Isatidis Radix

Figure 1 A photograph of Isatidis Radix

1. NAMES

Official Name: Isatidis Radix

Chinese Name: 板藍根

Chinese Phonetic Name: Banlangen

2. SOURCE

Isatidis Radix is the dried root of *Isatis indigotica* Fort. (Brassicaceae). The root is collected in autumn, foreign matter removed, then dried under the sun to obtain Isatidis Radix.

3. DESCRIPTION

Cylindrical, slightly twisted, 3-20 cm long, 3-20 mm in diameter. Externally pale greyish-yellow to brownish-yellow, with longitudinal wrinkles, transverse lenticels and branch roots or branch root scars. The top part of the root slightly expanded, marked by dark green to dark brown remnants of petiole bases arranged in whorls, and by dense tubercles. Texture compact or slightly soft, fracture yellowish-white to yellowish-brown in bark, and yellow in wood. Odour slight; taste slightly sweet, then bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells. Cortex narrow. Phloem broad, rays distinct, usually with clefts at the outer part. Cambium in a ring. Vessels subrounded, usually 2-3 in a group or singly scattered, arranged radially; xylem fibres in bundles. Parenchyma contains abundant starch granules (Fig. 2).

Powder

Colour pale brown to yellowish-brown. Starch granules numerous; simple granules subrounded, ovoid or subsquare, 2-21 μm in diameter, hilum distinct, dotted, slit-shaped or V-shaped, striations indistinct; black and cruciate in shape under the polarized microscope; compound granules composed of 2-5 units. Stone cells colourless to pale yellow, subsquare, subrectangular

or irregular in shape, sometimes slightly acute at one end, 24-185 μm long, 9-75 μm in diameter; pit canals and striations distinct; yellowish-white to greyish-white under the polarized microscope. Vessels mainly reticulate, 3-93 μm in diameter; spiral and bordered-pitted vessels occasionally visible. Xylem fibres mostly in bundles, 6-34 μm in diameter, sometimes with distinct pits and pit canals. Cork cells colourless to yellow, subpolygonal or elongated-polygonal in surface view (Fig. 3).

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

Standard solution

Epigoitrin standard solution

Weigh 0.5 mg of epigoitrin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), methanol and ethyl acetate (0.5:3:7, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of boiling water. Sonicate (140 W) the mixture for 1 h. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Evaporate the solvent 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 epigoitrin standard solution (1 μL) and the test solution (1.5 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. Examine the plate under UV light (254 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 epigoitrin.

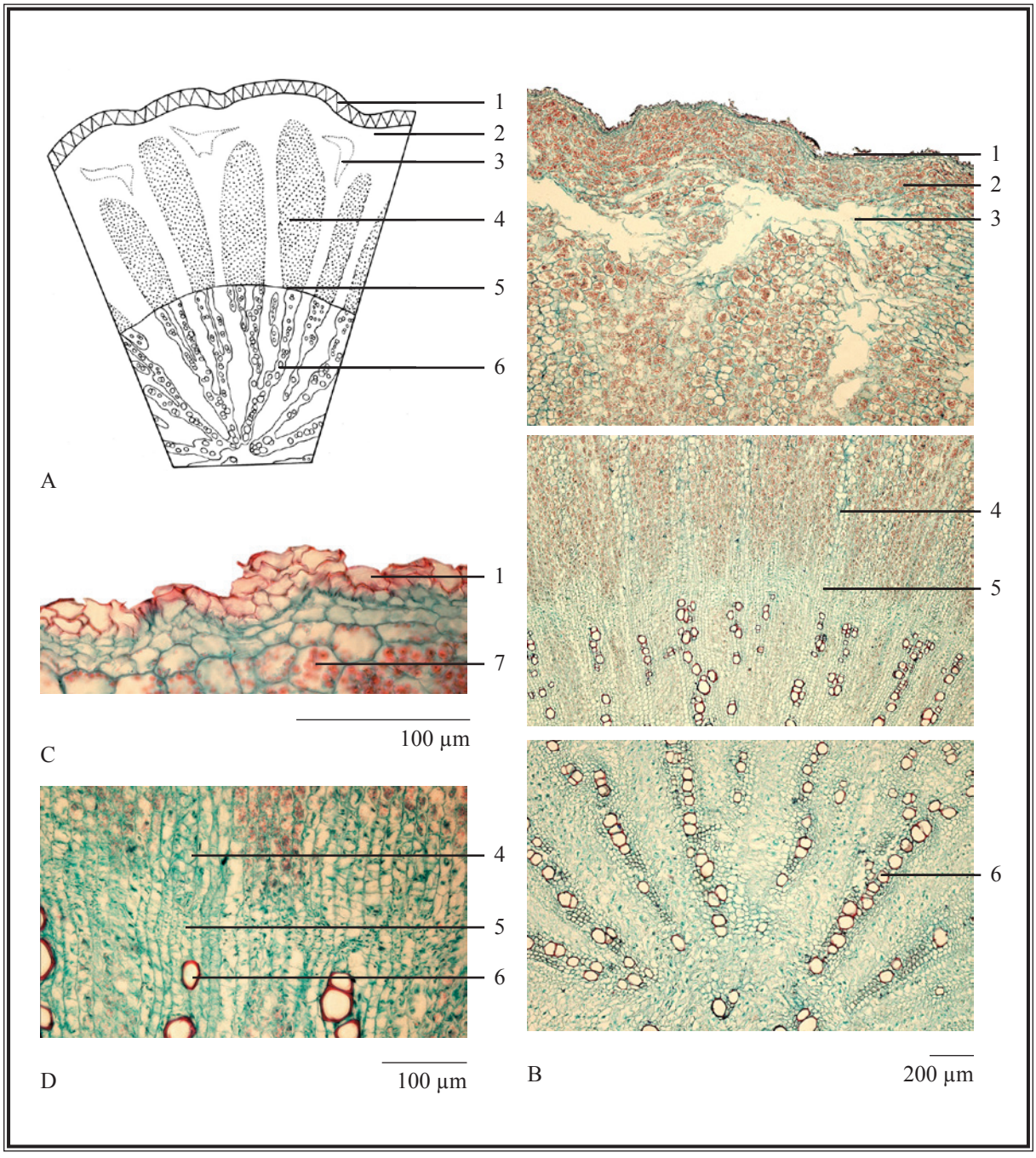

Figure 2 Microscopic features of transverse section of Isatidis Radix

A. Sketch B. Section illustration C. Cork and starch granules in parenchyma
D. Phloem, cambium and xylem

1. Cork 2. Cortex 3. Cleft 4. Phloem 5. Cambium 6. Xylem 7. Starch granules

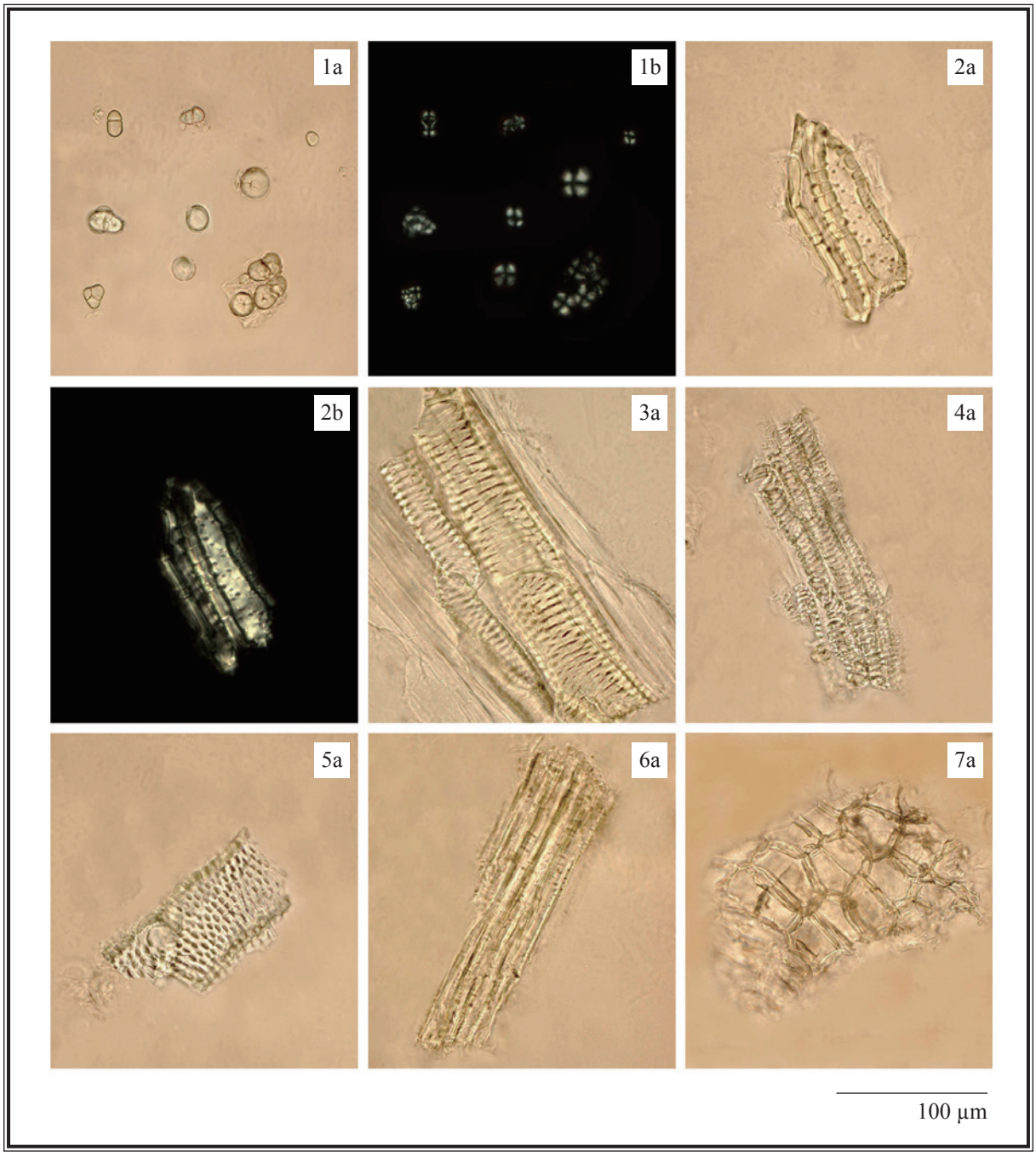

Figure 3 Microscopic features of powder of Isatidis Radix

1. Starch granules 2. Stone cells 3. Reticulate vessels 4. Spiral vessels
5. Bordered-pitted vessel 6. Xylem fibres 7. Cork cells

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

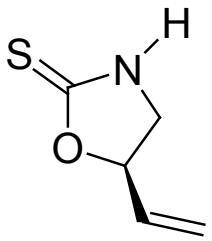

Figure 4 Chemical structure of epigoitrin

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

Standard solution

Epigoitrin standard solution for fingerprinting, Std-FP (7 mg/L)

Weigh 0.7 mg of epigoitrin CRS and dissolve in 100 mL of water.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of water. Immediately place the mixture in a boiling water bath for 1 h. Centrifuge at about 1800 × *g* for 15 min. Transfer the supernatant to a 25-mL volumetric flask. Wash the residue with water. Combine the solutions and make up to the mark with water. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size and 70 Å pore size). The column temperature is maintained at 35°C during the separation. The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	98	2	isocratic
10 – 40	98 → 85	2 → 15	linear gradient
40 – 60	85	15	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of epigoitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of epigoitrin should not be more than 5.0%; the RSD of the retention time of epigoitrin peak should not be more than 2.0%; the column efficiency determined from epigoitrin peak should not be less than 15000 theoretical plates.

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

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Isatidis Radix extract

Peak No.	RRT	Acceptable Range
1	0.25	± 0.03
2	0.29	± 0.03
3	0.53	± 0.03
4	0.74	± 0.03
5 (marker, epigoitrin)	1.00	-

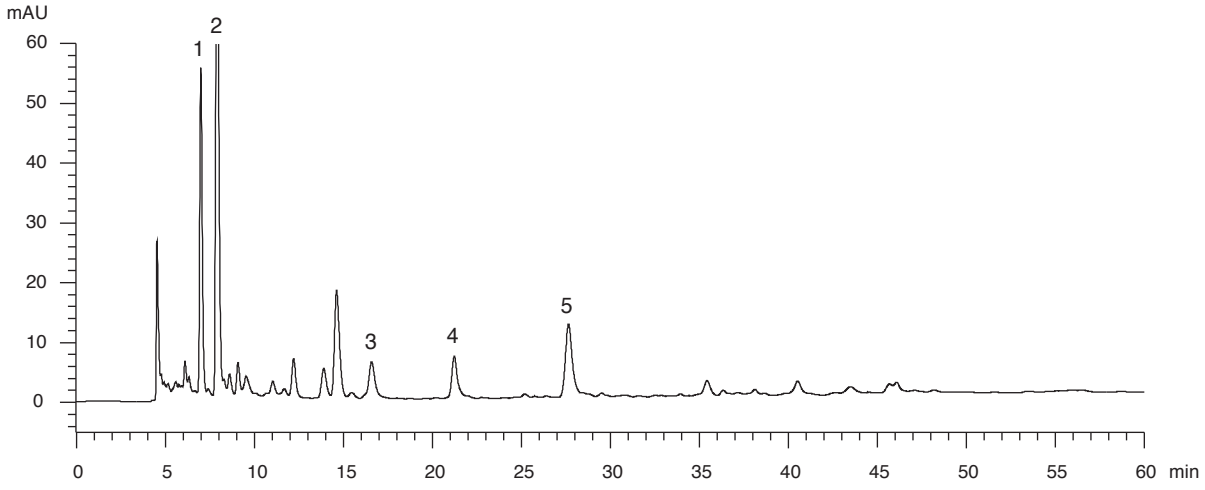

Figure 5 A reference fingerprint chromatogram of Isatidis Radix extract

For positive identification, the sample must give the above five 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 XVIII)*: 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)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 34.0%.
Ethanol-soluble extractives (hot extraction method): not less than 31.0%.

7. ASSAY

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

Standard solution

Epigoitrin standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 mg of epigoitrin CRS and dissolve in 10 mL of water.

Epigoitrin standard solution for assay, Std-AS

Measure accurately the volume of the epigoitrin Std-Stock, dilute with water to produce a series of solutions of 2, 5, 10, 20, 50 mg/L for epigoitrin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of water. Immediately place the mixture in a boiling water bath for 1 h. Centrifuge at about 1800 × *g* for 15 min. Transfer the supernatant to a 25-mL volumetric flask. Wash the residue with water. Combine the solutions and make up to the mark with water. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size and 70 Å pore size). The column temperature is maintained at 35°C during the separation. The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	98	2	isocratic
10 – 40	98 → 85	2 → 15	linear gradient
40 – 60	85	15	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the epigoitrin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of epigoitrin should not be more than 5.0%; the RSD of the retention time of epigoitrin peak should not be more than 2.0%; the column efficiency determined from epigoitrin peak should not be less than 15000 theoretical plates.

The *R* value between epigoitrin 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 epigoitrin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of epigoitrin against the corresponding concentrations of epigoitrin 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 epigoitrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of epigoitrin Std-AS. The retention times of epigoitrin 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 epigoitrin in the test solution, and calculate the percentage content of epigoitrin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.029% of epigoitrin (C_5H_7NOS), calculated with reference to the dried substance.