Acanthopanacis Cortex



Figure 1 A photograph of Acanthopanacis Cortex

A. Acanthopanacis Cortex B. Magnified outer surface of root bark
C. Magnified inner surface of root bark

1. NAMES

Official Name: Acanthopanacis Cortex

Chinese Name: 五加皮

Chinese Phonetic Name: Wujiapi

2. SOURCE

Acanthopanacis Cortex is the dried root bark of *Acanthopanax gracilistylus* W. W. Smith (Araliaceae). The root is collected in summer and autumn, washed clean, stripped off the root bark, then baked or dried under the sun to obtain Acanthopanacis Cortex.

3. DESCRIPTION

Irregularly quilled, 3.5-20.5 cm long, 3-16 mm in diameter, 1-3 mm thick. Externally greyish-brown, with slightly twisted longitudinal wrinkles and transverse lenticel-like scars; inner surface pale yellow or greyish-yellow, with fine longitudinal striations. Texture fragile and light in weight, easily broken. Fracture irregular, greyish-white. Odour slightly aromatic; taste slightly pungent and bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of suberized cells. Cortex narrow, scattered with a few secretory canals. Phloem broad; phloem rays 1-5 rows of cells wide; secretory canals scattered in phloem, abundant, surrounded by 4-15 secretory cells. Clusters of calcium oxalate abundant, located in the parenchymatous cells. Phloem fibres occasionally found at old root bark, scattered singly or 2-4 in bundles. Secretory canals subrounded; pale yellow oil droplets observed before stained (Fig. 2).

Powder

Colour greyish-white. Clusters of calcium oxalate scattered or present in parenchymatous cells, 10-72 μm in diameter, angles large and blunt, some short and sharp; polychromatic under the polarized microscope. Secretory cells and canal fragments contain pale yellow secretions and colourless oil droplets. Cork cells pale yellow or pale yellowish-brown, subpolygonal or subsquare in the surface view, walls thick. Phloem fibres present at old root bark, few, scattered singly or 2-4 in bundles, linear, straight, margin slightly wavy, endings blunt, short pointed or truncate, lignified, pits sparse (Fig. 3).

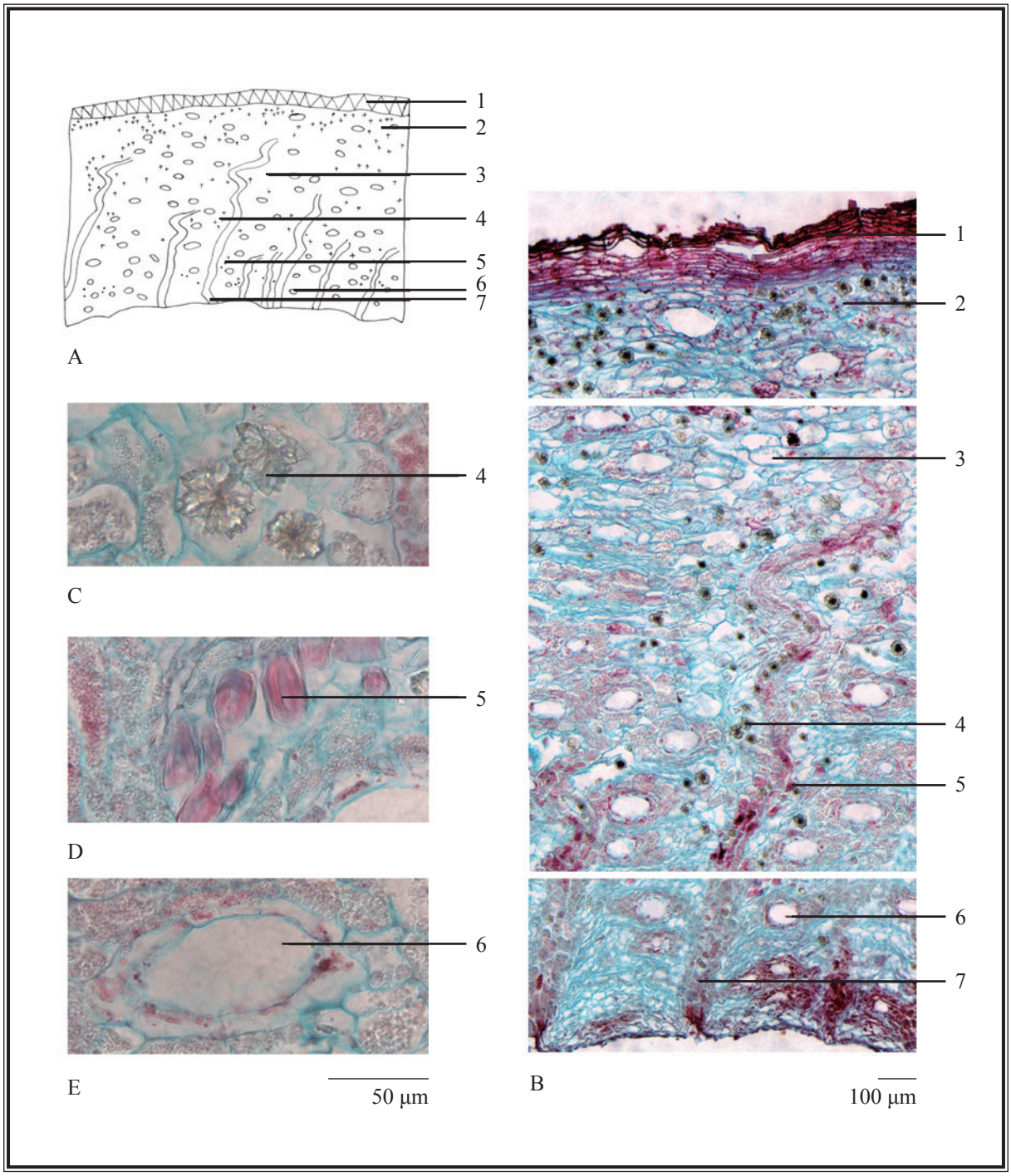


Figure 2 Microscopic features of transverse section of Acanthopanacis Cortex

A. Sketch B. Section illustration C. Clusters of calcium oxalate D. Phloem fibre E. Secretory canal

1. Cork 2. Cortex 3. Phloem 4. Clusters of calcium oxalate 5. Phloem fibre
6. Secretory canal 7. Phloem ray

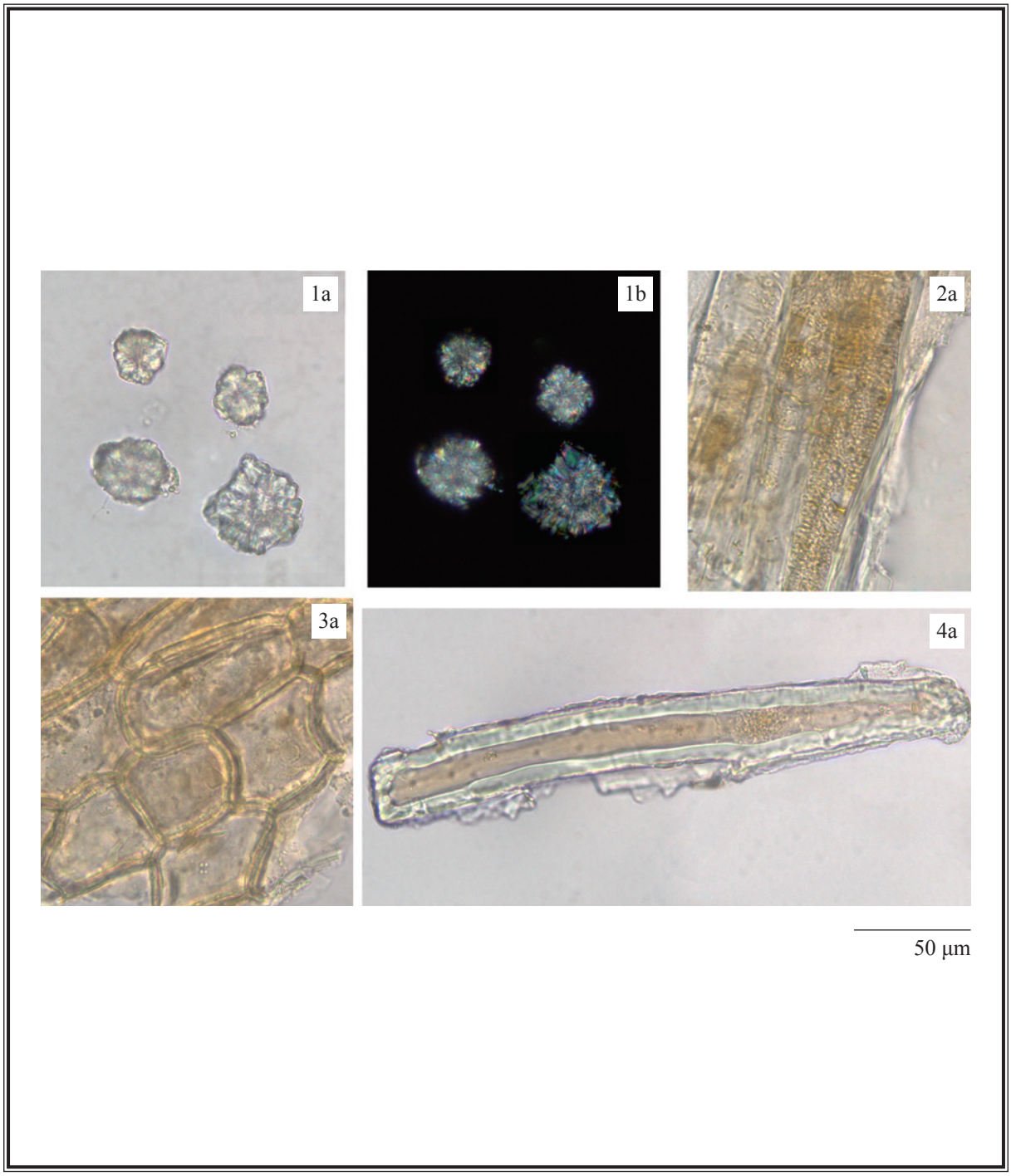


Figure 3 Microscopic features of powder of Acanthopanacis Cortex

1. Clusters of calcium oxalate 2. Secretory canal 3. Cork cells 4. Phloem fibre

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

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

Standard solution

Syringin (eleutheroside B) standard solution

Weigh 2.0 mg of syringin CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (10:2:0.1, v/v).

Test solution

Weigh 3.0 g of the powdered sample and place it in a 25-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 syringin standard solution (1 μL) and the test solution (10 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate 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 (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

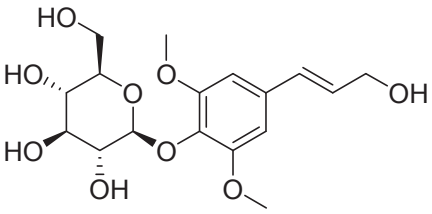


Figure 4 Chemical structure of syringin (eleutheroside B)



Figure 5 A reference HPTLC chromatogram of Acanthopanacis Cortex extract observed under UV light (254 nm)

1. Syringin standard solution 2. Test solution

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 syringin (Fig. 5).

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

Standard solution

Syringin (eleutheroside B) standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of syringin CRS and dissolve in 10 mL of methanol (50%).

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 (50%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (265 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)	Acetonitrile (%, v/v)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 13	11	89	isocratic
13 – 18	11 → 17	89 → 83	linear gradient
18 – 60	17	83	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of syringin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of syringin should not be more than 5.0%; the RSD of the retention time of syringin peak should not be more than 2.0%; the column efficiency determined from syringin peak should not be less than 10000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject syringin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of syringin peak in the chromatogram of syringin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify syringin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of syringin Std-FP. The retention times of syringin 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 Acanthopanacis Cortex extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Acanthopanacis Cortex extract

Peak No.	RRT	Acceptable Range
1 (marker, syringin)	1.00	-
2 (chlorogenic acid)	1.26	± 0.03
3	2.01	± 0.04
4	4.44	± 0.03
5 (1,5-dicaffeoylquinic acid)	4.61	± 0.04

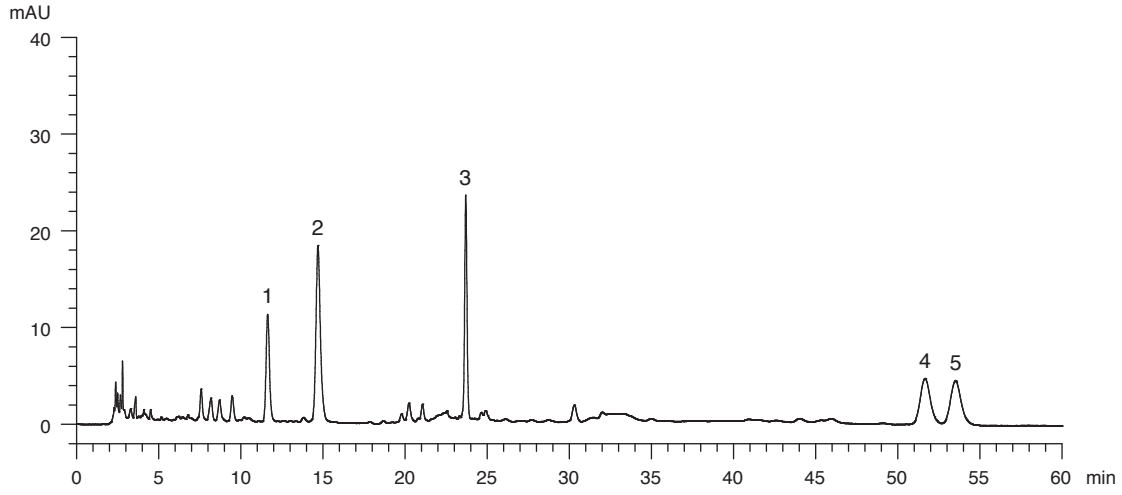


Figure 6 A reference fingerprint chromatogram of Acanthopanacis Cortex extract

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. 6).

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 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 12.0%.
Acid-insoluble ash: not more than 3.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 18.0%.
Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

7. ASSAY

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

Standard solution

Syringin (eleutheroside B) standard stock solution, Std-Stock (200 mg/L)
Weigh accurately 2.0 mg of syringin CRS and dissolve in 10 mL of methanol (50%).
Syringin standard solution for assay, Std-AS
Measure accurately the volume of the syringin Std-Stock, dilute with methanol (50%) to produce a series of solutions of 1, 4, 8, 40, 80 mg/L for syringin.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 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 0.05% trifluoroacetic acid and methanol (82:18, v/v). The elution time is about 35 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of syringin Std-AS (8 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of syringin should not be more than 5.0%; the RSD of the retention time of syringin peak should not be more than 2.0%; the column efficiency determined from syringin peak should not be less than 10000 theoretical plates.

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

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify syringin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of syringin Std-AS. The retention times of syringin peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of syringin in the test solution, and calculate the percentage content of syringin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.050% of syringin ($C_{17}H_{24}O_9$), calculated with reference to the dried substance.