Cortex Mori

Figure 1　A photograph of Cortex Mori

1. NAMES

Official Name: Cortex Mori

Chinese Name: 桑白皮

Chinese Phonetic Name: Sangbaipi

2. SOURCE

Cortex Mori is the dried root bark of *Morus alba* L. (Moraceae). The root is collected in late autumn, when the leaves fall off, and in early spring, before the start of new growth, cut longitudinally, the root bark is stripped off, then dried under the sun to obtain Cortex Mori.

3. DESCRIPTION

Quilled, channelled, flattened or segmented, varying in length and width, 1-4 mm thick. Outer surface mostly with orangish-yellow or yellowish-brown scales bark; inner surface yellowish-white to brownish-yellow, with fine longitudinal striations. Texture light and tenacious, strongly fibrous, uneasily broken, but easily stripped longitudinally, dusting on stripping. Odour slight; taste slightly sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork cells tangentially elongated and orderly arranged, slightly or fully lignified. Phloem rays 2-6 cells wide. Laticiferous tubes scattered. Fibres scattered singly or in bundles, unlignified or slightly lignified. Parenchyma cells contain starch grains, some contain prisms of calcium oxalate. Groups of sclerenchyma cells mixed with stone cells are scattered in the older root bark, most cells contain prisms of calcium oxalate (Fig. 2).

Powder

Colour pale greyish-yellow to pale brownish-yellow. Fibres numerous, 10-26 μm in diameter, wall thickened, showing a polychrome when examined under a polarized microscope. Prisms of calcium oxalate 7-35 μm in diameter, showing a polychrome when examined under a polarized microscope. Cork cells yellow, golden-yellow or yellowish-brown, polygonal or subsquare in surface view. Crystal-containing sclerenchyma cells suborbicular, subsquare or irregular in shape,

sometimes annular striations observed. Stone cells suborbicular, subsquare, rectangular or irregular in shape, 17-56 μm in diameter, wall relatively thickened or extremely thickened, with distinct pits and pit-canals, some contain prisms of calcium oxalate. Simple starch granules subglobose to ovoid, 2-16 μm in diameter, with dotted, cleft or asteroidal hilum in larger granules; compound starch grains mostly composed of 2-5 units, showing a black, cross shape when examined under a polarized microscope (Fig. 3).

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

Standard solution

Morusin standard solution

Weigh 1.0 mg of morusin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of dichloromethane and ethanol (10:1, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of ethanol. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Collect the supernatant.

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 morusin standard solution and the test solution (4 μL each) 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 morusin.

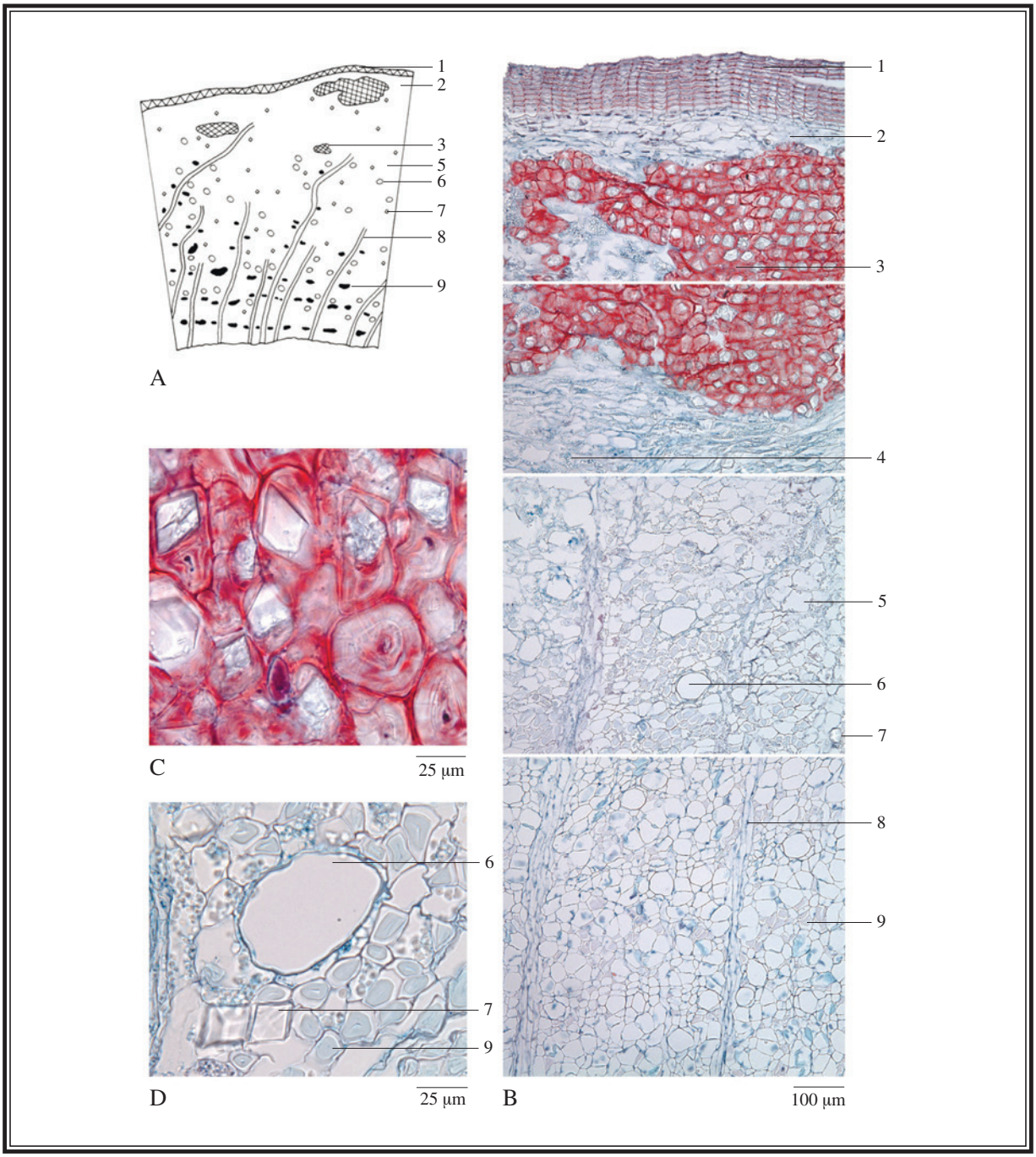


Figure 2 Microscopic features of transverse section of Cortex Mori

A. Sketch B. Section illustration

C. A group of crystal-containing sclerenchyma cells mixed with stone cells

D. Magnified image of laticifer, prisms of calcium oxalate and fibres

1. Cork 2. Cortex

3. A group of crystal-containing sclerenchyma cells mixed with stone cells

4. Starch grains 5. Phloem 6. Laticiferous tubes

7. Prisms of calcium oxalate 8. Phloem ray 9. Phloem fibres

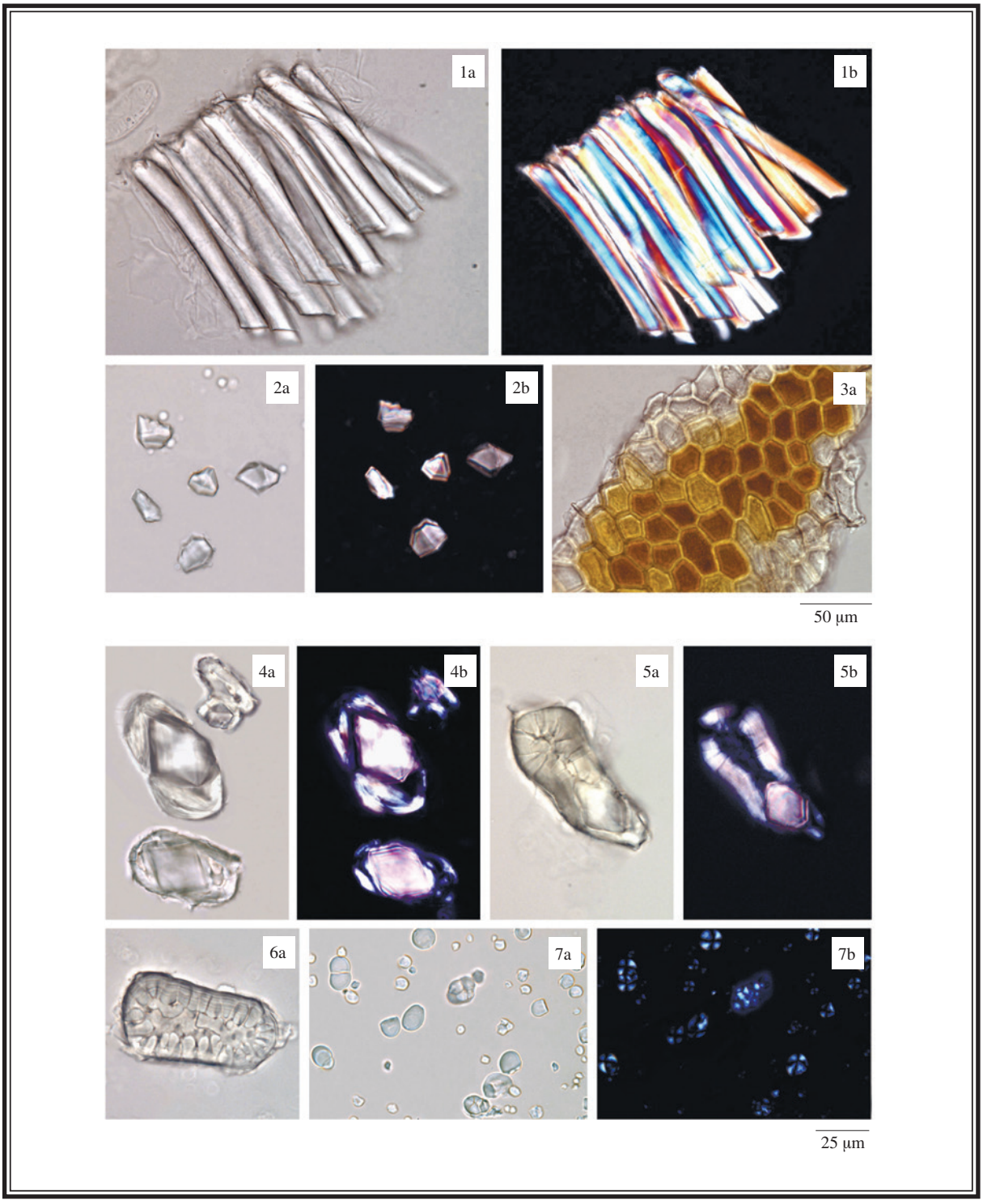


Figure 3 Microscopic features of powder of Cortex Mori

1. Phloem fibres 2. Prisms of calcium oxalate 3. Cork cells
4. Sclerenchyma cells with prisms of calcium oxalate
5. Stone cell with prisms of calcium oxalate 6. Stone cell 7. Starch grains

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

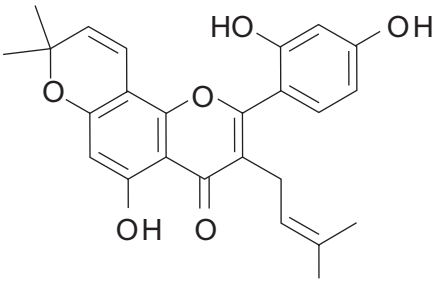


Figure 4 Chemical structure of morusin

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

Standard solution

Morusin standard stock solution, Std-Stock (40 mg/L)

Weigh 2.0 mg of morusin CRS and dissolve in 50 mL of ethanol (95%).

Morusin standard solution for fingerprinting, Std-FP (8 mg/L)

Pipette 5 mL of morusin Std-Stock into a 25-mL volumetric flask and make up to the mark with ethanol (95%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL test tube, then add 25 mL of ethanol (95%). Sonicate (560 W) the mixture for 30 min. Filter through a 0.45-μm RC filter. Make appropriate dilution where necessary.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 nm) and a column (4.6 × 150 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 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 25	57	43	isocratic
25 – 38	57→46	43→54	linear gradient
38 – 65	46	54	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of morusin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of morusin should not be more than 5.0%; the RSD of the retention time of morusin peak should not be more than 2.0%; the column efficiency determined from morusin peak should not be less than 30000 theoretical plates.

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

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.19	±0.03
2	0.37	±0.03
3	0.60	±0.03
4 (marker, morusin)	1.00	-

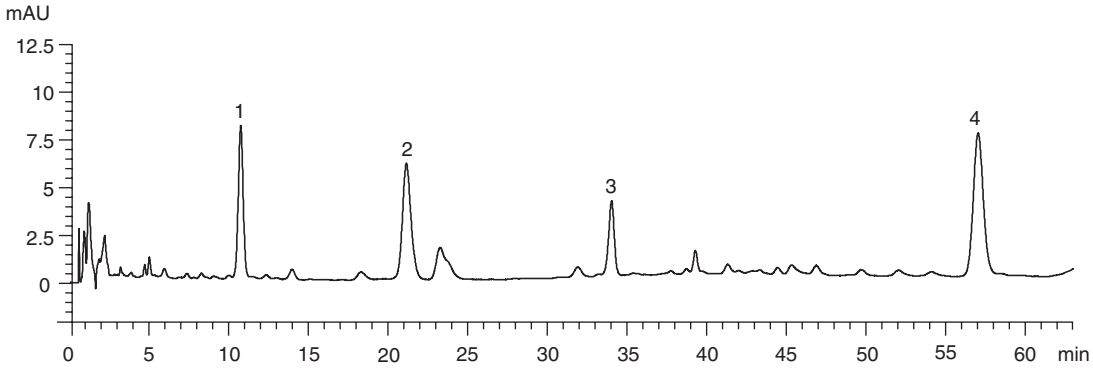


Figure 5 A reference fingerprint chromatogram of Cortex Mori extract

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 10.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 17.0%.
Ethanol-soluble extractives (hot extraction method): not less than 16.0%.

7. ASSAY

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

Standard solution

Morusin standard stock solution, Std-Stock (40 mg/L)

Weigh accurately 10.0 mg of morusin CRS and dissolve in 250 mL of ethanol (95%).

Morusin standard solution for assay, Std-AS

Measure accurately the volume of the morusin Std-Stock, dilute with ethanol (95%) to produce a series of solutions of 1, 4, 8, 12, 16 mg/L for morusin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (95%). Macerate for 60 min. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times without macerating. Combine the extracts and make up to the mark with ethanol (95%). Filter through a 0.45-μm RC filter. Make appropriate dilution where necessary.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 nm) and a column (4.6 × 150 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 – 35	42	58	isocratic
35 – 45	42 → 0	58 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of morusin Std-AS (4 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of morusin should not be more than 5.0%; the RSD of the retention time of morusin peak should not be more than 2.0%; the column efficiency determined from morusin peak should not be less than 8000 theoretical plates.

The *R* value between morusin 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 morusin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of morusin against the corresponding concentrations of morusin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

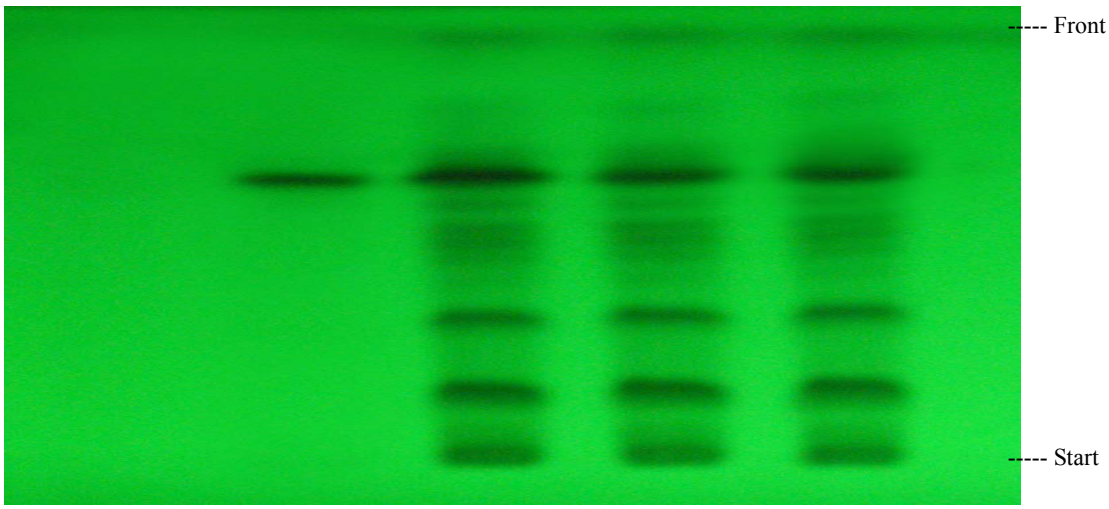
Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify morusin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of morusin Std-AS. The retention times of morusin 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 morusin in the test solution, and calculate the percentage content of morusin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.10% of morusin ($C_{25}H_{24}O_6$), calculated with reference to the dried substance.

Cortex Mori (桑白皮)



Lane	Sample	Results
1	Blank (Ethanol)	Negative
2	Standard (Morusin)	Morusin positive
3	Spiked sample (Sample plus morusin)	Morusin positive
4	Sample (Cortex Mori)	Morusin positive
5	Sample duplicate (Cortex Mori)	Morusin positive

Figure 1 TLC results of Cortex Mori extract observed under UV light (254 nm)