Cortex Magnoliae Officinalis

Figure 1(i) A photograph of dried stem bark of *Magnolia officinalis* Rehd. et Wils.

Figure 1(ii) A photograph of dried stem bark of *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils.

1. NAMES

Official Name: Cortex Magnoliae Officinalis

Chinese Name: 厚樸

Chinese Phonetic Name: Houpo

2. SOURCE

Cortex Magnoliae Officinalis is the dried stem bark of *Magnolia officinalis* Rehd. et Wils. or *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. (Magnoliaceae). The stem bark is collected in the spring and summer, and then boiled in water until soft, then piled in a wet place until its inner surface and the surface of the transverse section purplish-brown or brown in colour and shiny or showing luster, then further softened by steaming, rolled and dried to obtain Cortex Magnoliae Officinalis.

3. DESCRIPTION

***Magnolia officinalis* Rehd. et Wils. :** The stem bark quilled singly or double-quilled, 25-75 cm long and 1-4 mm thick (known as Tongpo); the stem bark near the root has one end expanding like the mouth of a bell, 13-25 cm long and 3-8 mm thick (known as Xuetongpo). The outer surface greyish-yellow or greyish-brown, rough, sometimes scaly, easily exfoliated, with distinct elliptical lenticels and longitudinal wrinkles. The bark with the cork scraped off exhibits a yellowish-brown colour, the inner surface purplish-brown or dark brown, smooth, with fine and dense longitudinal striations and exhibits an oily trace on scratching. Texture hard, not easily broken, fracture granular, outer layer greyish-brown, inner layer purplish-brown or brown, oily, sometimes with numerous small bright spots visible. Odour aromatic; taste pungent and slightly bitter [Fig. 1(i)].

Compared with *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils., *Magnolia officinalis* Rehd. et Wils. has a coarser outer surface, a less fibrous fracture, and a more oily and aromatic odour.

***Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. :** The processing procedure of stem bark is the same as that of *Magnolia officinalis* Rehd. et Wils., and stem bark is often classified to "Tongpo" and "Xuetongpo". The outer surface greyish-brown, with distinct elliptical lenticels and longitudinal wrinkles. The inner surface purplish-brown or dark brown, relatively smooth, with fine and dense

longitudinal striations; arid, less oily, with the fracture much more fibrous. Odour slightly aromatic; taste slightly pungent and slightly bitter [Fig. 1(ii)].

Compared with *Magnolia officinalis* Rehd. et Wils., *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. has a finer outer surface, with the fracture being much more fibrous, dry and less oily.

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

***Magnolia officinalis* Rehd. et Wils. :** The cork consists of more than 10 layers of cells, cells rectangular, with suberized and slightly lignified wall, rhytidome tissue visible. The outer side of cortex showing a ring of stone cells consisting of several layers of tangentially elongated stone cells, cells rectangular or elongated-rounded, 7-65 μm in diameter; the inner side scattered with single or groups of stone cells, some cells polygonal or irregularly branched, large in size, nearly up to 300 μm long, with distinct pit canals and striations; oil cells numerous, scattered, rounded or elliptical; fibre bundles occasionally observed. Phloem rays narrow; fibres mostly in several bundles, alternately arranged with the parenchyma cells of the sieve vessels, slightly tangentially arranged intermittently in layers; oil cells numerous, scattered. Parenchyma cells contain prismatic or polyhedral crystals of calcium oxalate [Fig. 2(i)].

***Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. :** The transverse section shows cork, cortex, and phloem from the outer to the inner side. The outer side of cortex shows a ring of stone cells consisting of 3-7 layers of tangentially elongated stone cells, cells rectangular or elongated-rounded, 9-50 μm in diameter; the inner side of cortex scattered with numerous irregular stone cells, nearly up to 220 μm long [Fig. 2(ii)].

Powder

***Magnolia officinalis* Rehd. et Wils. :** Colour brown. Stone cells numerous, some of them subsquare, elongated-rounded or ovate, 7-65 μm in diameter, and some of the cells irregularly branched, nearly up to 300 μm long, sometimes pit canals and striations visible. Fibres numerous, rather long, mostly broken, with strongly thickened wall, lignified; bright polychrome when observed under the polarized microscope. Oil cells ellipsoid or subround, containing yellowish-brown oily contents. Cork cells yellowish-brown, polygonal in surface view. Prismatic or polyhedral crystals of calcium oxalate rare [Fig. 3(i)].

***Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. :** Colour brown. Stone cells numerous, some of them sub-square, elongated-rounded or ovate, 9-50 µm in diameter, some others irregularly branched, nearly up to 220 µm long, sometimes pit canals and striations visible. Fibres mostly broken, with strongly thickened wall, lignified; bright polychrome when observed under the polarized microscope. Oil cells few, ellipsoid or subround, containing yellowish-brown oily contents. Cork cells yellowish-brown, polygonal in surface view. Prismatic or polyhedral crystals of calcium oxalate rare [Fig. 3(ii)].

4.2 Physicochemical Identification

Procedure

Weigh 0.5 g of the powdered sample and put into a test tube, then add 4 mL of ethanol. Heat the mixture in a water bath (80-90˚C) for 5 min. Cool down to room temperature. Filter and transfer the filtrate to a test tube. Add two drops of aqueous iron (III) chloride solution (9%, w/v). A dark green solution is observed.

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

Standard solutions

Magnolol standard solution

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

Honokiol standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane, toluene and ethyl acetate (5:4:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

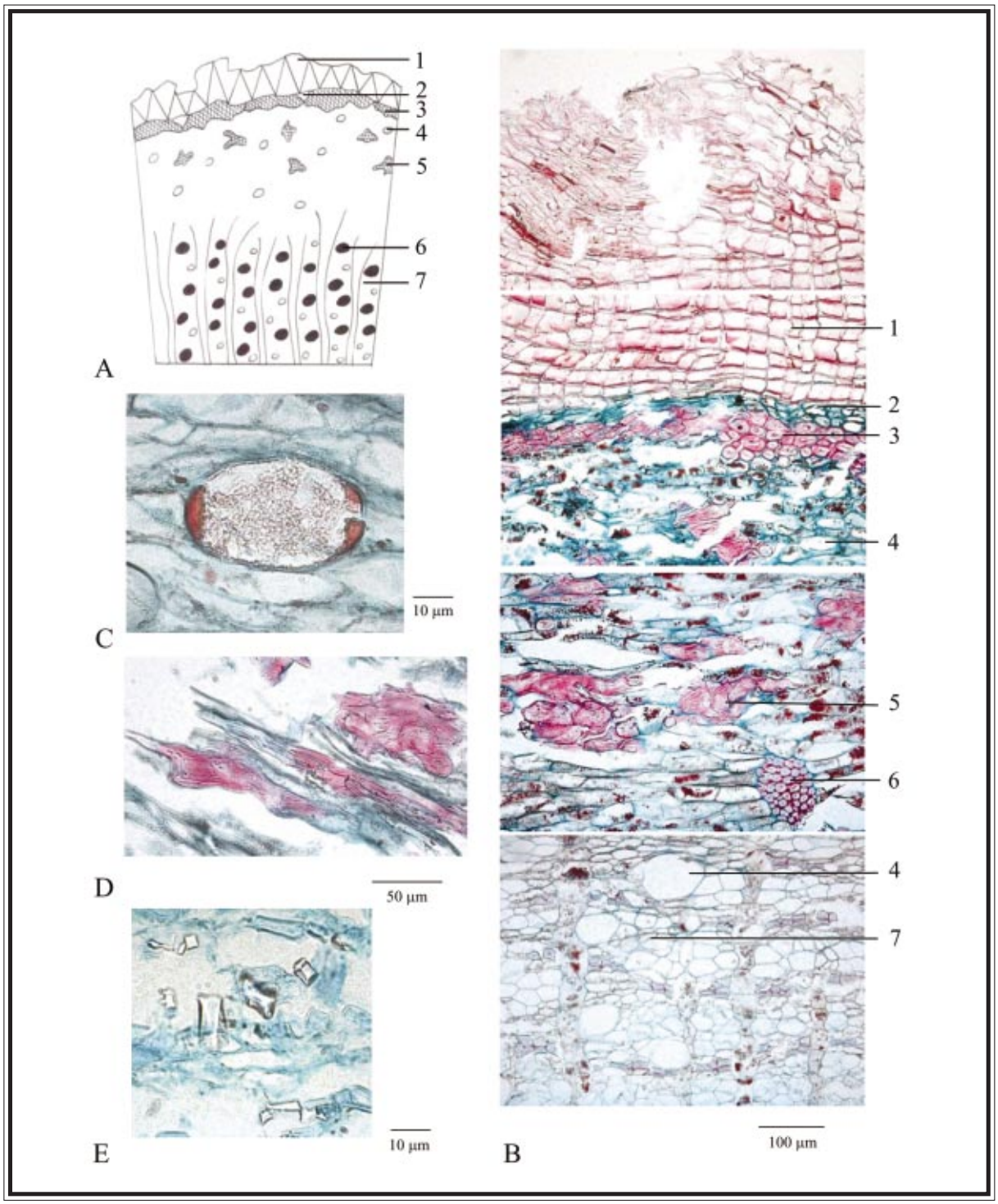


Figure 2(i) Microscopic features of transverse section of dried stem bark of *Magnolia officinalis* Rehd. et Wils.

A. Sketch B. Section illustration C. Oil cell D. Stone cells E. Crystals of calcium oxalate

1. Cork 2. Cortex 3. The ring of stone cells 4. Oil cells 5. Stone cells 6. Fibre bundles 7. Phloem

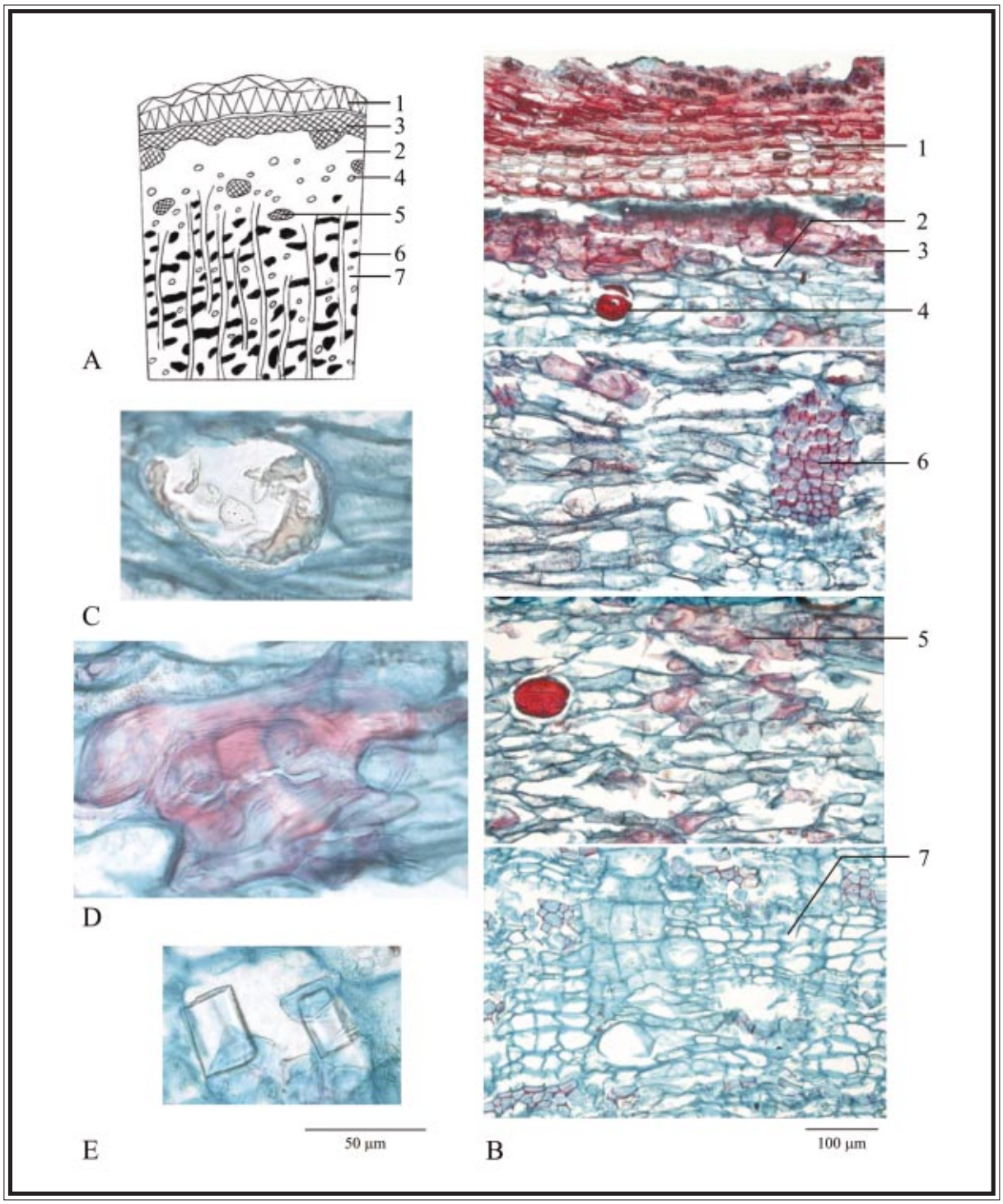


Figure 2(ii) Microscopic features of transverse section of dried stem bark of *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils.

A. Sketch B. Section illustration C. Oil cell D. Stone cells E. Crystals of calcium oxalate

1. Cork 2. Cortex 3. The ring of stone cells 4. Oil cells 5. Stone cells 6. Fibre bundles 7. Phloem

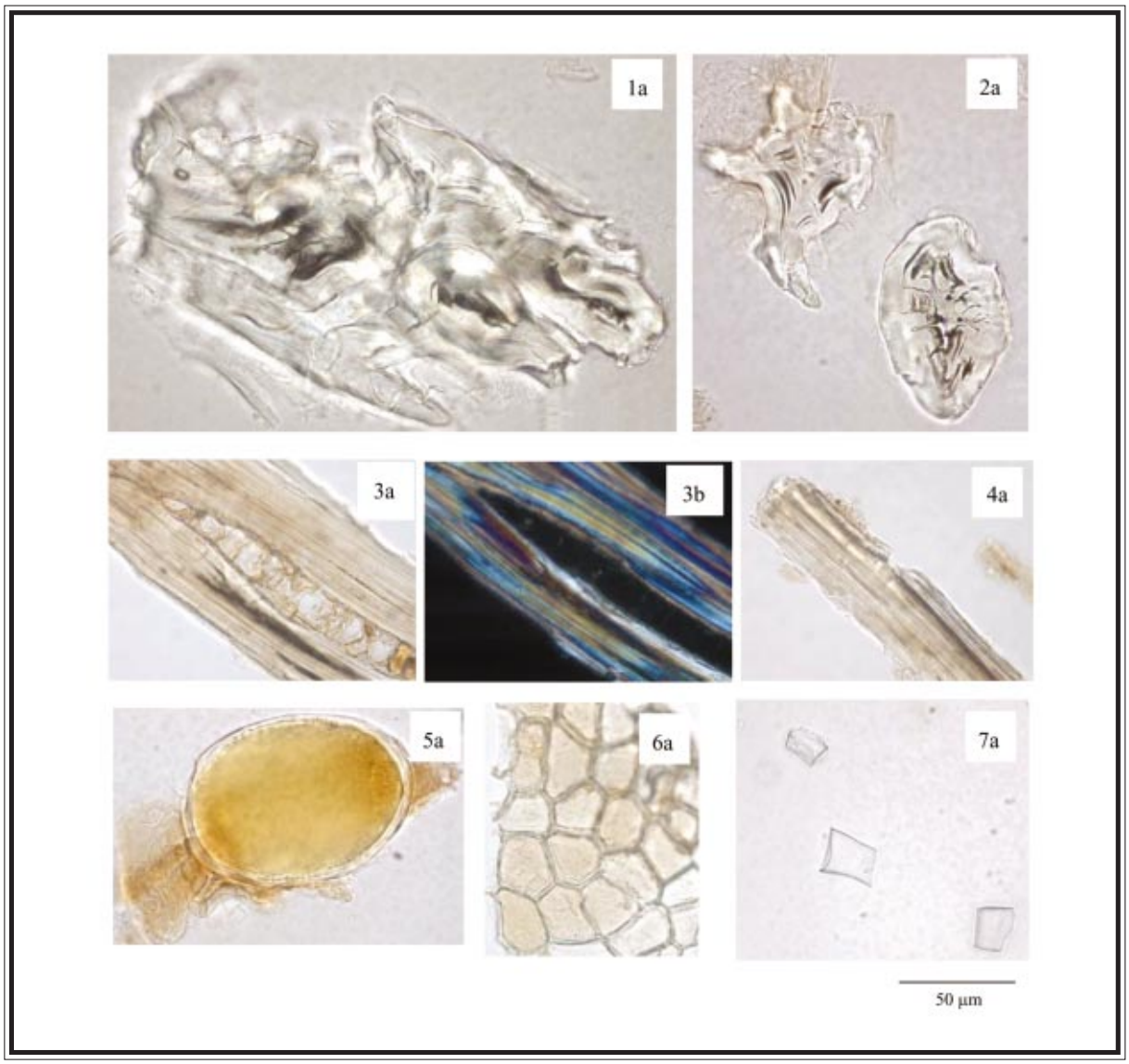


Figure 3(i) Microscopic features of powder of dried stem bark of *Magnolia officinalis* Rehd. et Wils.

1. Group of large irregular stone cells 2. Stone cells 3. Fibre bundles alternated with parenchyma ray cells 4. Fibre bundles 5. Oil cell 6. Cork cells 7. Crystals of calcium oxalate

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

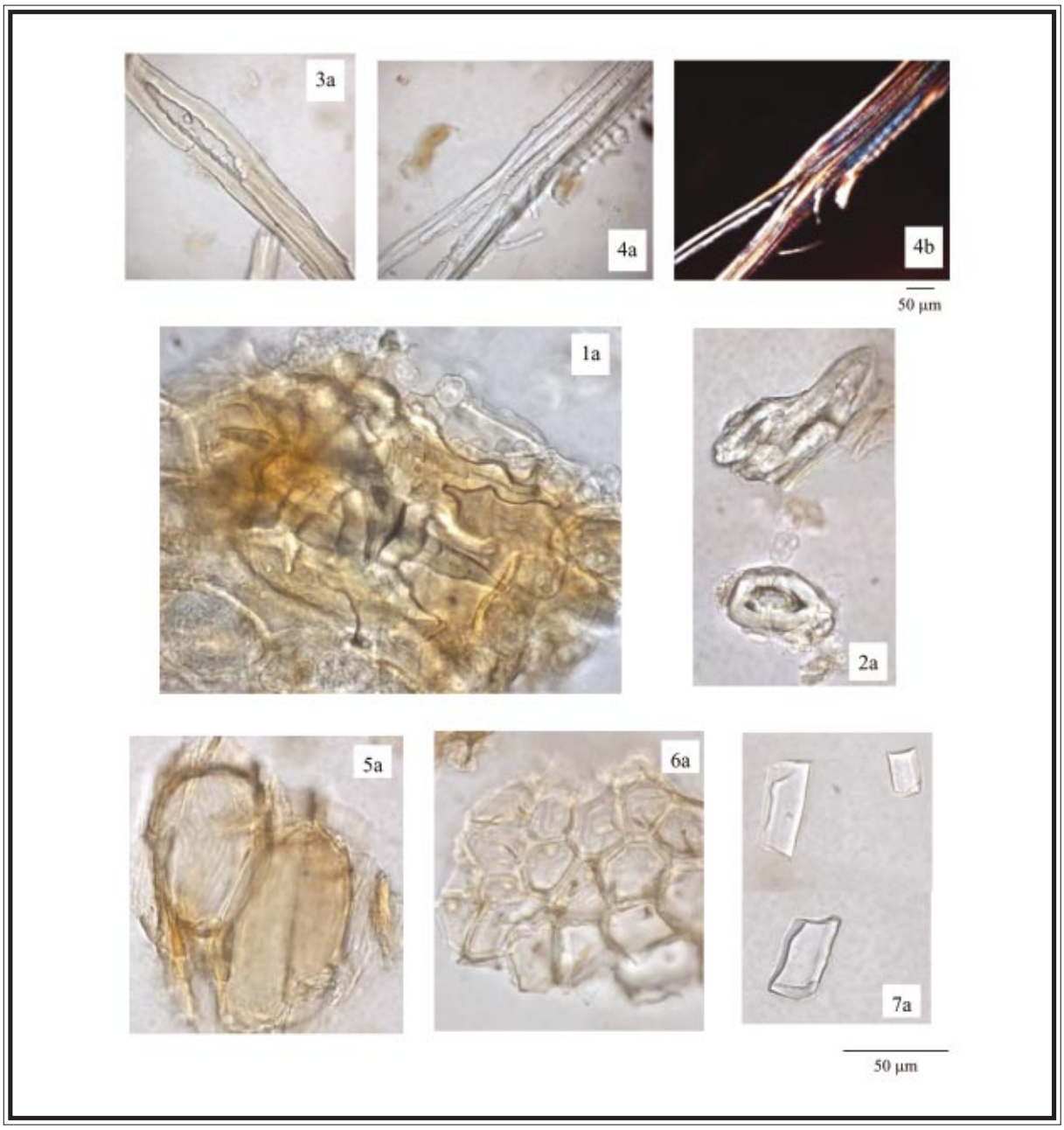


Figure 3(ii) Microscopic features of powder of dried stem bark of *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils.

1. Group of large irregular stone cells 2. Stone cells 3. Fibre bundles alternated with parenchyma ray cells 4. Fibre bundles 5. Oil cells 6. Cork cells 7. Crystals of calcium oxalate

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

Test solution

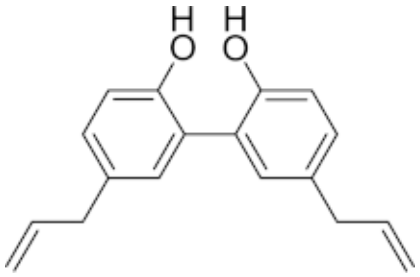
Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to another tube. Evaporate the solvent to dryness with a gentle stream of nitrogen. Dissolve 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 magnolol standard solution, honokiol standard solution (2 µL each) and the test solution (0.5-1 µL) 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. Spray the plate evenly with the spray reagent and heat at about 105°C until the spots or bands become visible (about 5-10 min). Examine the plate under UV light (254 nm) and 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 magnolol and honokiol.

(i)



(ii)

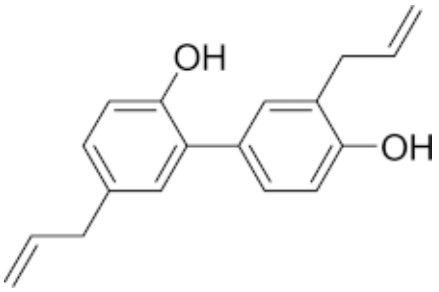


Figure 4 Chemical structures of (i) magnolol and (ii) honokiol

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

Standard solution

Magnolol standard solution for fingerprinting, Std-FP (500 mg/L)

Weigh 2.5 mg of magnolol CRS and dissolve in 5 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (320 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 –

Time (min)	0.4% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	50	50	isocratic
20 – 55	50 → 0	50 → 100	linear gradient
55 – 60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 20 μL of magnolol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of magnolol should not be more than 3.0%; the RSD of the retention time of magnolol peak should not be more than 2.0%; the column efficiency determined from magnolol peak should not be less than 80000 theoretical plates.

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

Procedure

Separately inject magnolol Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of magnolol peak in the chromatogram of magnolol Std-FP and the retention times of the six characteristic peaks [Fig. 5(i) or (ii)] in the chromatogram of the test solution. Under the same HPLC conditions, identify magnolol peak in

the chromatogram of the test solution by comparing its retention time with that in the chromatogram of magnolol Std-FP. The retention times of magnolol 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 six characteristic peaks of Cortex Magnoliae Officinalis extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the six characteristic peaks of Cortex Magnoliae Officinalis extract

Peak No.	RRT	Acceptable Range
1	0.24	±0.02
2	0.31	±0.02
3	0.40	±0.03
4 (honokiol)	0.84	±0.03
5	0.90	±0.03
6 (marker, magnolol)	1.00	-

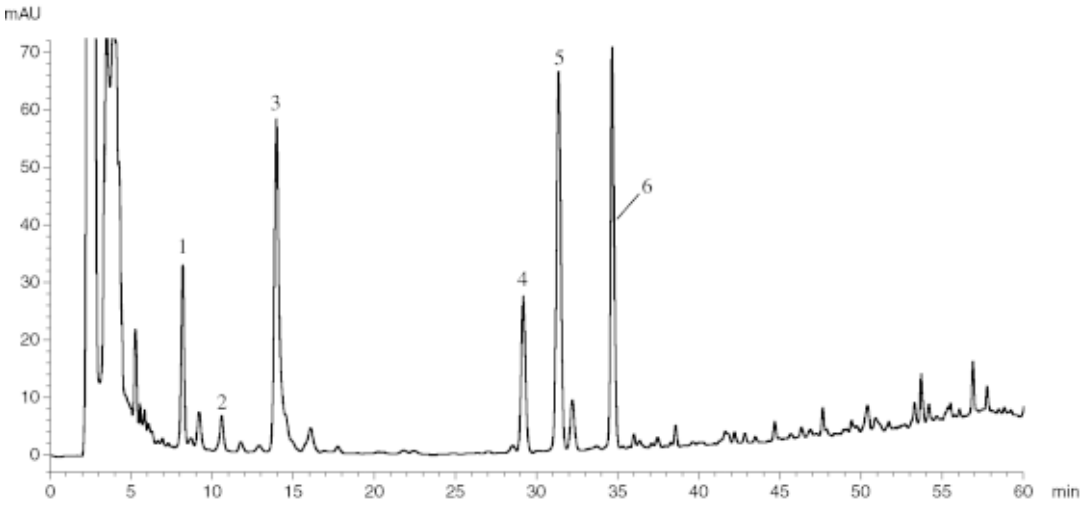


Figure 5(i) A reference fingerprint chromatogram of dried stem bark of *Magnolia officinalis* Rehd. et Wils. extract

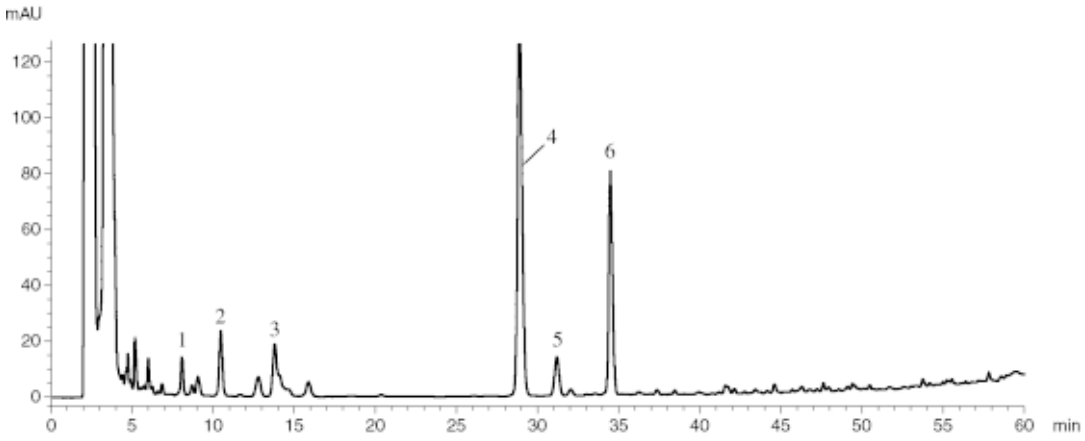


Figure 5(ii) A reference fingerprint chromatogram of dried stem bark of *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. extract

For positive identification, the sample must give the above six characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram [Fig. 5(i) or (ii)].

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 8.0%.
Acid-insoluble ash: not more than 3.5%.

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

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 3.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

Mixed magnolol and honokiol standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 10.0 mg of magnolol CRS and 10.0 mg of honokiol CRS, and dissolve in 10 mL of methanol.

Mixed magnolol and honokiol standard solution for assay, Std-AS

Measure accurately the volume of the mixed magnolol and honokiol Std-Stock, dilute with methanol to produce a series of solutions of 1, 10, 30, 60, 100 mg/L for both magnolol and honokiol.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of methanol. Sonicate (490 W) the mixture for 15 min. Centrifuge at about 1800×*g* for 5 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction twice. Combine the extracts and make up to the mark with methanol. Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (294 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 0.4% formic acid (65:35, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections each with 20 μL of the mixed magnolol and honokiol Std-AS (30 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak area of magnolol should not be more than 3.0%; the RSD of the retention time of magnolol peak should not be more than 2.0%; the column efficiency determined from magnolol peak should not be less than 10000 theoretical plates.

The *R* value between magnolol peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

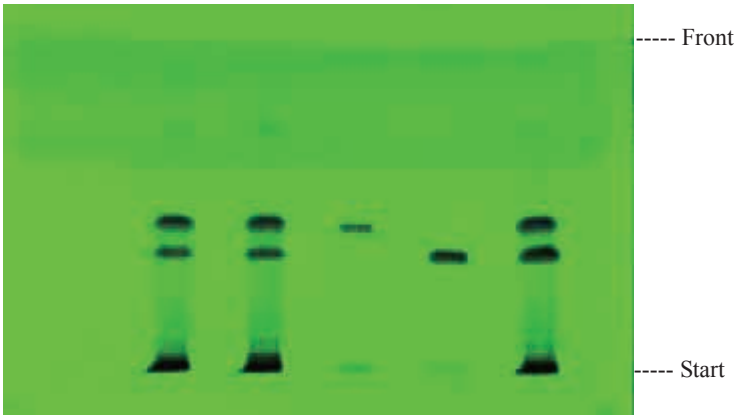
Inject a series of mixed magnolol and honokiol Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of magnolol and honokiol against the corresponding concentrations of mixed magnolol and honokiol Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify magnolol peak and honokiol peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of mixed magnolol and honokiol Std-AS. The retention times of magnolol peaks and honokiol peaks from the two chromatograms should not differ from their counterparts by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of magnolol and honokiol in the test solution, and calculate the percentage contents of magnolol and honokiol in the sample by using the equations indicated in Appendix IV(B).

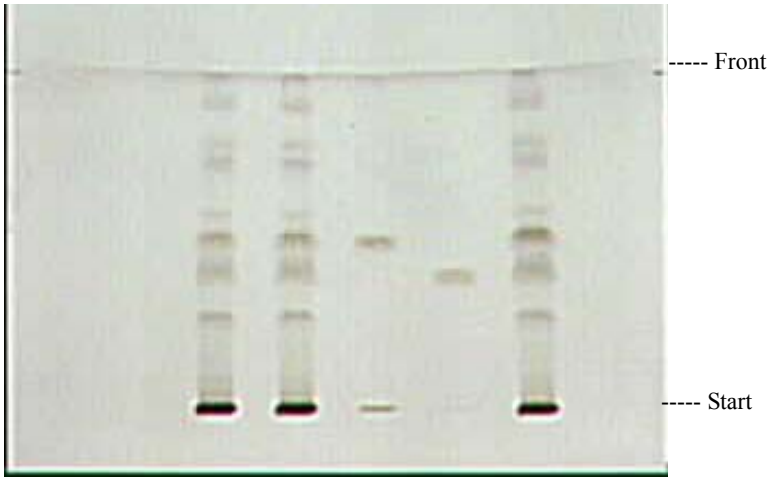
Limits

The sample contains not less than 2.0% of the total content of magnolol ($C_{18}H_{18}O_2$) and honokiol ($C_{18}H_{18}O_2$), calculated with reference to the dried substance.



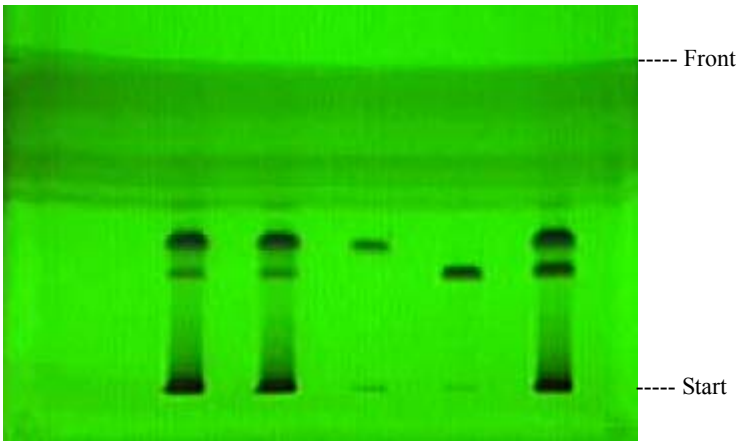
Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (*Magnolia officinalis* Rehd. et Wils.)	Magnolol and honokiol positive
3	Sample duplicate (*Magnolia officinalis* Rehd. et Wils.)	Magnolol and honokiol positive
4	Standard (Magnolol)	Magnolol positive
5	Standard (Honokiol)	Honokiol positive
6	Spiked sample (Sample plus magnolol and honokiol)	Magnolol and honokiol positive

Figure 1 TLC results of stem bark of *Magnolia officinalis* Rehd. et Wils. extract observed under UV light (254 nm) after staining



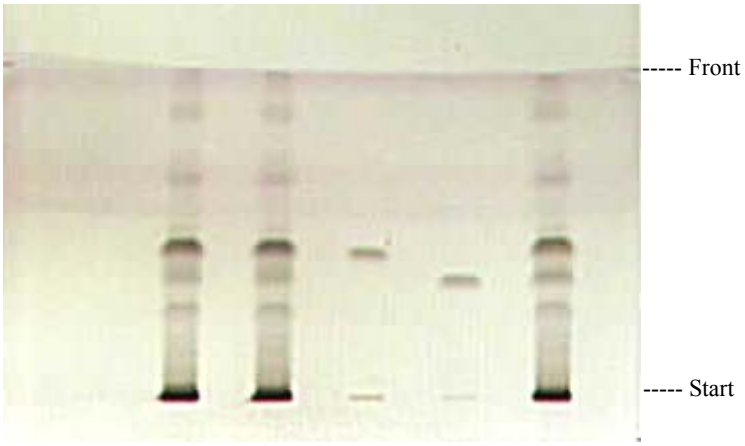
Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (*Magnolia officinalis* Rehd. et Wils.)	Magnolol and honokiol positive
3	Sample duplicate (*Magnolia officinalis* Rehd. et Wils.)	Magnolol and honokiol positive
4	Standard (Magnolol)	Magnolol positive
5	Standard (Honokiol)	Honokiol positive
6	Spiked sample (Sample plus magnolol and honokiol)	Magnolol and honokiol positive

Figure 2 TLC results of stem bark of *Magnolia officinalis* Rehd. et Wils. extract observed under visible light after staining



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (*Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils.)	Magnolol and honokiol positive
3	Sample duplicate (*Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils.)	Magnolol and honokiol positive
4	Standard (Magnolol)	Magnolol positive
5	Standard (Honokiol)	Honokiol positive
6	Spiked sample (Sample plus magnolol and honokiol)	Magnolol and honokiol positive

Figure 3 TLC results of stem bark of *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. extract observed under UV light (254 nm) after staining



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Sample (*Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils.)	Magnolol and honokiol positive
3	Sample duplicate (*Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils.)	Magnolol and honokiol positive
4	Standard (Magnolol)	Magnolol positive
5	Standard (Honokiol)	Honokiol positive
6	Spiked sample (Sample plus magnolol and honokiol)	Magnolol and honokiol positive

Figure 4 TLC results of stem bark of *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. extract observed under visible light after staining