Baphicacanthis Cusiae Rhizoma et Radix

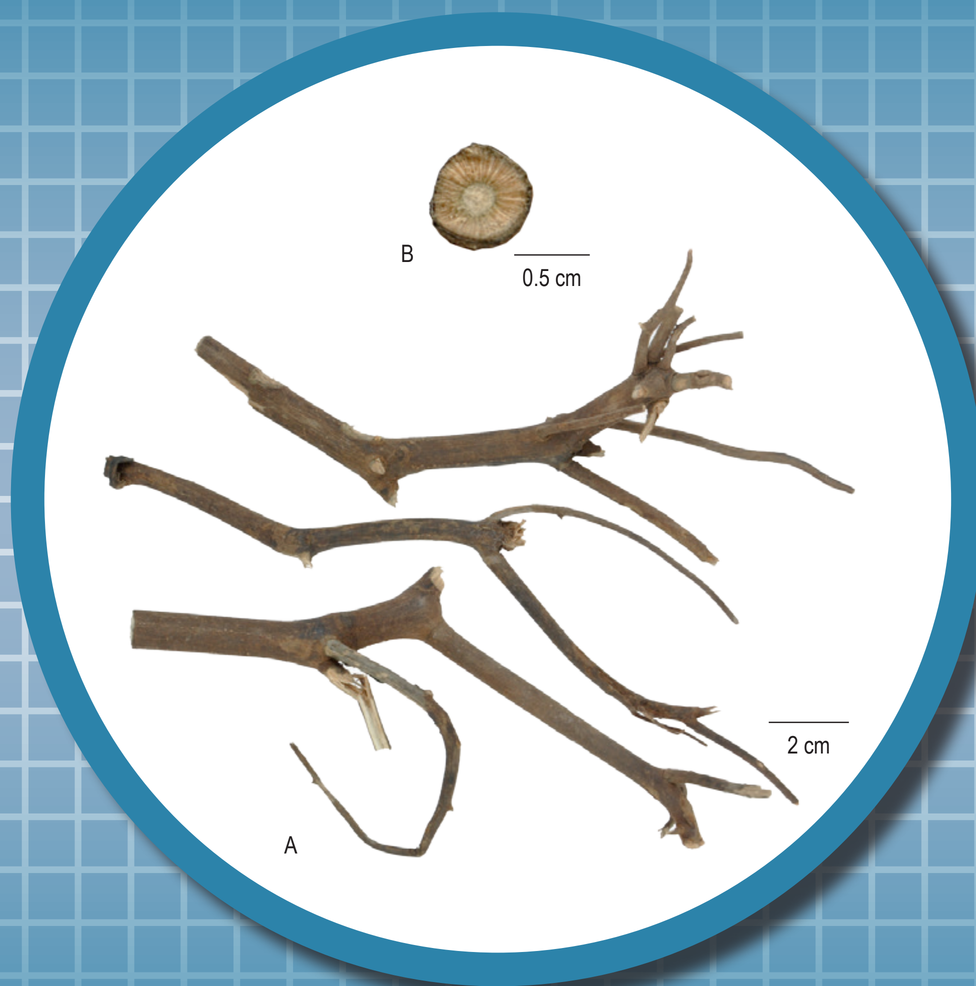

Figure 1 A photograph of Baphicacanthis Cusiae Rhizoma et Radix

A. Baphicacanthis Cusiae Rhizoma et Radix B. Transverse section of rhizome

1. NAMES

Official Name: Baphicacanthis Cusiae Rhizoma et Radix

Chinese Name: 南板藍根

Chinese Phonetic Name: Nanbanlangen

2. SOURCE

Baphicacanthis Cusiae Rhizoma et Radix is the dried rhizome and root of *Baphicacanthus cusia* (Nees) Bremek. (Acanthaceae). The rhizome and root are collected in summer and autumn, the aerial part and foreign matter removed, then dried under the sun to obtain Baphicacanthis Cusiae Rhizoma et Radix.

3. DESCRIPTION

Rhizome cylindrical, mostly tortuous, branched, 5-28 cm long, 2-11 mm in diameter. Externally greyish-brown or reddish-brown, nodes swollen, sometimes with slender and curved rootlets, stem remnants at the upper parts of nodes. Texture hard and fragile. Fracture uneven, bark bluish-grey, wood yellowish-white to yellow, pith bluish-grey. Odour slight; taste weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists several layers of cells, containing brown contents. Cortex broad, consisting of several layers of sclerenchymatous cells at outer side; Stone cells present. Endodermis distinct. Phloem relatively narrow, with numerous phloem fibres. Xylem vessels singly scattered or several in groups, arranged radially; xylem rays relatively broad. Pith consists of parenchymatous cells; some cells contain cystoliths (Fig. 2).

Powder

Colour greyish-green or brown. Cystoliths yellow, pale yellow or colourless, elongated-elliptical or elliptical, 21-225 µm long (occasionally up to 295 µm), 14-84 µm in diameter; yellowish-white, white or polychromatic under the polarized light microscope. Phloem fibres elongated-fusiform, 6-27 µm in diameter, walls thick, lumen linear; bright yellow or white under the polarized light microscope. Xylem fibres 10-44 µm in diameter, wall relatively thick and lumen large, pits and pit canals distinct; yellowish-white or white under the polarized light microscope. Stone cells subrectangular, subsquare, subrounded or irregular in shape, 29-271 µm long and 14-123 µm in diameter, wall thick and lumen large, pits and pit canals distinct; bright yellow or white under the polarized light microscope. Vessels mainly bordered-pitted, 11-69 µm in diameter. Cork cells subpolygonal or elongated-polygonal in surface view. Simple starch granules rounded, subrounded or ovoid, 2-23 µm in diameter, hilum slit-shaped, dotted or V-shaped, striations indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-9 units (Fig. 3).

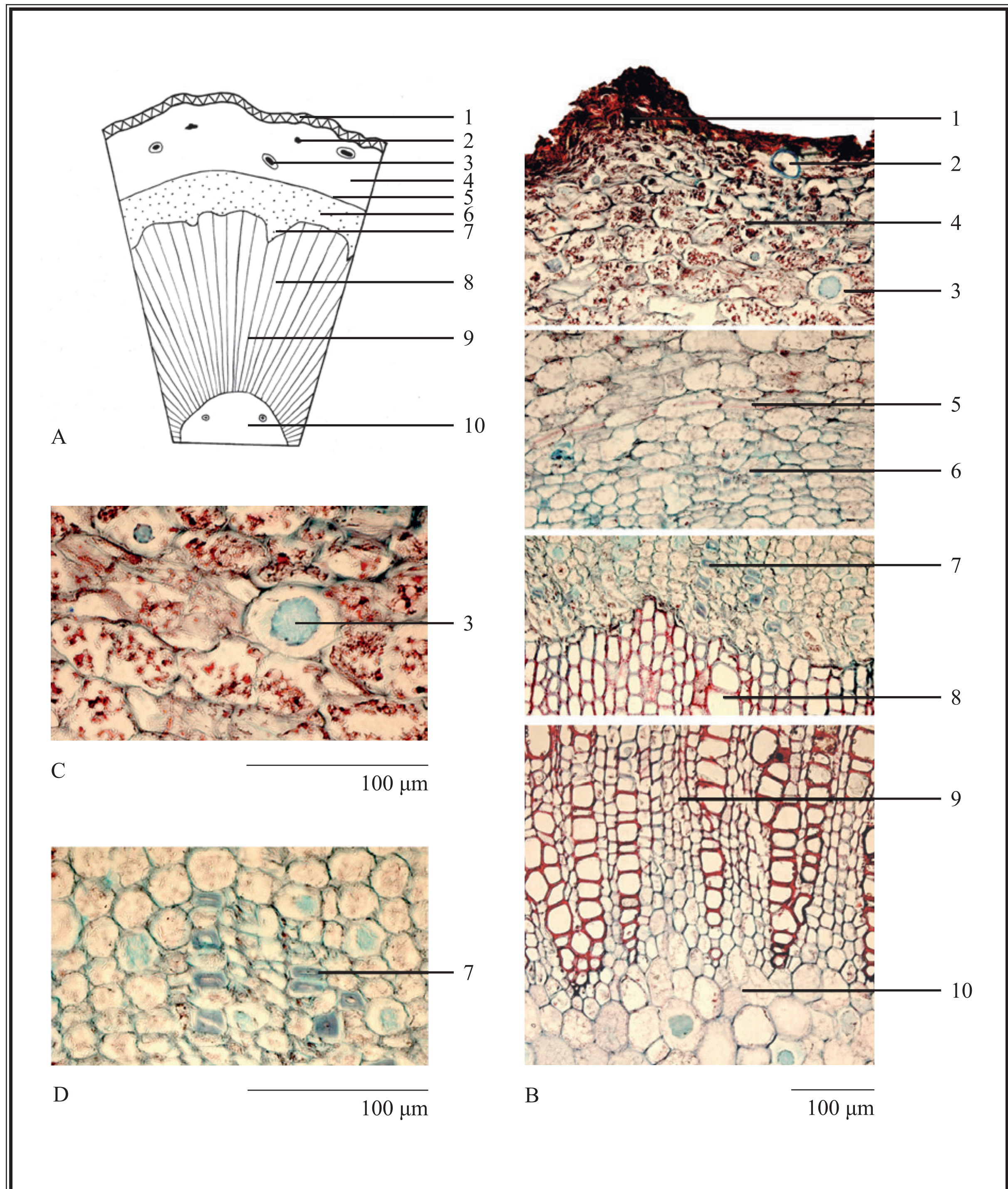

Figure 2 Microscopic features of transverse section of rhizome of Baphicacanthis Cusiae Rhizoma et Radix

A. Sketch B. Section illustration C. Cystoliths in parenchymatous cells
D. Phloem fibres

1. Cork 2. Stone cell 3. Cystolith 4. Cortex 5. Endodermis
6. Phloem 7. Phloem fibre 8. Xylem 9. Xylem ray 10. Pith

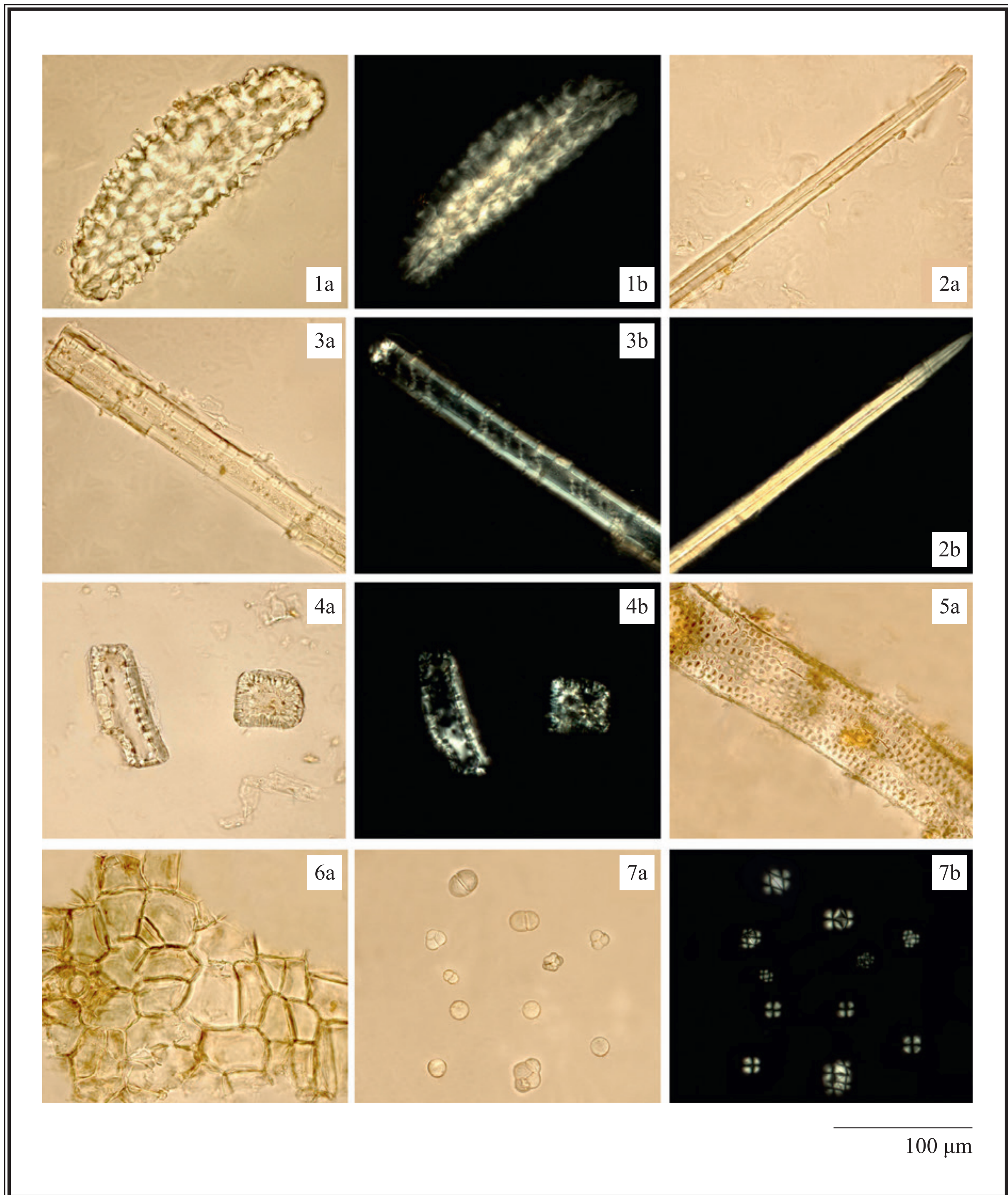

Figure 3 Microscopic features of powder of Baphicacanthis Cusiae Rhizoma et Radix

1. Cystolith 2. Phloem fibre 3. Xylem fibre 4. Stone cells 5. Bordered-pitted vessel
6. Cork cells 7. Starch granules

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

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

Standard solutions

Indigo standard solution

Weigh 0.1 mg of indigo CRS (Fig. 4) and dissolve in 1 mL of a mixture of methanol and dichloromethane (1:9, v/v).

Indirubin standard solution

Weigh 0.1 mg of indirubin CRS (Fig. 4) and dissolve in 1 mL of a mixture of methanol and dichloromethane (1:9, v/v).

Developing solvent system

Prepare a mixture of dichloromethane and acetone (97:3, v/v).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of dichloromethane. Sonicate (140 W) the mixture for 15 min. Centrifuge at about 1800 × *g* for 10 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of a mixture of methanol and dichloromethane (1:9, v/v).

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 indigo standard solution (60 µL), indirubin standard solution (10 µL) and the test solution (5 µL) to the plate. Develop over a path of about 4 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. 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 indigo and indirubin.

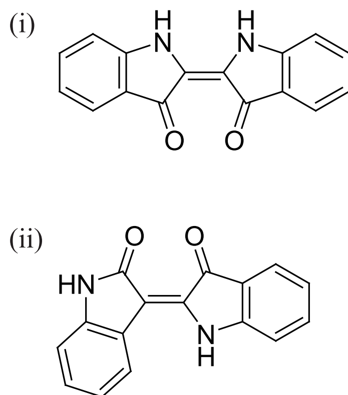

Figure 4 Chemical structures of (i) indigo and (ii) indirubin

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

Standard solutions

Indigo standard solution for fingerprinting, Std-FP (2 mg/L)

Weigh 0.2 mg of indigo CRS and dissolve in 100 mL of *N,N*-dimethylformamide.

Indirubin standard solution for fingerprinting, Std-FP (0.6 mg/L)

Weigh 0.15 mg of indirubin CRS and dissolve in 250 mL of *N,N*-dimethylformamide.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 8 mL of *N,N*-dimethylformamide. 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 two more times. Combine the supernatants and make up to the mark with *N,N*-dimethylformamide. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	70 → 45	30 → 55	linear gradient
20 – 30	45 → 40	55 → 60	linear gradient
30 – 40	40 → 10	60 → 90	linear gradient
40 – 60	10 → 0	90 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of indigo Std-FP and indirubin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of indigo and indirubin should not be more than 5.0%; the RSD of the retention times of indigo and indirubin peaks should not be more than 2.0%; the column efficiencies determined from indigo and indirubin peaks should not be less than 20000 theoretical plates.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject indigo Std-FP, indirubin Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention times of indigo and indirubin peaks in the chromatograms of indigo Std-FP, indirubin Std-FP and the retention times of the three characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify indigo and indirubin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of indigo Std-FP and indirubin Std-FP. The retention times of indigo and indirubin 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 three characteristic peaks of Baphicacanthis Cusiae Rhizoma et Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Baphicacanthis Cusiae Rhizoma et Radix extract

Peak No.	RRT	Acceptable Range
1	0.82	± 0.03
2 (marker, indigo)	1.00	-
3 (indirubin)	1.11	± 0.03

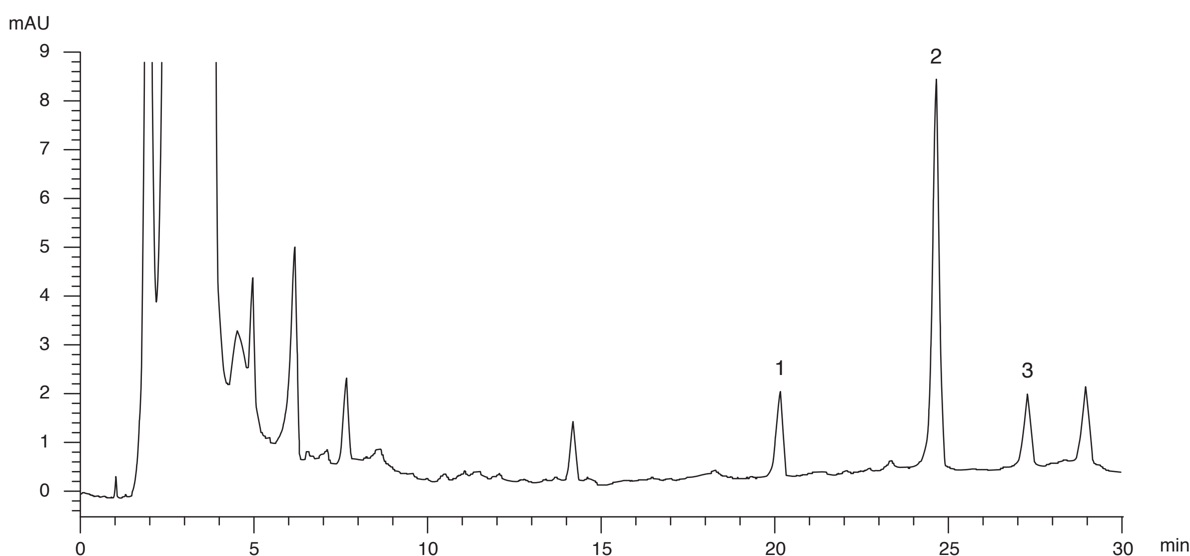

Figure 5 A reference fingerprint chromatogram of Baphicacanthis Cusiae Rhizoma et Radix extract

For positive identification, the sample must give the above three 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 9.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

Carry out the method as directed in Appendix XV.

Reagent
Anthrone sulphuric acid solution
Weigh accurately 0.1 g of anthrone and dissolve in 100 mL of sulphuric acid (80%, v/v).

Standard solution
Anhydrous glucose standard stock solution, Std-Stock (200 mg/L)
Weigh accurately 10.0 mg of anhydrous glucose CRS and dissolve in 50 mL of water.
Anhydrous glucose standard solution for assay, Std-AS
Measure accurately the volume of the anhydrous glucose Std-Stock, dilute with water to produce a series of solutions of 5, 10, 30, 50, 70 mg/L for anhydrous glucose.

Test solution
Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of water. Place the mixture in a water bath for 1 h. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with 5 mL of water. Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and make up to the mark with water. Pipette 3 mL of the solution into a 50-mL centrifuge tube. Add 30 mL of ethanol. Place the mixture at 4ºC for 12 h. Centrifuge at about 1800 × g for 10 min. Discard the supernatant. Dissolve the residue in water. Transfer the solution to a 25-mL volumetric flask and make up to the mark with water.

Ultraviolet/Visible spectrophotometeric system

The spectrophotometer is set at 625 nm.

Colourimetric method

Pipette 2 mL of the standard solution or test solution into a 10-mL test tube, then pipette 6 mL of anthrone sulphuric acid solution. Place the mixture in a water bath for 15 min. Cool the mixture in an ice water bath for 15 min. Using the corresponding anthrone sulphuric acid solution as the blank. Proceed to UV/Visible analysis at 625 nm.

System suitability requirements

Perform at least five replicate determinations, each using 2 mL of anhydrous glucose Std-AS (30 mg/L) by colourimetric method. The requirement of the system suitability parameters is as follows: the RSD of the absorbance of anhydrous glucose should not be more than 5.0%.

Calibration curve

Determine a series of anhydrous glucose Std-AS (2 mL each) in the ultraviolet/visible spectrophotometeric system and record the absorbance by colourimetric method. Plot the absorbances of anhydrous glucose against the corresponding concentrations of anhydrous glucose Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Measure the absorbance and calculate the concentration (in milligram per litre) of anhydrous glucose in the test solution, and calculate the percentage content of anhydrous glucose in the sample by using the equations as indicated in Appendix XV.

Limits

The sample contains not less than 1.1% of polysaccharides [calculated as anhydrous glucose ($C_6H_{12}O_6$)], calculated with reference to the dried substance.