate evenly with the spray reagent, then heat at about 60˚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 saikosaponin A, saikosaponin C and saikosaponin D.

(i)



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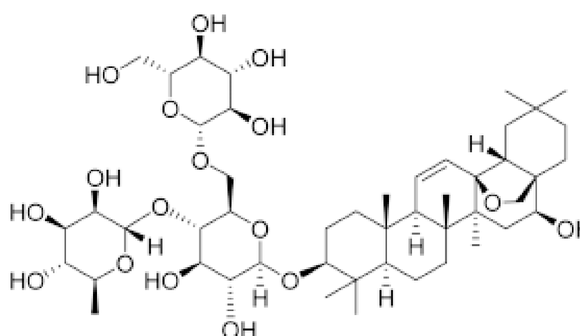

(iii)

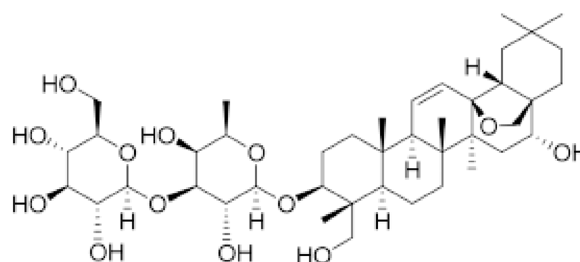

Figure 4 Chemical structures of (i) saikosaponin A (ii) saikosaponin C and (iii) saikosaponin D

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

Standard solution

Saikosaponin D standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of saikosaponin D CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.25 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of ammonium hydroxide solution in methanol [add 1 volume of ammonium hydroxide (25%, v/v) to 4 volumes of methanol] and mix well. Sonicate (220 W) the mixture for 1 h. Centrifuge at about 3200 × *g* for 10 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (210 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	70 → 50	30 → 50	linear gradient
20 – 30	50 → 40	50 → 60	linear gradient
30 – 45	40 → 10	60 → 90	linear gradient
45 – 60	10 → 0	90 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of saikosaponin D Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of saikosaponin D should not be more than 5.0%; the RSD of the retention time of saikosaponin D peak should not be more than 5.0%; the column efficiency determined from saikosaponin D peak should not be less than 20000 theoretical plates.

The *R* value between peak 3 and peak 4 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject saikosaponin D Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of saikosaponin D peak in the chromatogram of saikosaponin D Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify saikosaponin D peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of saikosaponin D Std-FP. The retention times of saikosaponin D peaks from the two chromatograms should not differ by more than 3.0%. 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 Bupleuri extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Radix Bupleuri extract

Peak No.	RRT	Acceptable Range
1 (saikosaponin A)	0.70	±0.03
2 (marker, saikosaponin D)	1.00	-
3	2.34	±0.09
4	2.37	±0.09

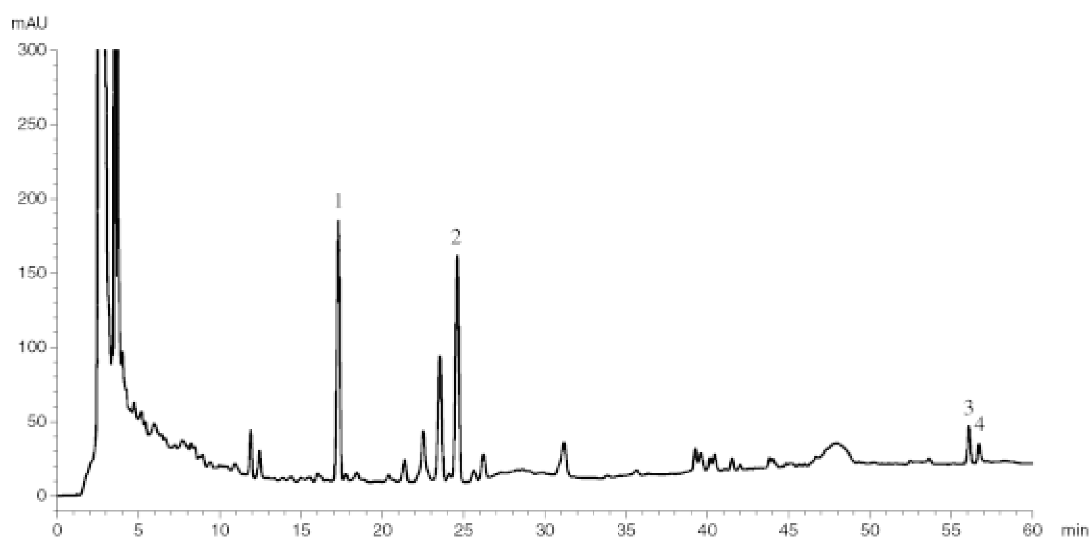

Figure 5 A reference fingerprint chromatogram of Radix Bupleuri extract

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. 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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*:

Non-medicinal part: not more than 8.0%
Other foreign matter: not more than 2.0%

5.6 Ash *(Appendix IX)*

Total ash: not more than 9.0%.
Acid-insoluble ash: not more than 3.5%.

5.7 Water Content *(Appendix X)*: not more than 5.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 5.0 mg of saikosaponin A CRS and dissolve in 5 mL of methanol.

Saikosaponin A standard solution for assay, Std-AS

Measure accurately the volume of the saikosaponin A Std-Stock, dilute with methanol to produce a series of solutions of 25, 50, 100, 200, 300 mg/L for saikosaponin A.

Test solution

Weigh accurately 0.25 g of the powdered sample and put into a 50-mL centrifuge tube, then add 15 mL of ammonium hydroxide solution in methanol [add 1 volume of ammonium hydroxide (25%, v/v) to 4 volumes of methanol]. Sonicate (220 W) the mixture for 30 min. Centrifuge at about 3200 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Repeat the extraction twice. 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 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (210 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 water (38:62, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections each with 20 µL of saikosaponin A Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of saikosaponin A should not be more than 5.0%; the RSD of the retention time of saikosaponin A peak should not be more than 5.0%; the column efficiency determined from saikosaponin A peak should not be less than 4000 theoretical plates.

The *R* value between saikosaponin A 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 saikosaponin A Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of saikosaponin A against the corresponding concentrations of saikosaponin A Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify saikosaponin A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of saikosaponin A Std-AS. The retention times of saikosaponin A peaks from the two chromatograms should not differ by more than 3.0%. Measure the peak area and calculate the concentration (in milligram per litre) of saikosaponin A in the test solution, and calculate the percentage content of saikosaponin A in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.16% of saikosaponin A ($C_{42}H_{68}O_{13}$), calculated with reference to the dried substance.