Zanthoxyli Radix

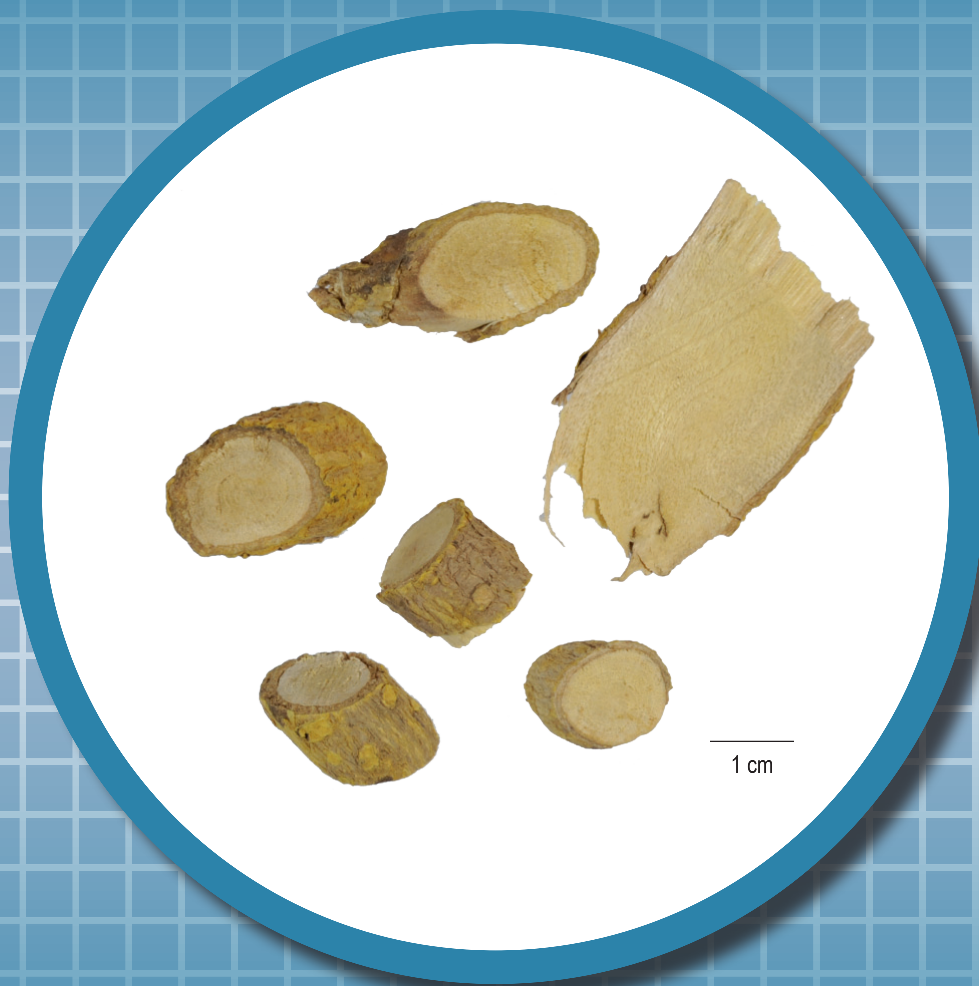

Figure 1 A photograph of Zanthoxyli Radix

1. NAMES

Official Name: Zanthoxyli Radix

Chinese Name: 兩面針

Chinese Phonetic Name: Liangmianzhen

2. SOURCE

Zanthoxyli Radix is the dried root of *Zanthoxylum nitidum* (Roxb.) DC. (Rutaceae). The root is collected throughout the year, washed clean, sliced or sectioned, then dried under the sun to obtain Zanthoxyli Radix.

3. DESCRIPTION

Thick slices or cylindrical and curved sections, 0.4-11 cm long, 0.4-5 cm wide, 1-34 mm thick. Externally pale brownish-yellow or pale yellow, with thick longitudinal wrinkles, sometimes with transverse clefts; lenticels protuberant, bright yellow or yellowish-brown, suborbicular. Cut surface rather smooth; bark pale brown, xylem pale yellow, concentric annulations and dense pores visible. Texture hard. Odour slightly aromatic; taste pungent, tongue-numb and bitter.

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 10-15 layers of cells. Oil cells scattered at phloem, 28-122 µm in diameter. Lignified fibres outside of phloem, single or 2-5 in groups. Stone cells present at phloem of stout root. Phloem scatters with few prisms of calcium oxalate and oil cells. Xylem vessels scattered singly or 2-4 in groups, arranged radially; xylem rays 1-3 rows of cells wide (Fig. 2).

Powder

Colour yellowish-brown. Simple starch granules broadly ovate or subrounded, 3-25 μm in diameter; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-3 units. Stone cells pale yellowish-green, subsquare, subrectangular, subrounded, fusiform or strip-shaped, relatively large, 16-225 μm long, 12-131 μm wide, wall varying in thickness, cells with thick wall had distinct striations, cells with thin wall had fine pits. Prisms of calcium oxalate scattered singly or presented in parenchymatous cells, shapes various, short rod-shaped, rhombic, or irregularly polygonal; bright orange under the polarized microscope. Vessels mainly bordered-pitted, 14-182 μm in diameter. Cork cells brown, polygonal or long polygonal in surface view, wall slightly thickened, some lumen containing brown masses. Fibres relatively long, wall relatively thick, lignified; polychromatic under the polarized microscope (Fig. 3).

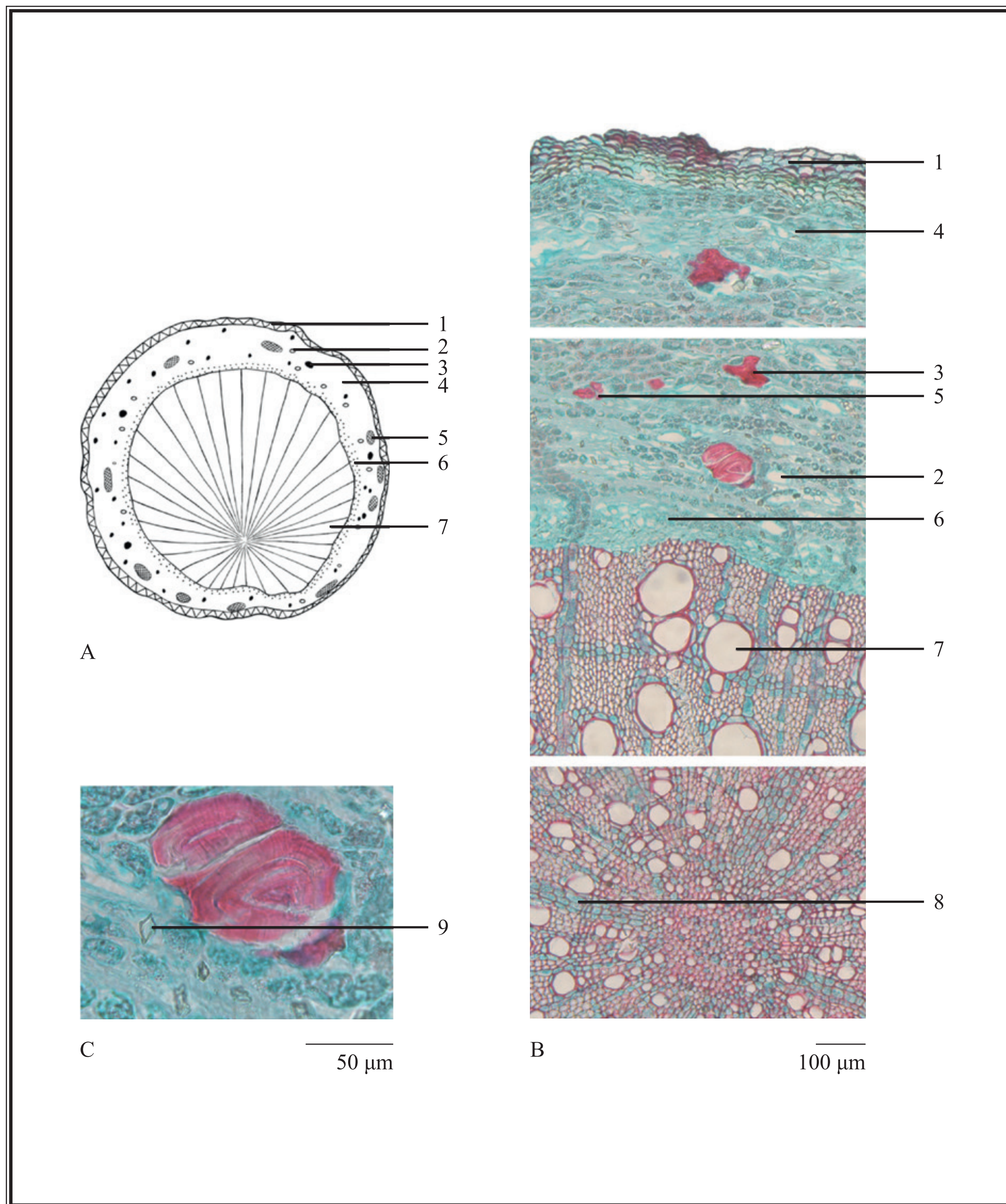

Figure 2 Microscopic features of transverse section of Zanthoxyli Radix

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

1. Cork 2. Oil cell 3. Stone cells 4. Cortex 5. Fibre
6. Phloem 7. Xylem 8. Xylem rays 9. Prisms of calcium oxalate

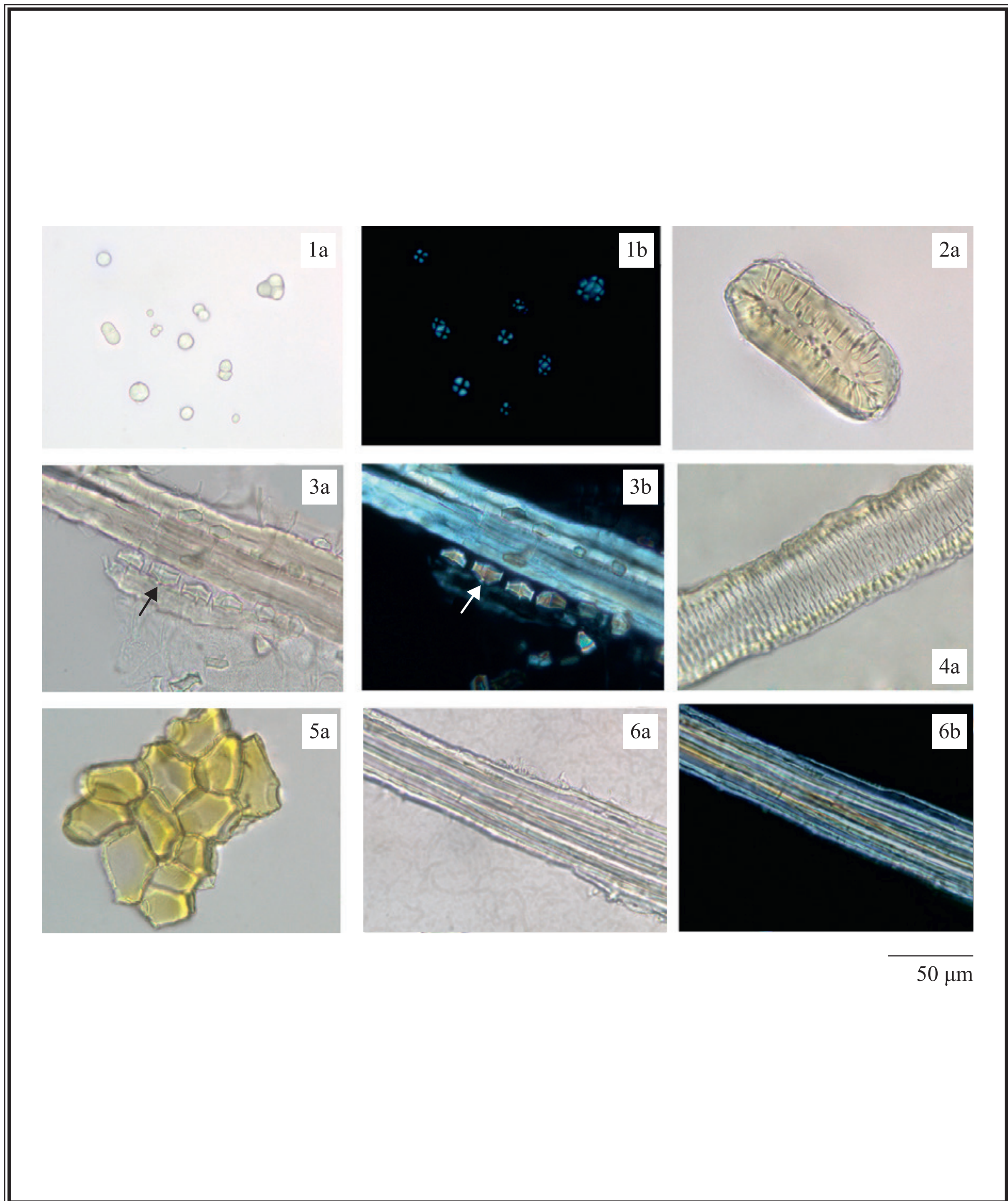

Figure 3 Microscopic features of powder of Zanthoxyli Radix

1. Starch granules 2. Stone cell 3. Prisms of calcium oxalate
4. Bordered-pitted vessel 5. Cork cells 6. Fibres

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

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

Standard solutions

Chelerythrine chloride standard solution

Weigh 1.0 mg of chelerythrine chloride CRS (Fig. 4) and dissolve in 20 mL of ethanol (70%).

Nitidine chloride standard solution

Weigh 1.0 mg of nitidine chloride CRS (Fig. 4) and dissolve in 20 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of cyclohexane and ethyl acetate (1:1, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (150 W) the mixture for 10 min. Filter the mixture.

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 chelerythrine standard solution (0.5 μL), nitidine standard solution (1 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 add 10 mL of ammonia hydroxide solution (25%, v/v) to the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully place the HPTLC plate in the trough containing the developing solvent for development. 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 (366 nm). 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 chelerythrine chloride and nitidine chloride.

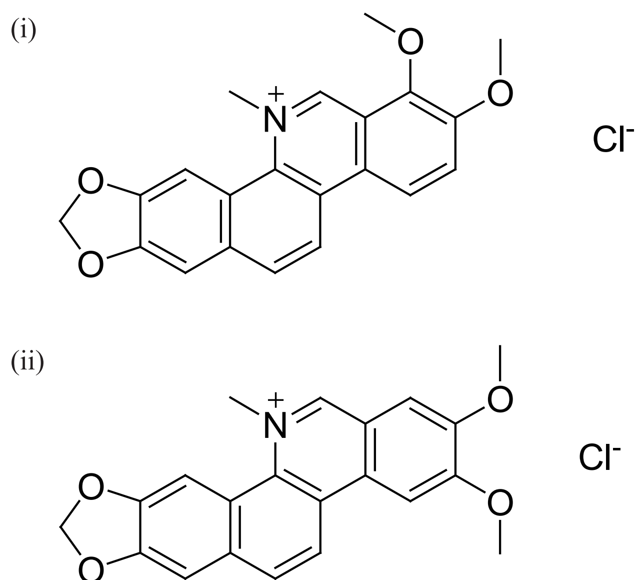

Figure 4 Chemical structures of (i) chelerythrine chloride and (ii) nitidine chloride

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

Standard solutions

Chelerythrine chloride standard solution for fingerprinting, Std-FP (6 mg/L)

Weigh 0.6 mg of chelerythrine chloride CRS and dissolve in 100 mL of ethanol (70%)

Nitidine chloride standard solution for fingerprinting, Std-FP (6 mg/L)

Weigh 0.6 mg of nitidine chloride CRS and dissolve in 100 mL of ethanol (70%)

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (273 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 0.05% trifluoroacetic acid with 0.05% triethylamine (27:73, v/v). The elution time is about 55 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of chelerythrine chloride Std-FP and nitidine chloride Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chelerythrine chloride and nitidine chloride should not be more than 5.0%; the RSD of the retention times of chelerythrine chloride and nitidine chloride peaks should not be more than 2.0%; the column efficiencies determined from chelerythrine chloride and nitidine chloride peaks should not be less than 8700 theoretical plates.

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

Procedure

Separately inject chelerythrine chloride Std-FP, nitidine chloride Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of chelerythrine chloride and nitidine chloride peaks in the chromatograms of chelerythrine chloride Std-FP, nitidine chloride Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify chelerythrine chloride and nitidine chloride peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chelerythrine chloride Std-FP and nitidine chloride Std-FP. The retention times of chelerythrine chloride and nitidine chloride 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 five characteristic peaks of Zanthoxyli Radix extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the five characteristic peaks of Zanthoxyli Radix extract

Peak No.	RRT	Acceptable Range
1	0.60	± 0.03
2	0.65	± 0.03
3	0.81	± 0.03
4 (marker, nitidine chloride)	1.00	-
5 (chelerythrine chloride)	1.33	± 0.03

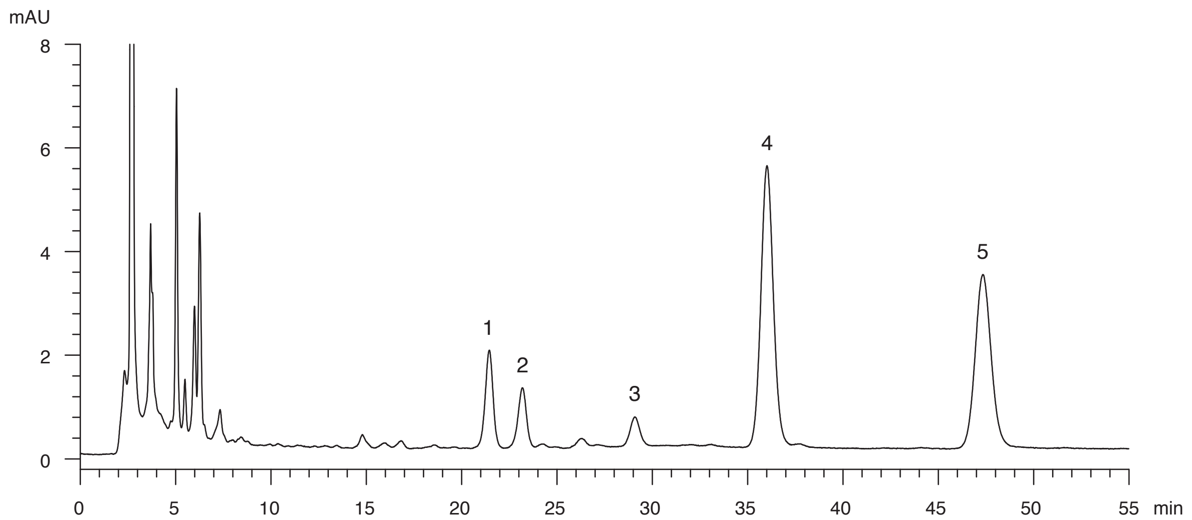

Figure 5 A reference fingerprint chromatogram of Zanthoxyli Radix extract

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 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 4.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 6.0%.
Ethanol-soluble extractives (cold extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Mixed chelerythrine chloride and nitidine chloride standard stock solution, Std-Stock (50 mg/L each)

Weigh accurately 0.5 mg of chelerythrine chloride CRS and 0.5 mg of nitidine chloride CRS, and dissolve in 10 mL of ethanol (70%).

Mixed chelerythrine chloride and nitidine chloride standard solution for assay, Std-AS

Measure accurately the volume of the mixed chelerythrine chloride and nitidine chloride Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 1, 2, 4, 10, 16 mg/L for both chelerythrine chloride and nitidine chloride.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (273 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 0.05% trifluoroacetic acid with 0.05% triethylamine (27:73, v/v). The elution time is about 55 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed chelerythrine chloride and nitidine chloride Std-AS (4 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chelerythrine chloride and nitidine chloride should not be more than 5.0%; the RSD of the retention times of chelerythrine chloride and nitidine chloride peaks should not be more than 2.0%; the column efficiencies determined from chelerythrine chloride and nitidine chloride peaks should not be less than 8700 theoretical plates.

The *R* value between chelerythrine chloride peak and the closest peak; and the *R* value between nitidine chloride 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 chelerythrine chloride and nitidine chloride Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of chelerythrine chloride and nitidine chloride against the corresponding concentrations of the mixed chelerythrine chloride and nitidine chloride Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify chelerythrine chloride and nitidine chloride peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed chelerythrine chloride and nitidine chloride Std-AS. The retention times of chelerythrine chloride and nitidine chloride 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 chelerythrine chloride and nitidine chloride in the test solution, and calculate the percentage contents of chelerythrine and nitidine (the percentage contents of chelerythrine chloride and nitidine chloride × 0.907) in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.30% of the total content of chelerythrine ($C_{21}H_{18}NO_4$) and nitidine ($C_{21}H_{18}NO_4$), calculated with reference to the dried substance.