# Mori Ramulus

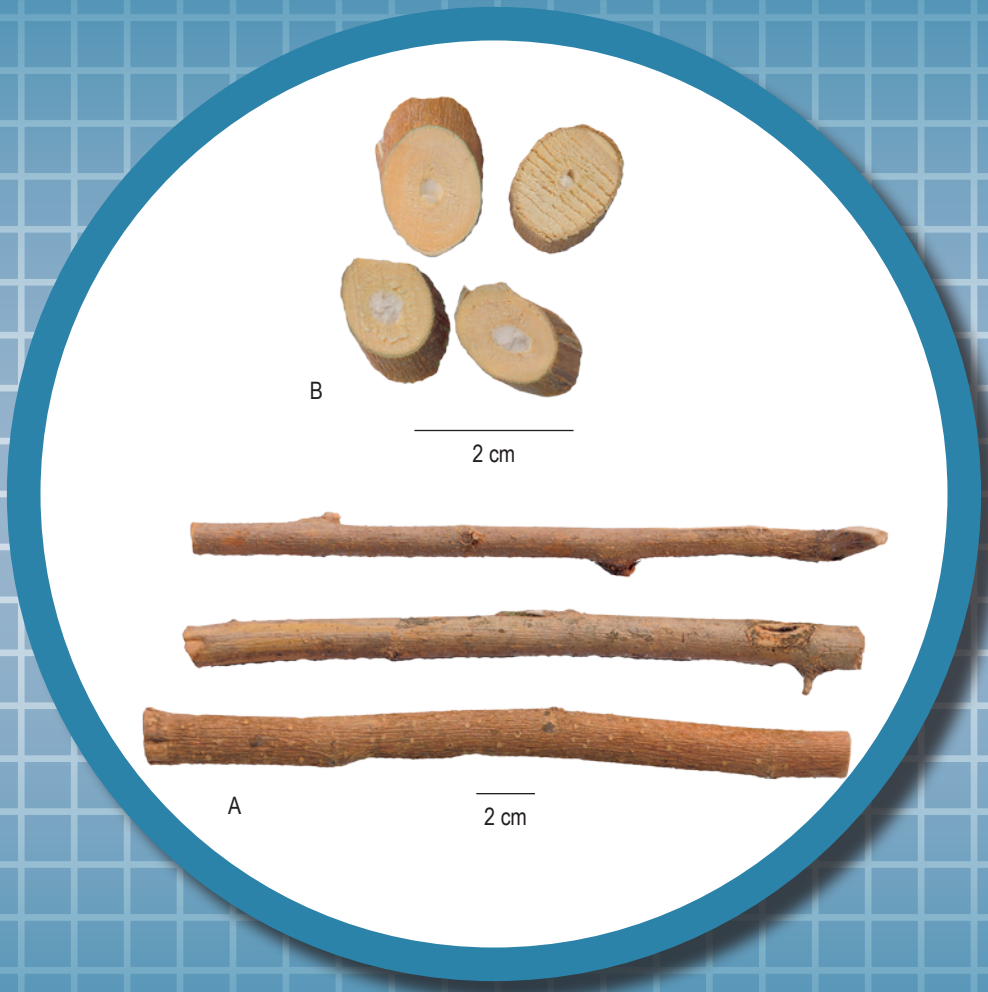



**Figure 1** A photograph of Mori Ramulus

A. Mori Ramulus B. Slices

## 1. NAMES

Official Name: Mori Ramulus

Chinese Name: 桑枝

Chinese Phonetic Name: Sangzhi

## 2. SOURCE

Mori Ramulus is the dried slender branch (new sprout) of *Morus alba* L. (Moraceae). The branch is collected in late spring to early summer, the leaves removed, then dried under the sun; or the branch sliced while fresh, then dried under the sun to obtain Mori Ramulus.

## 3. DESCRIPTION

Slender and cylindrical, occasionally branched, 5-25 mm in diameter. Externally greyish-yellow to yellowish-brown, with numerous yellowish-brown dotted lenticels and fine longitudinal striations, nodes usually swollen, with leaf scars and axillary buds. Texture hard and tough, uneasily broken; fracture fibrous. Slices 1-4 mm thick, the bark relatively narrow; the xylem broad, with pale yellow to yellow radial striations and cracks; the pith white to pale yellow. Odour slight; taste weak (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification *(Appendix III)*

**Transverse section**

Cork consists of several layers of cells, the cells rectangular, arranged densely, with relatively thick wall, slightly lignified. Cortex relatively narrow, containing stone cells and prisms of calcium oxalate, arranged in an interrupted ring. Fibre bundles present in the outside of phloem. Phloem ray distinct, consisting 1-3 rows of cells. Cambium in a ring. Xylem broad; xylem ray distinct, arranged radially; large xylem vessels scattered singly or 2-3 in groups. Pith consists of parenchymatous cells (Fig. 2).

**Powder**

Colour greyish-yellow. Starch granules abundant, simple starch granules subrounded or polygonal, 2-11 μm in diameter, hilum pointed; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-10 units. Stone cells subellipitical or triangular, 13-61 μm in diameter, with fine pit canals and thickened wall; blue-white under the polarized microscope. Prisms of calcium oxalate scattered singly, occasionally found in stone cells, polyhedral, cubical, and rhombic, 7-36 μm in diameter; polychromatic under the polarized microscope. Vessels mainly bordered-pitted, 13-90 μm in diameter, pits arranged closely. Phloem fibres scattered singly, slender, 5-36 μm in diameter, wall thick, pit indistinct, lumen narrow, slit-shaped; xylem fibres in bundles, numerous, fusiform, tapering slightly towards the end; polychromatic under the polarized microscope. Cork cells brownish-yellow, subrectangular or irregular (Fig. 3).

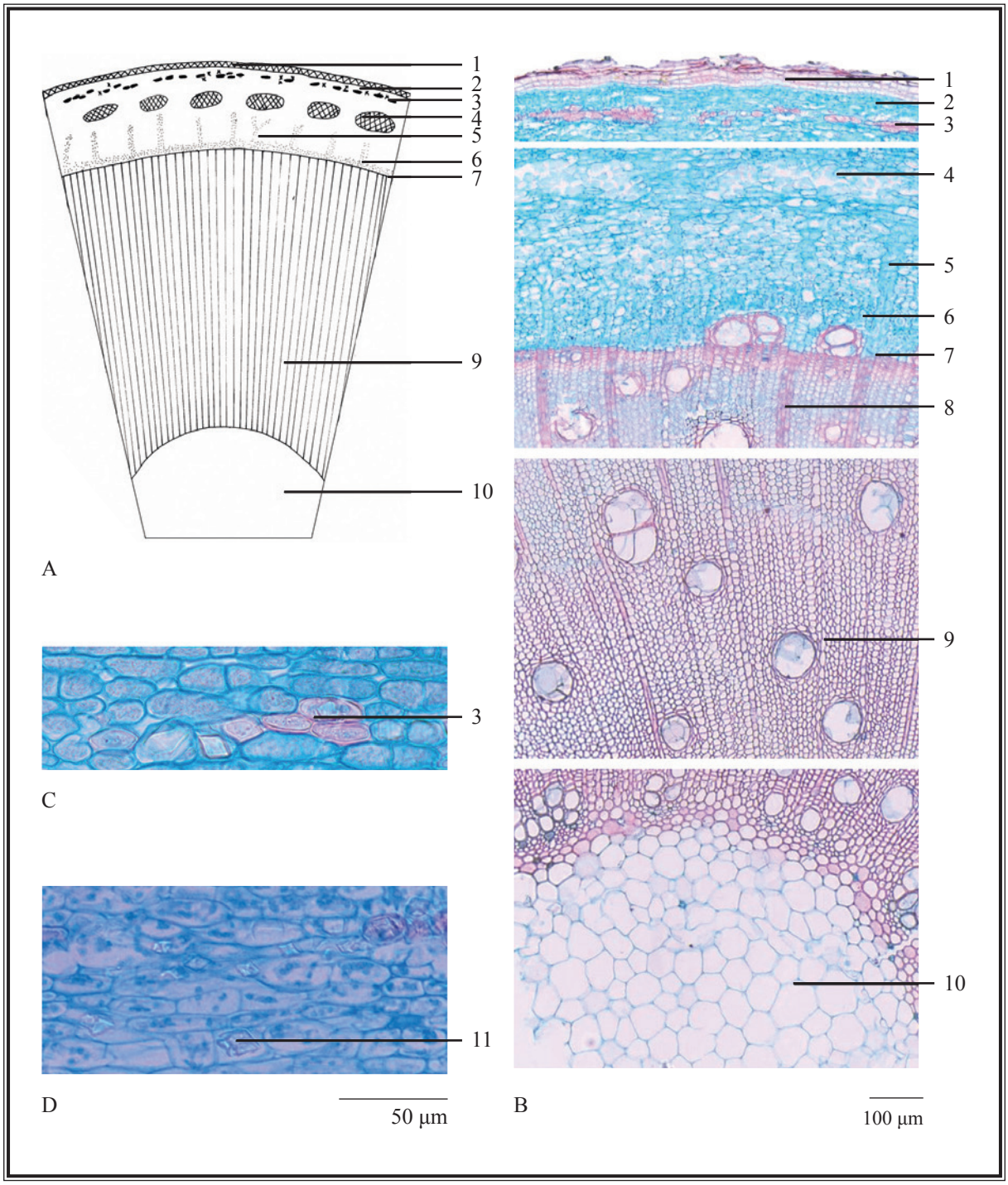



**Figure 2** Microscopic features of transverse section of Mori Ramulus

A. Sketch B. Section illustration C. Stone cells
D. Prisms of calcium oxalate

1. Cork 2. Cortex 3. Stone cells 4. Phloem fibres 5. Phloem ray 6. Phloem
7. Cambium 8. Xylem ray 9. Xylem 10. Pith 11. Prisms of calcium oxalate

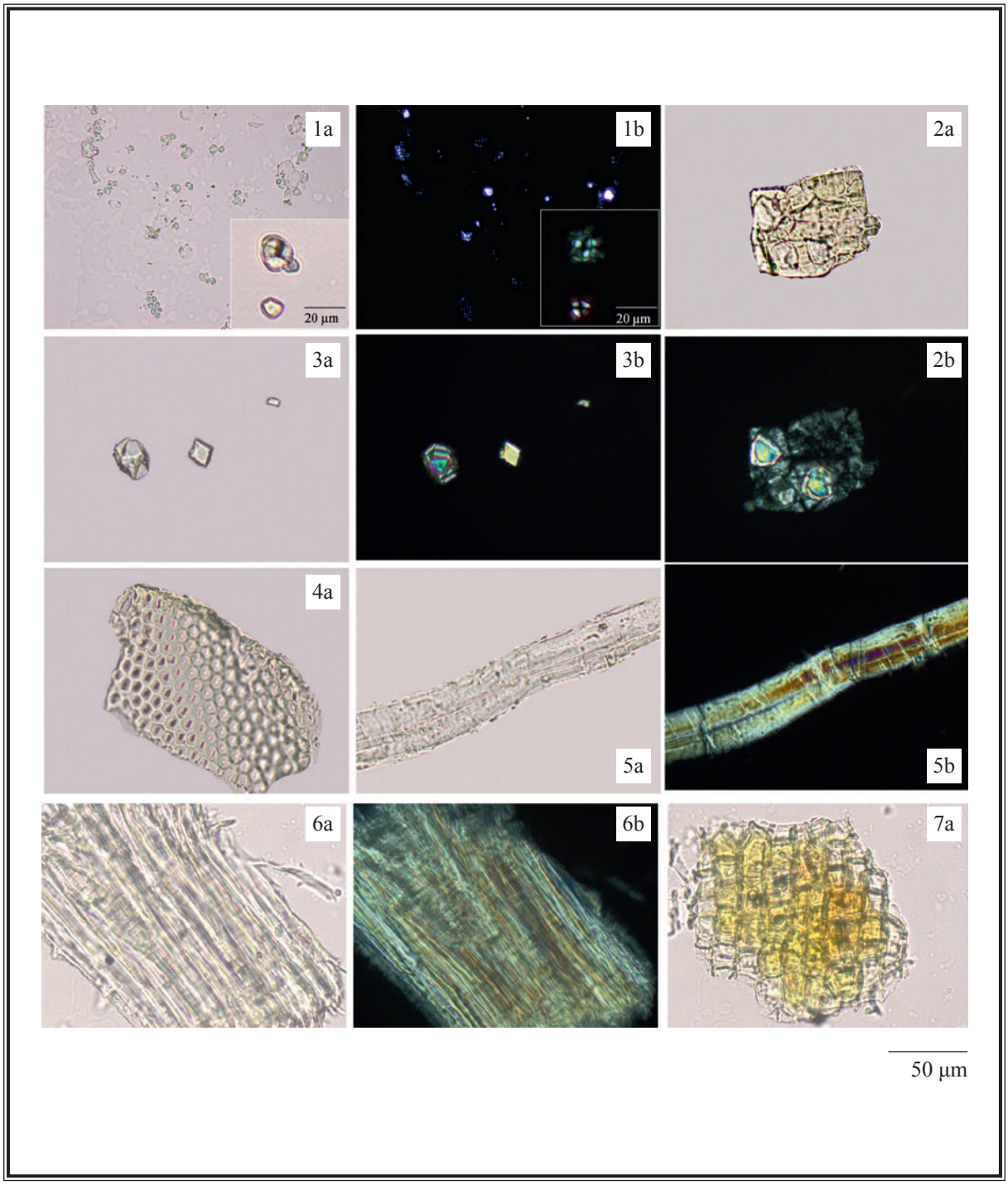



**Figure 3** Microscopic features of powder of Mori Ramulus

1. Starch granules 2. Stone cells 3. Prisms of calcium oxalate
4. Bordered-pitted vessels 5. Phloem fibre 6. Xylem fibres 7. Cork cells

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

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

**Standard solution**

*Mulberrin standard solution*

Weigh 0.5 mg of mulberrin CRS (Fig. 4) and dissolve in 5 mL of methanol.

**Developing solvent system**

Prepare a mixture of cyclohexane, ethyl acetate, acetone and glacial acetic acid (5:2:1:0.2, v/v).

**Spray reagent**

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (200 W) the mixture for 15 min. Centrifuge at about 4000 × *g* 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 mulberrin standard solution (2 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 8.5 cm. After the development, remove the plate from the chamber, mark the solvent front and heat at about 105ºC (about 2 min). Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 2 min). Examine the plate under UV light (366 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 mulberrin.

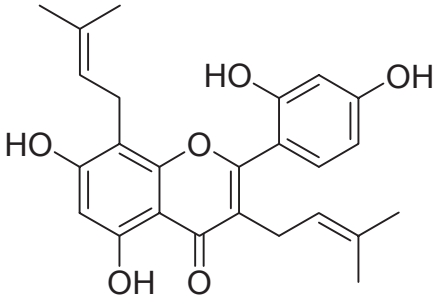



**Figure 4** Chemical structure of mulberrin

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

### Standard solution

*Mulberrin standard solution for fingerprinting, Std-FP (50 mg/L)*

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

### Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (200 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon 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 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 | 80 → 58 | 20 → 42 | linear gradient |
| 20 – 45 | 58 → 35 | 42 → 65 | linear gradient |
| 45 – 60 | 35 → 15 | 65 → 85 | linear gradient |

### System suitability requirements

Perform at least five replicate injections, each using 10 μL of mulberrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of mulberrin should not be more than 5.0%; the RSD of the retention time of mulberrin peak should not be more than 2.0%; the column efficiency determined from mulberrin peak should not be less than 120000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

**Procedure**

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

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

| Peak No. | RRT | Acceptable Range |
|---|---|---|
| 1 (oxyresveratrol) | 0.28 | ± 0.03 |
| 2 | 0.86 | ± 0.03 |
| 3 (marker, mulberrin) | 1.00 | - |
| 4 (morusin) | 1.28 | ± 0.03 |

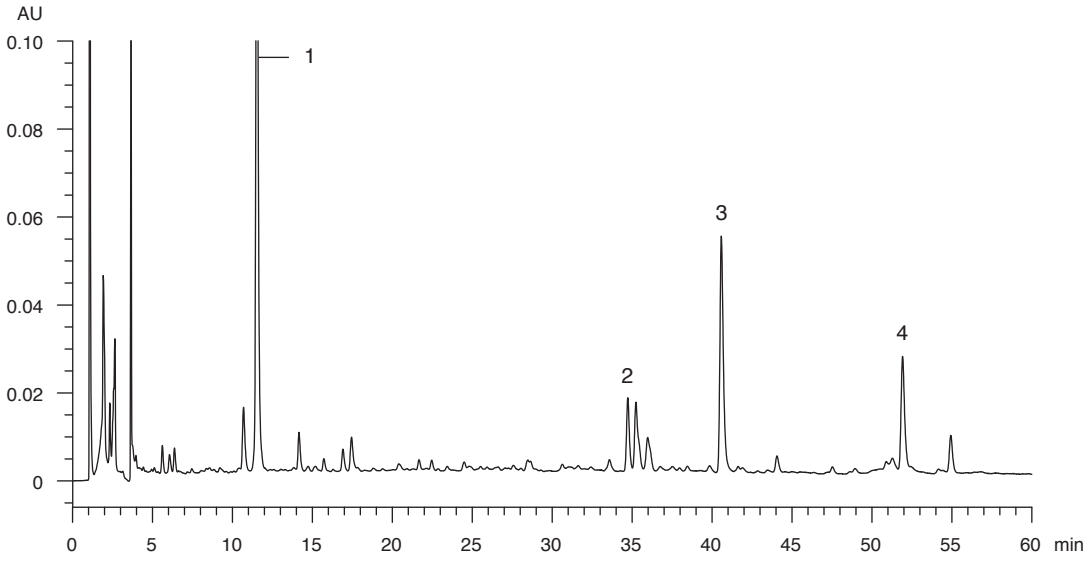



**Figure 5** A reference fingerprint chromatogram of Mori Ramulus extract

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 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 0.5%.

**5.7 Water Content** *(Appendix X)*

Oven dried method: not more than 11.0%.

## 6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.
Ethanol-soluble extractives (hot extraction method): not less than 6.0%.

## 7. ASSAY

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

**Standard solution**

*Mulberrin standard stock solution, Std-Stock (100 mg/L)*

Weigh accurately 1.0 mg of mulberrin CRS and dissolve in 10 mL of ethanol.

*Mulberrin standard solution for assay, Std-AS*

Measure accurately the volume of mulberrin Std-Stock, dilute with ethanol to produce a series of solutions of 1, 2.5, 5, 10, 20 mg/L for mulberrin.

**Test solution**

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 10 mL of ethanol. Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. Add 8 mL of ethanol to the residue and reflux the mixture for 15 min. Cool down to room temperature. Filter and combine the filtrates. Wash the residue with ethanol. Combine the solutions and make up to the mark with ethanol. Filter through a 0.45-μm nylon filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (262 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 water (55:45, v/v). The elution time is about 25 min.

**System suitability requirements**

Perform at least five replicate injections, each using 10 μL of mulberrin Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of mulberrin should not be more than 5.0%; the RSD of the retention time of mulberrin peak should not be more than 2.0%; the column efficiency determined from mulberrin peak should not be less than 10000 theoretical plates.

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

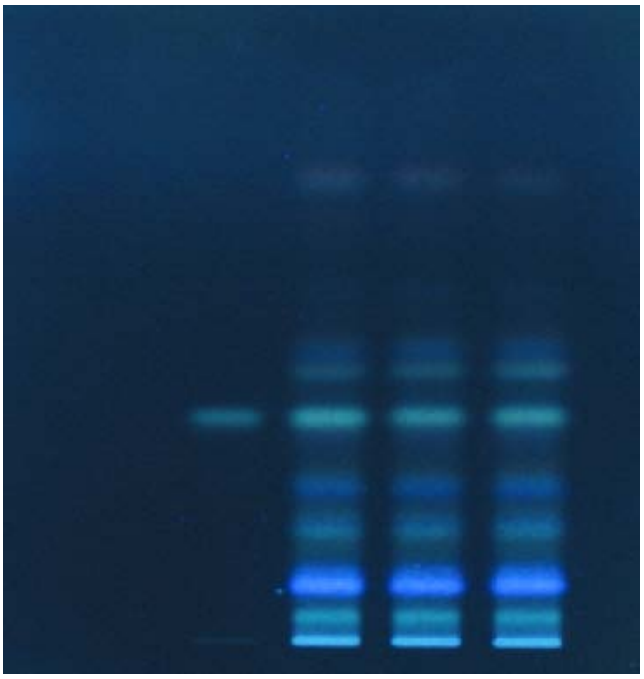
**Procedure**

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify mulberrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of mulberrin Std-AS. The retention times of mulberrin peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of mulberrin in the test solution, and calculate the percentage content of mulberrin in the sample by using the equations as indicated in Appendix IV(B).

**Limits**

The sample contains not less than 0.017% of mulberrin ($C_{25}H_{26}O_6$), calculated with reference to the dried substance.

**Mori Ramulus (桑枝)**




| Lane | Sample | Results |
|---|---|---|
| 1 | Blank (Methanol) | Negative |
| 2 | Standard (Mulberrin) | Mulberrin positive |
| 3 | Spiked sample (Sample plus mulberrin) | Mulberrin positive |
| 4 | Sample (Mori Ramulus) | Mulberrin positive |
| 5 | Sample duplicate (Mori Ramulus) | Mulberrin positive |

**Figure 1** TLC results of Mori Ramulus extract observed under UV light (366 nm) after staining