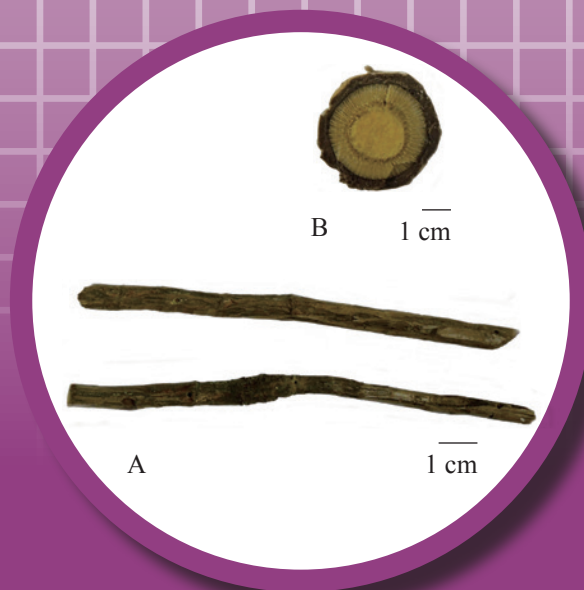


# Mahoniae Caulis



**Figure 1 (i)** A photograph of the dried stem of *Mahonia bealei* (Fort.) Carr.

A. Stems B. Magnified image of transverse section of stem



**Figure 1 (ii)** A photograph of the dried stem of *Mahonia fortunei* (Lindl.) Fedde

A. Stems B. Magnified image of transverse section of stem

## 1. NAMES

Official Name: Mahoniae Caulis

Chinese Name: 功勞木

Chinese Phonetic Name: Gonglaomu

## 2. SOURCE

Mahoniae Caulis is the dried stem of *Mahonia bealei* (Fort.) Carr. or *Mahonia fortunei* (Lindl.) Fedde (Berberidaceae). The stems are collected all year round, leaves and foreign matter removed, then dried under the sun to obtain Mahoniae Caulis.

## 3. DESCRIPTION

***Mahonia bealei* (Fort.) Carr.:** Cylindrical in shape, varying in length, 6-40 mm in diameter, externally greyish-brown or brown, with distinct longitudinal furrows and fine transverse fissures, sometimes outer bark relatively smooth. Texture hard. In transverse section, cortex narrow, easily stripped with the fibres, xylem yellow, several concentric rings and closely arranged radial striations. Deeper colour in pith, ray white and distinct. Odour slight; taste bitter [Fig. 1 (i)].

***Mahonia fortunei* (Lindl.) Fedde:** 3-12 mm in diameter, with fine longitudinal furrows [Fig. 1 (ii)].

## 4. IDENTIFICATION

### 4.1 Microscopic Identification (Appendix III)

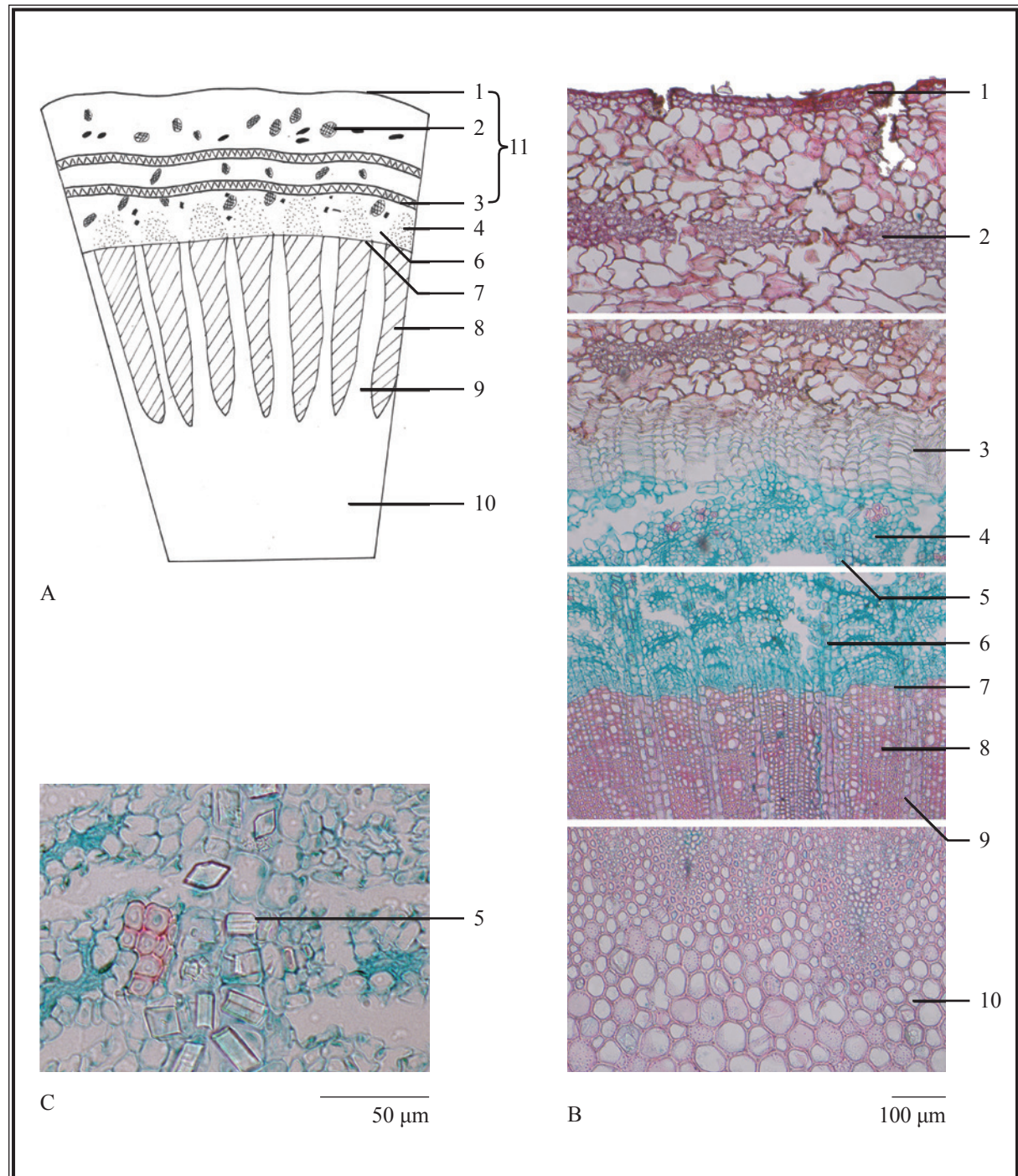
#### Transverse section

***Mahonia bealei* (Fort.) Carr.:** Epidermis consists of 1 layer of cells, covered with cuticle. Rhytidome scattered with numerous fibre bundles and 2 cork layers. Phloem narrow, with fibre bundles located at the outer side. Phloem ray cells contain prisms of calcium oxalate. Cambium in a ring. Xylem broad, vessels occurring singly or in groups, arranged radially. Xylem ray consists of 2-4 rows of cells. Pith composes of parenchymatous cells, prisms of calcium oxalate occasionally found [Fig. 2 (i)].

***Mahonia fortunei* (Lindl.) Fedde:** Rhytidome narrow, with 1 cork layer inside. Phloem fibre bundles and prisms of calcium oxalate relatively few [Fig. 2 (ii)].

**Powder**

Colour yellowish-brown to brown. Phloem fibres scattered singly or in bundles, yellow, 9-40  $\mu\text{m}$  in diameter, pit canals distinct, walls relatively thickened and lignified; yellowish-green under the polarized microscope. Stone cells fairly abundant, occasionally adhere with cork cells, polygonal, subrounded or subrectangular, 16-88  $\mu\text{m}$  in diameter, walls extremely thickened. Xylem fibres scattered or in bundles, 7-38  $\mu\text{m}$  in diameter, walls thickened; pale yellowish-green to greenish-white under the polarized microscope. Bordered-pitted vessels 7-40  $\mu\text{m}$  in diameter. Prisms of calcium oxalate numerous, 7-50  $\mu\text{m}$  in diameter; polychromatic under the polarized microscope. Cork cells subpolygonal to subrectangular [Fig. 3 (i) and (ii)].

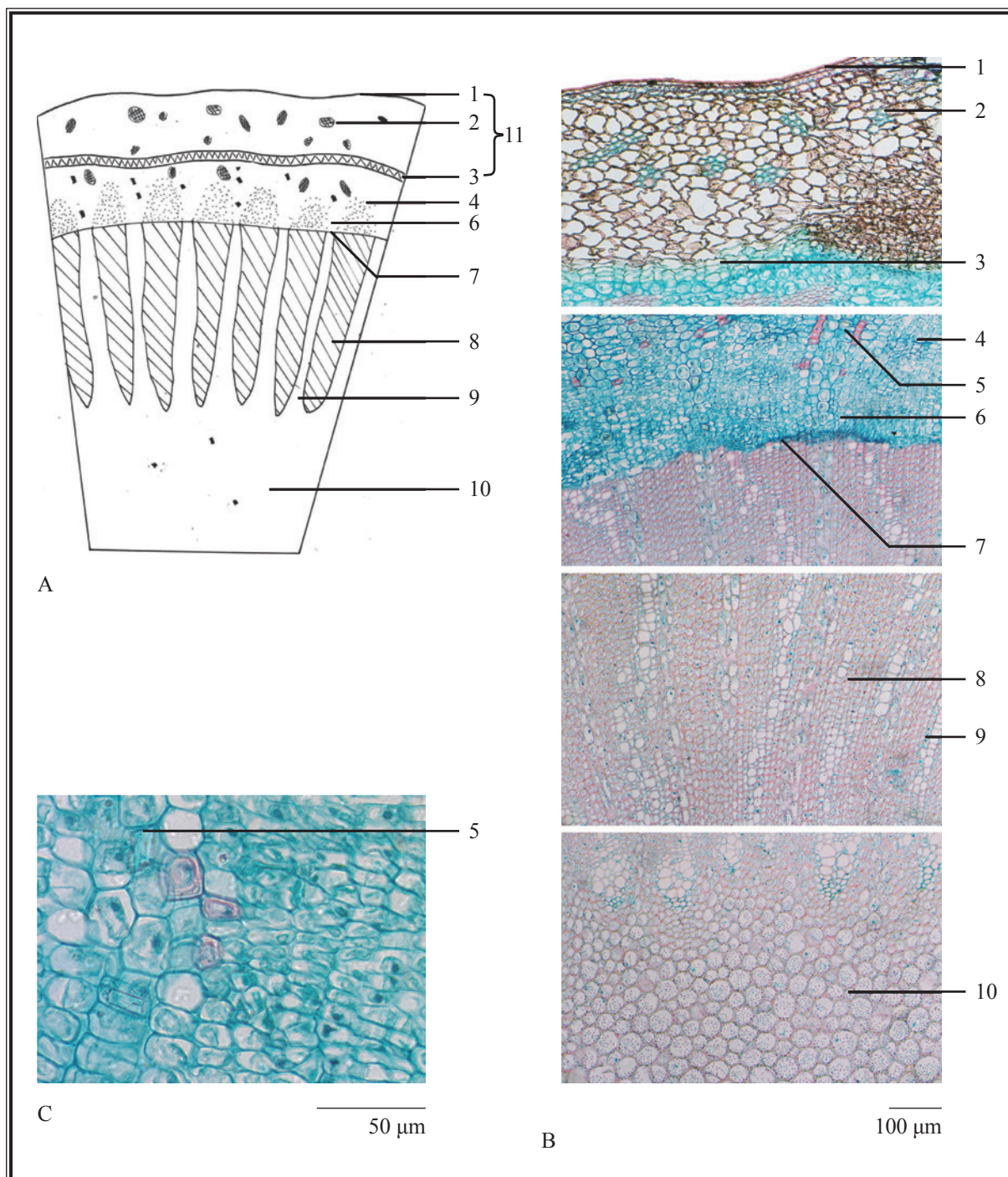


**Figure 2 (i)** Microscopic features of transverse section of dried stem of *Mahonia bealei* (Fort.) Carr.

A. Sketch B. Section illustration C. Prisms of calcium oxalate

1. Epidermis 2. Fibre bundles 3. Cork layer 4. Phloem 5. Prisms of calcium oxalate 6. Phloem ray  
7. Cambium 8. Xylem 9. Xylem ray 10. Pith 11. Rhytidome

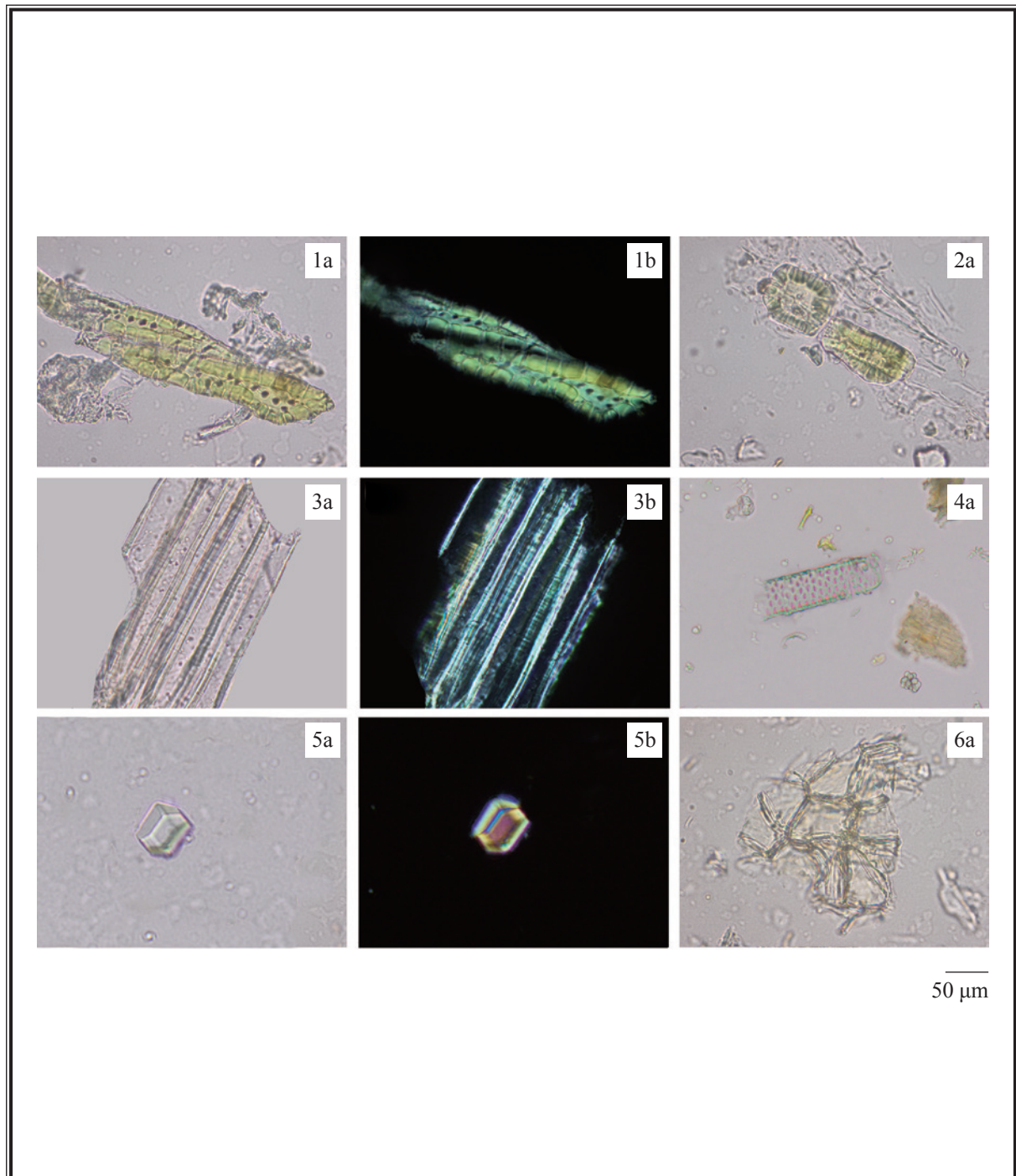




**Figure 2 (ii)** Microscopic features of transverse section of dried stem of *Mahonia fortunei* (Lindl.) Fedde

A. Sketch B. Section illustration C. Prisms of calcium oxalate

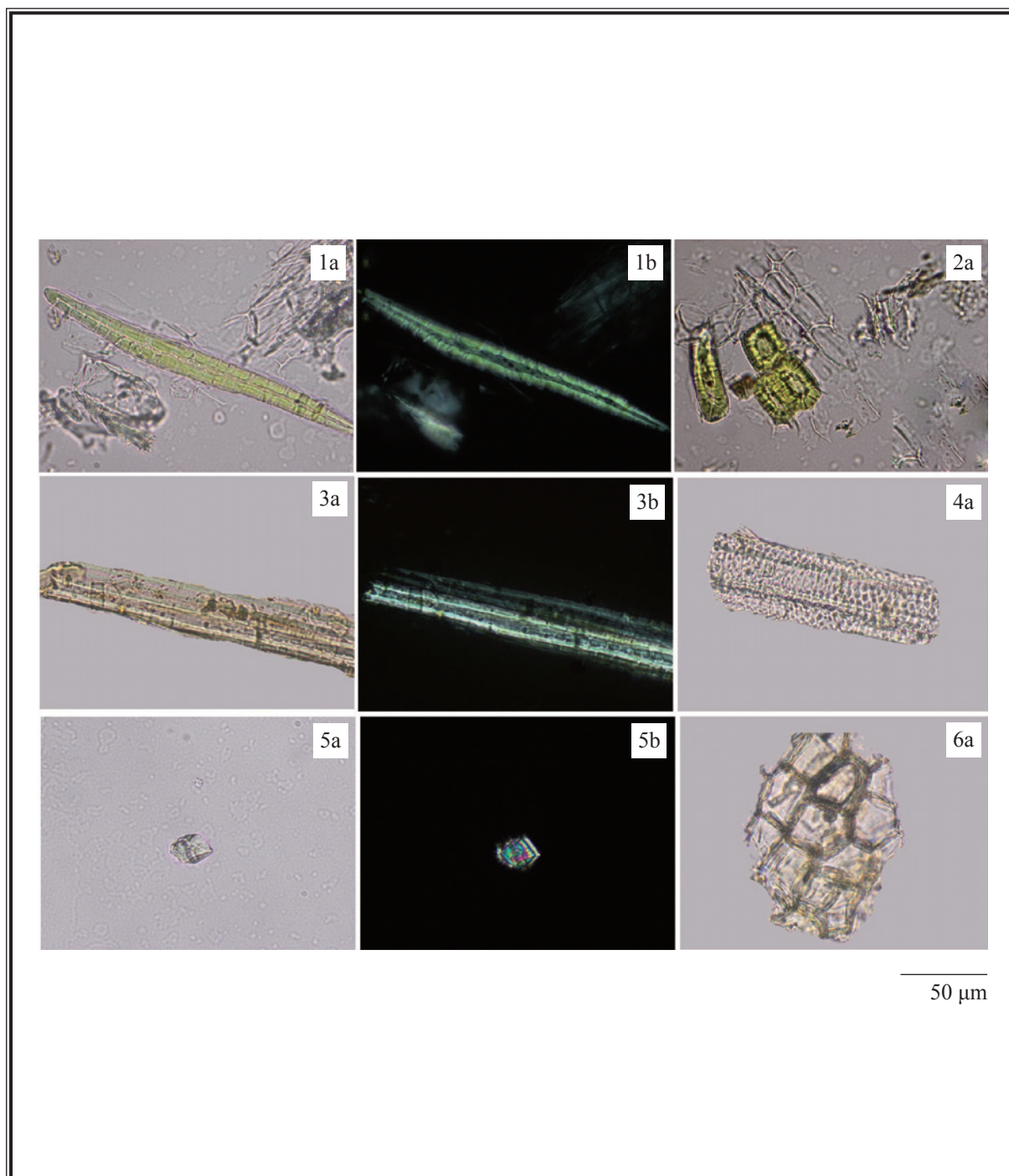
1. Epidermis 2. Fibre bundles 3. Cork layer 4. Phloem 5. Prisms of calcium oxalate 6. Phloem ray  
7. Cambium 8. Xylem 9. Xylem ray 10. Pith 11. Rhytidome



**Figure 3 (i)** Microscopic features of powder of dried stem of *Mahonia bealei* (Fort.) Carr.

1. Phloem fibres    2. Stone cells    3. Xylem fibres    4. Vessel    5. Prisms of calcium oxalate    6. Cork cells  
 a. Features under the light microscope    b. Features under the polarized microscope





**Figure 3 (ii)** Microscopic features of powder of dried stem of *Mahonia fortunei* (Lindl.) Fedde

1. Phloem fibre    2. Stone cells    3. Xylem fibres    4. Vessel    5. Prism of calcium oxalate    6. Cork cells  
 a. Features under the light microscope    b. Features under the polarized microscope

Footnote: Microscopic features of powder have no significant differences between the dried stem of *Mahonia bealei* (Fort.) Carr. and *Mahonia fortunei* (Lindl.) Fedde

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

### Standard solutions

#### *Berberine chloride standard solution*

Weigh 1.0 mg of berberine chloride CRS (Fig. 4) and dissolve in 10 mL of ethanol (90%).

#### *Jatrorrhizine chloride standard solution*

Weigh 10.0 mg of jatrorrhizine chloride CRS (Fig. 4) and dissolve in 10 mL of ethanol (90%).

#### *Palmatine chloride standard solution*

Weigh 1.0 mg of palmatine chloride CRS (Fig. 4) and dissolve in 10 mL of ethanol (90%).

### Developing solvent system

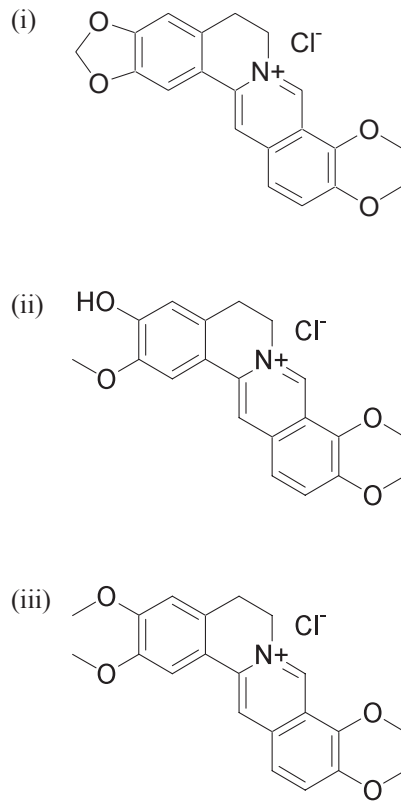
Prepare a mixture of ethyl acetate, ethanol, ammonium hydroxide solution (25%, v/v) and water (10:3.5:3.5:1, v/v).

### Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of ethanol (90%). Sonicate (350 W) the mixture for 15 min. Centrifuge at about  $4000 \times g$  for 5 min. Filter through a 0.45- $\mu\text{m}$  nylon filter. Transfer the filtrate to a 5-mL volumetric flask and make up to the mark with ethanol (90%).

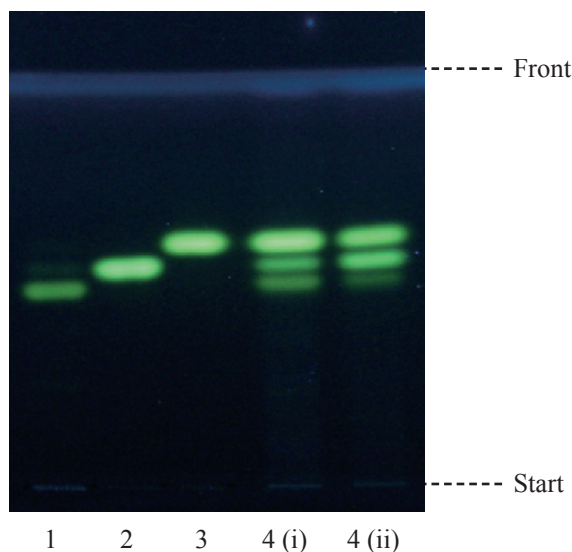
### Procedure

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 berberine chloride standard solution (1  $\mu\text{L}$ ), jatrorrhizine chloride standard solution (2  $\mu\text{L}$ ), palmatine chloride standard solution (1  $\mu\text{L}$ ) and the test solution (1  $\mu\text{L}$ ) 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 30 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 6 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 nm). Calculate the  $R_f$  values by using the equation as indicated in Appendix IV (A).



**Figure 4** Chemical structures of (i) berberine chloride (ii) jatrorrhizine chloride and (iii) palmatine chloride





**Figure 5** A reference HPTLC chromatogram of Mahoniae Caulis extract observed under UV light (366 nm)

1. Jatrorrhizine chloride standard solution
2. Palmatine chloride standard solution
3. Berberine chloride standard solution
4. Test solution of
  - (i) dried stem of *Mahonia fortunei* (Lindl.) Fedde
  - (ii) dried stem of *Mahonia bealei* (Fort.) Carr.

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 berberine, jatrorrhizine and palmatine (Fig. 5).

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

#### Standard solutions

*Berberine chloride standard solution for fingerprinting, Std-FP (20 mg/L)*

Weigh 0.2 mg of berberine chloride CRS and dissolve in 10 mL of methanol (80%).

*Jatrorrhizine chloride standard solution for fingerprinting, Std-FP (25 mg/L)*

Weigh 0.25 mg of jatrorrhizine chloride CRS and dissolve in 10 mL of methanol (80%).

*Palmatine chloride standard solution for fingerprinting, Std-FP (5 mg/L)*

Weigh 0.25 mg of palmatine chloride CRS and dissolve in 50 mL of methanol (80%).

#### Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (80%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about  $4000 \times g$  for

10 min. Pipette 1 mL of supernatant to a 2-mL volumetric flask and make up to the mark with methanol (80%). Filter through a 0.45- $\mu$ m nylon filter.

### Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu$ m 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)	0.5% Triethylamine* (% v/v)	Acetonitrile (% v/v)	Elution
0 – 20	73 $\rightarrow$ 70	27 $\rightarrow$ 30	linear gradient

\*Adjust the pH to 2.5 with phosphoric acid

### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of berberine chloride Std-FP, jatrorrhizine chloride Std-FP and palmatine chloride Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of berberine, jatrorrhizine and palmatine should not be more than 5.0%; the RSD of the retention times of berberine, jatrorrhizine and palmatine peaks should not be more than 2.0%; the column efficiencies determined from berberine, jatrorrhizine and palmatine peaks should not be less than 18000, 15000 and 18000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; the *R* value between peak 3 and the closest peak; and 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. 6 (i) or (ii)].

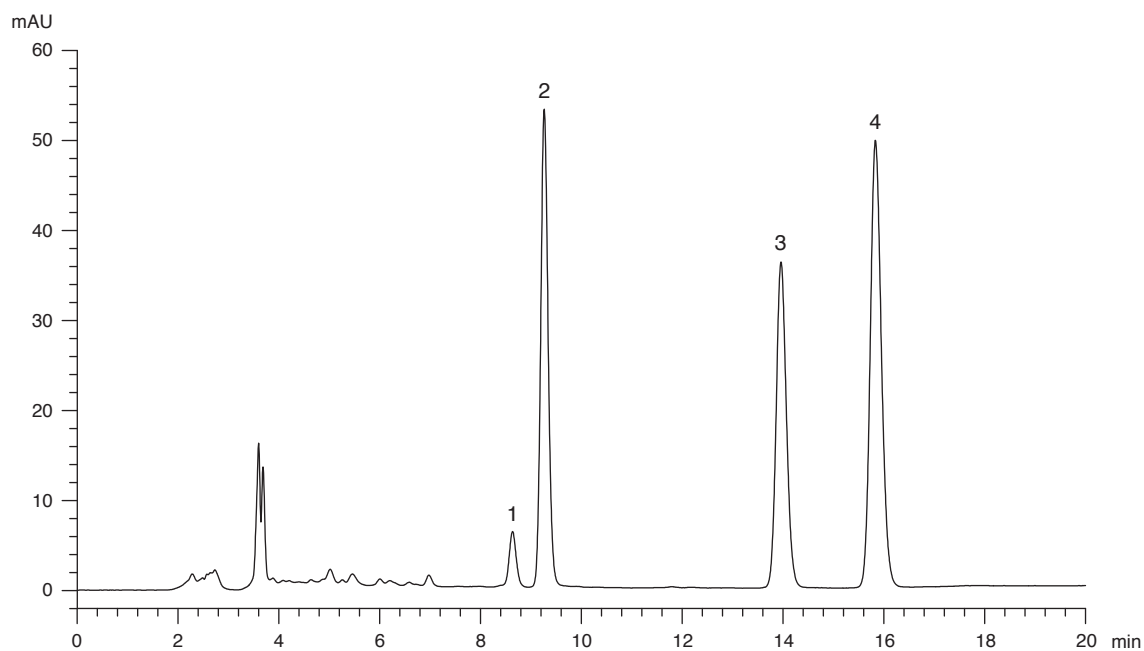
### Procedure

Separately inject berberine chloride Std-FP, jatrorrhizine chloride Std-FP, palmatine chloride Std-FP and the test solution (10  $\mu$ L each) into the HPLC system and record the chromatograms. Measure the retention times of berberine, jatrorrhizine and palmatine peaks in the chromatograms of berberine chloride Std-FP, jatrorrhizine chloride Std-FP and palmatine chloride Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify berberine, jatrorrhizine and palmatine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of berberine chloride Std-FP, jatrorrhizine chloride Std-FP and palmatine chloride Std-FP. The retention times of berberine, jatrorrhizine and palmatine peaks in the chromatograms of the test solution and the corresponding Std-FP should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

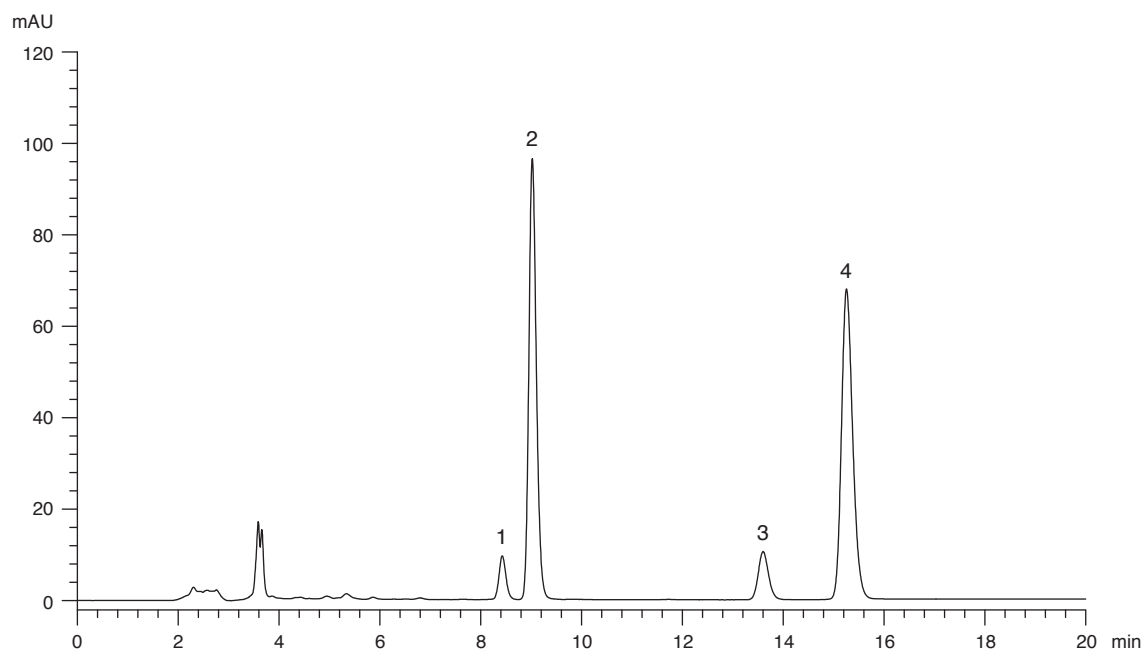
The RRTs and acceptable ranges of the four characteristic peaks of Mahoniae Caulis extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the four characteristic peaks of Mahoniae Caulis extract

Peak No.	RRT	Acceptable Range
1	0.55	$\pm 0.03$
2 (jatrorrhizine)	0.58	$\pm 0.03$
3 (palmatine)	0.88	$\pm 0.03$
4 (marker, berberine)	1.00	-



**Figure 6 (i)** A reference fingerprint chromatogram of dried stem of *Mahonia bealei* (Fort.) Carr. extract



**Figure 6 (ii)** A reference fingerprint chromatogram of dried stem of *Mahonia fortunei* (Lindl.) Fedde 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 respective reference fingerprint chromatograms [Fig. 6 (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** (*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 3.5%.

Acid-insoluble ash: not more than 1.0%.

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

Oven dried method: not more than 12.0%.

## 6. EXTRACTIVES (Appendix XI)

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

Ethanol-soluble extractives (hot extraction method): not less than 7.0%.

## 7. ASSAY

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

### Standard solution

*Mixed berberine chloride, jatrorrhizine chloride and palmatine chloride standard stock solution, Std-Stock (300 mg/L for berberine chloride, 220 mg/L for jatrorrhizine chloride and 260 mg/L for palmatine chloride)*

Weigh accurately 3.0 mg of berberine chloride CRS, 2.2 mg of jatrorrhizine chloride CRS and 2.6 mg of palmatine chloride CRS, and dissolve in 10 mL of methanol (80%).

*Mixed berberine chloride, jatrorrhizine chloride and palmatine chloride standard solution for assay, Std-AS*

Measure accurately the volume of the mixed berberine chloride, jatrorrhizine chloride and palmatine chloride Std-Stock, dilute with methanol (80%) to produce a series of solutions of 0.6, 3, 9, 37.5, 75 mg/L for berberine chloride, 0.4, 2.2, 6.6, 28, 56 mg/L for jatrorrhizine chloride and 0.5, 2.6, 7.8, 32.5, 65 mg/L for palmatine chloride.

### Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol (80%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about  $4000 \times g$  for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (80%). Filter through a 0.45- $\mu\text{m}$  nylon filter.

### Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu\text{m}$  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)	0.5% Triethylamine* (% v/v)	Acetonitrile (% v/v)	Elution
0 – 20	73 → 70	27 → 30	linear gradient

\*Adjust the pH to 2.5 with phosphoric acid

### System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed berberine chloride, jatrorrhizine chloride and palmatine chloride Std-AS (9 mg/L for berberine chloride, 6.6 mg/L for jatrorrhizine chloride and 7.8 mg/L for palmatine chloride). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of berberine, jatrorrhizine and palmatine should not be more than 5.0%; the RSD of the retention times of berberine, jatrorrhizine and palmatine peaks should not be more than 2.0%; the column efficiencies determined from berberine, jatrorrhizine and palmatine peaks should not be less than 18000, 15000 and 18000 theoretical plates respectively.

The *R* value between berberine peak and the closest peak; the *R* value between jatrorrhizine peak and the closest peak; and the *R* value between palmatine 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 the mixed berberine chloride, jatrorrhizine chloride and palmatine chloride Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of berberine, jatrorrhizine and palmatine against the corresponding concentrations of the mixed berberine chloride, jatrorrhizine chloride and palmatine chloride Std-AS. Obtain the slopes, y-intercepts and the *r*<sup>2</sup> values from the corresponding 5-point calibration curves.

### Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify berberine, jatrorrhizine and palmatine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed berberine chloride, jatrorrhizine chloride and palmatine chloride Std-AS. The retention times of berberine, jatrorrhizine and palmatine peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of berberine, jatrorrhizine and palmatine in the test solution, and calculate the percentage contents of berberine (the percentage content of berberine chloride × 0.91, where 0.91 is the molar mass ratio of berberine and berberine chloride), jatrorrhizine (the percentage content of jatrorrhizine chloride × 0.91, where 0.91 is the molar mass ratio of jatrorrhizine and jatrorrhizine chloride) and palmatine (the percentage content of palmatine chloride × 0.91, where 0.91 is the molar mass ratio of palmatine and palmatine chloride) in the sample by using the equations as indicated in Appendix IV (B).

Strychni Semen (unprocessed)

馬錢子(生)

Ginseng Folium

人參葉

Aconiti Lateralis Radix (unprocessed) 附子(生)

Litsea Fructus

Pseudolaricis Cortex 土荊皮

Bolbostemmatis Rhizoma

Bufois Venenum 蟾酥

華澄茄

Mahoniae Caulis

橘紅

Magnoliae Officinalis Flos

土貝母

Lonicerae Japonicae Flos

功勞木

Citri Exocarpium Rubrum

厚朴花

月季花

金銀花

*Mahoniae Caulis*

Rosae Chinensis Flos

### Limits

The sample contains not less than 0.76% of the total content of berberine ( $C_{20}H_{18}NO_4$ ), jatrorrhizine ( $C_{20}H_{20}NO_4$ ) and palmatine ( $C_{21}H_{22}NO_4$ ), calculated with reference to the dried substance.