Cortex Eucommiae

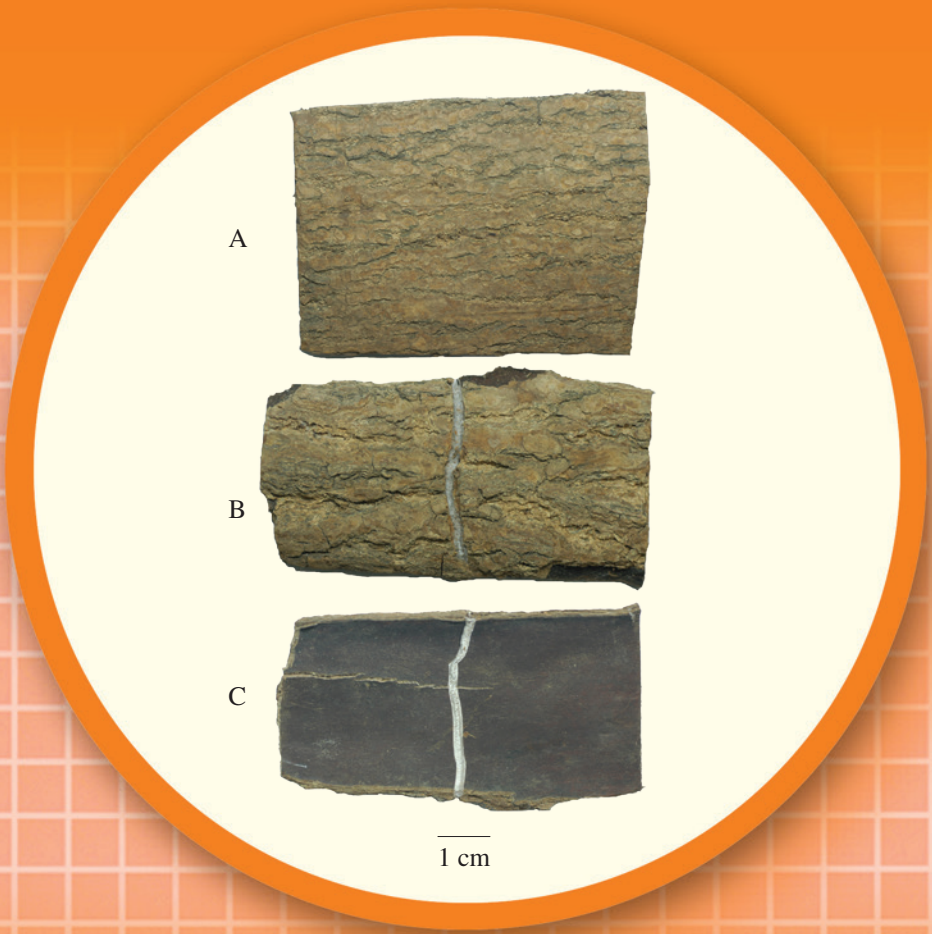


Figure 1 A photograph of Cortex Eucommiae

A. Outer surface

B. Outer surface with fracture showing resinous threads

C. Inner surface with fracture showing resinous threads

1. NAMES

Official Name: Cortex Eucommiae

Chinese Name: 杜仲

Chinese Phonetic Name: Duzhong

2. SOURCE

Cortex Eucommiae is the dried stem bark of *Eucommia ulmoides* Oliv. (Eucommiaceae). The bark is collected from April to June, the coarse outer portion removed, piled up for 5-7 days until the inner surface becomes purplish-brown, then dried under the sun to obtain Cortex Eucommiae.

3. DESCRIPTION

Flat pieces, or the two edges somewhat curved inward, varying in size, 2-7 mm thick. Outer surface pale brown or greyish-brown, markedly wrinkled or fissured and channelled longitudinally; some pieces relatively thin, showing distinct lenticels when the coarse bark is unscraped. Inner surface dark purple, smooth. Texture fragile, easily broken; fracture linked by fine, dense, silvery, elastic and resinous threads. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The transverse section sometimes shows evidence of rhytidome outside the cork cells. Cork consists of several rows of cells. Phloem consists of 5-7 bands of lignified stone cells, each band contains 2-6 rows of stone cells, with fibres nearby. Phloem rays 2-3 cells wide. Resinous masses scattered (Fig. 2).

Powder

Colour brown. Resinous threads stripe-shaped or twisted into masses, the surface granular. Stone cells numerous, mostly in groups, subrectangular to subrounded, elongated-rectangular or irregular, 15-80 μm in diameter, up to 180 μm long, thick-walled, some containing resinous masses. Cork cells polygonal in surface view, 12-40 μm in diameter, with unevenly thickened, lignified and finely pitted walls; rectangular in lateral view, wall thickened on three sides and relatively thin on one side, pit canals distinct (Fig. 3).

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

Standard solution

Pinoresinol diglucoside standard solution

Weigh 1.0 mg of pinoresinol diglucoside CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and formic acid (3:1:0.1, v/v).

Spray reagent

Add slowly 20 mL of sulphuric acid to 80 mL of water.

Test solution

Weigh 2.5 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of methanol. Sonicate (560 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 mL of water. Transfer the aqueous solution to a separatory funnel. Extract the solution with 50 mL of dichloromethane and discard the dichloromethane layer. Extract the aqueous layer with 50 mL of 1-butanol. Evaporate the 1-butanol extract to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

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 pinoresinol diglucoside standard solution (5 μL) and the test solution (1 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. Spray the plate evenly with the spray reagent and heat at about 120ºC until the spots or bands become visible (about 15 min). Examine the plate under visible light. 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 pinoresinol diglucoside.

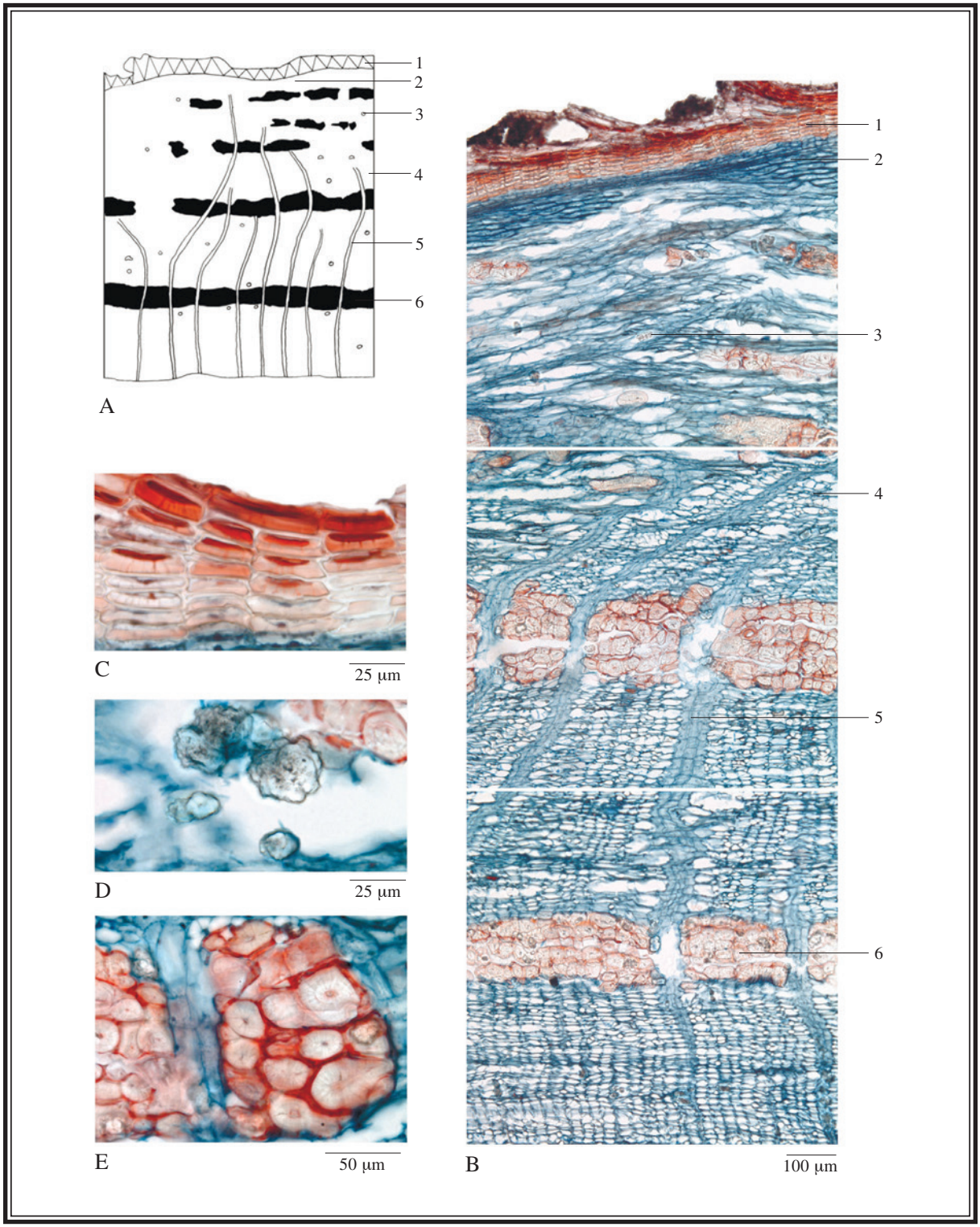


Figure 2 Microscopic features of transverse section of Cortex Eucommiae

A. Sketch B. Section illustration C. Cork cells D. Resinous masses
E. Groups of stone cells

1. Cork 2.Cortex 3. Resinous masses 4. Phloem 5. Phloem ray
6. Groups of stone cells

Figure 3 Microscopic features of powder of Cortex Eucommiae

1. Resinous threads 2. Elongated-rectangular stone cells 3. Subrectangular stone cells
4. Irregular stone cell 5. Cork cells

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

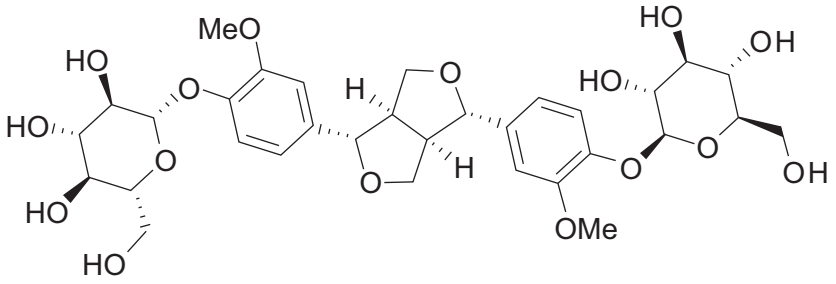


Figure 4 Chemical structure of pinoresinol diglucoside

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

Standard solution

Pinoresinol diglucoside standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 1.0 mg of pinoresinol diglucoside CRS and dissolve in 20 mL of methanol.

Test solution

Weigh 0.4 g of the powdered sample and place it in a 50-mL test tube, then add 10 mL of ethanol (50%). Sonicate (560 W) the mixture for 30 min. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–30	90→80	10→20	linear gradient
30–60	80→60	20→40	linear gradient
60–70	60→0	40→100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of pinoresinol diglucoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of pinoresinol diglucoside should not be more than 5.0%; the RSD of the retention time of pinoresinol diglucoside peak should not be more than 2.0%; the column efficiency determined from pinoresinol diglucoside peak should not be less than 40000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Cortex Eucommiae extract

Peak No.	RRT	Acceptable Range
1 (marker, pinoresinol diglucoside)	1.00	-
2	1.13	±0.03
3	1.59	±0.03
4	2.23	±0.06

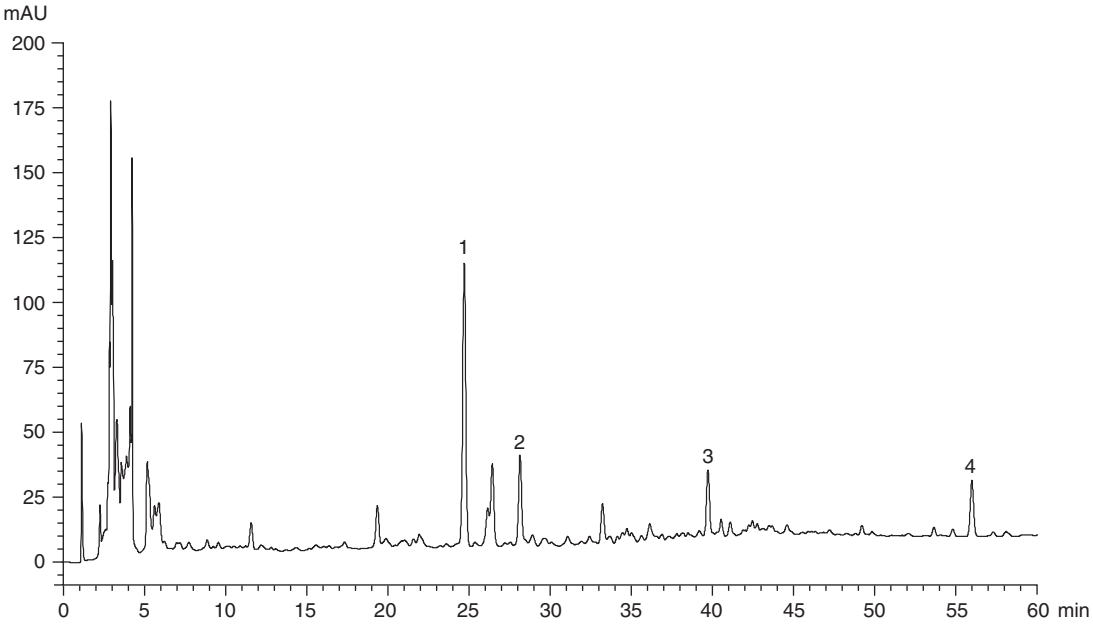


Figure 5 A reference fingerprint chromatogram of Cortex Eucommiae extract

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 – Aflatoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XV)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.5%.
Acid-insoluble ash: not more than 6.0%.

5.7 Water Content *(Appendix X)*: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 10.0%.
Ethanol-soluble extractives (hot extraction method): not less than 13.0%.

7. ASSAY

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

Standard solution

Pinoresinol diglucoside standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 10.0 mg of pinoresinol diglucoside CRS and dissolve in 10 mL of methanol.

Pinoresinol diglucoside standard solution for assay, Std-AS

Measure accurately the volume of the pinoresinol diglucoside Std-Stock, dilute with methanol to produce a series of solutions of 1, 10, 50, 100, 200 mg/L for pinoresinol diglucoside.

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 ethanol (50%). Sonicate (560 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter and transfer the filtrate to a 25-mL volumetric flask.

Repeat the extraction for one more time. Combine the filtrate and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (228 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 (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–20	90→80	10→20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of pinoresinol diglucoside Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of pinoresinol diglucoside should not be more than 5.0%; the RSD of the retention time of pinoresinol diglucoside peak should not be more than 2.0%; the column efficiency determined from pinoresinol diglucoside peak should not be less than 50000 theoretical plates.

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

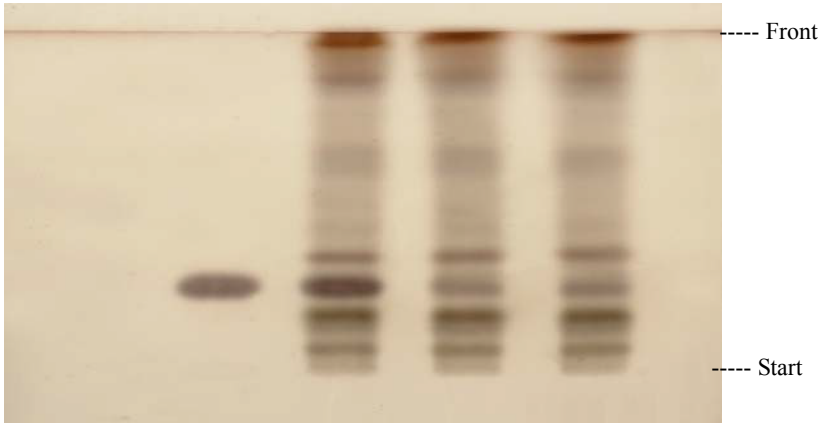
Procedure

Inject 5 μL of the test solution into the HPLC system and record the chromatogram. Identify pinoresinol diglucoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of pinoresinol diglucoside Std-AS. The retention times of pinoresinol diglucoside 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 pinoresinol diglucoside in the test solution, and calculate the percentage content of pinoresinol diglucoside in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.10% of pinoresinol diglucoside ($C_{32}H_{42}O_{16}$), calculated with reference to the dried substance.

Cortex Eucommiae (杜仲)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Pinoresinol diglucoside)	Pinoresinol diglucoside positive
3	Spiked sample (Sample plus pinoresinol diglucoside)	Pinoresinol diglucoside positive
4	Sample (Cortex Eucommiae)	Pinoresinol diglucoside positive
5	Sample duplicate (Cortex Eucommiae)	Pinoresinol diglucoside positive

Figure 1 TLC results of Cortex Eucommiae extract observed under visible light after staining