Ilicis Rotundae Cortex

Figure 1 A photograph of Ilicis Rotundae Cortex

A. Ilicis Rotundae Cortex B. Magnified image of outer surface of bark
C. Magnified image of inner surface of bark

1. NAMES

Official Name: Ilicis Rotundae Cortex

Chinese Name: 救必應

Chinese Phonetic Name: Jiubiying

2. SOURCE

Ilicis Rotundae Cortex is the dried bark of *Ilex rotunda* Thunb. (Aquifoliaceae). The bark is collected in summer and autumn, then dried under the sun to obtain Ilicis Rotundae Cortex.

3. DESCRIPTION

Quilled, semi-quilled or slightly curved flat pieces, varying in size, 1.5-25.7 cm long, 0.4-8.2 cm wide, the bark 1-25 mm thick. Outer surface greyish-white to greyish-brown, rough, usually wrinkled. Inner surface yellowish-brown to blackish-brown, with shallow longitudinal striations. Texture hard and brittle. Fracture slightly even. Odour slightly fragrant; taste bitter and slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 5-12 layers of tangentially prolonged cells. Stone cells arranged singly or in groups, scattered in cortex, formed an interrupted ring. Phloem broad, scattered with stone cells and prisms of calcium oxalate. Phloem rays consist of 1-5 rows of radially prolonged cells. Prisms of calcium oxalate present in the parenchymatous cells of cortex and phloem (Fig. 2).

Powder

Colour pale brown to brown. Stone cells numerous, scattered singly or in groups, pale yellowish-green or pale yellow, subsquare, elliptical or irregular in shape, 13-138 μm in diameter, pit canals obvious, some contain prisms of calcium oxalate; prisms of calcium oxalate polychromatic under the polarized microscope. Prisms of calcium oxalate numerous, scattered in parenchymatous cells, polyhedral, square, fusiform or subbiconical, 9-58 μm long, 6-43 μm wide; polychromatic under the polarized microscope. Cork cells scattered singly or in groups, colourless or pale

brown, polygonal, walls thick, small pits canals visible. Clusters of calcium oxalate occasionally found, scattered singly. Fibres rare and broken (Fig. 3).

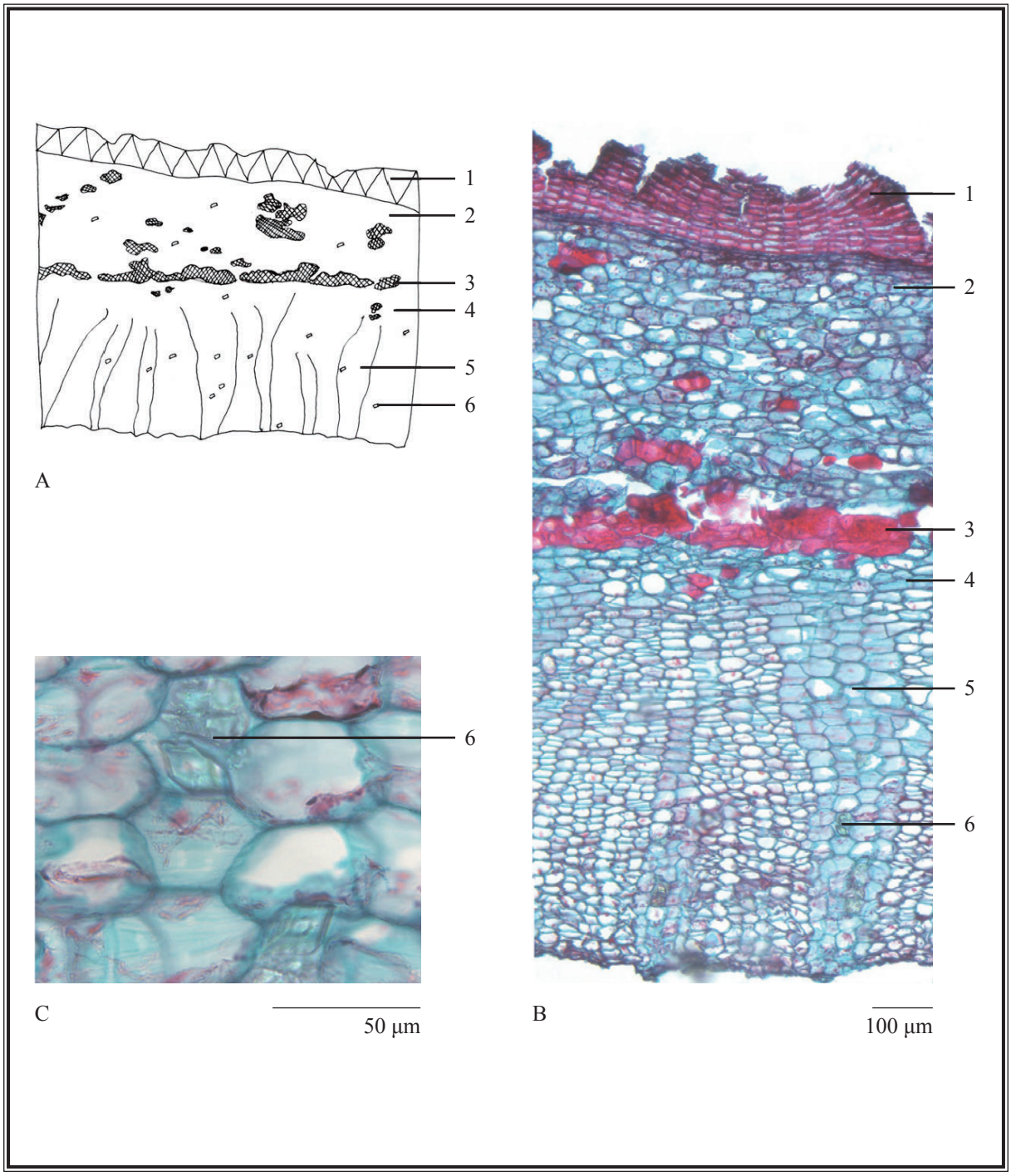


Figure 2 Microscopic features of transverse section of Ilicis Rotundae Cortex

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

1. Cork 2. Cortex 3. Stone cells 4. Phloem 5. Phloem ray 6. Prisms of calcium oxalate

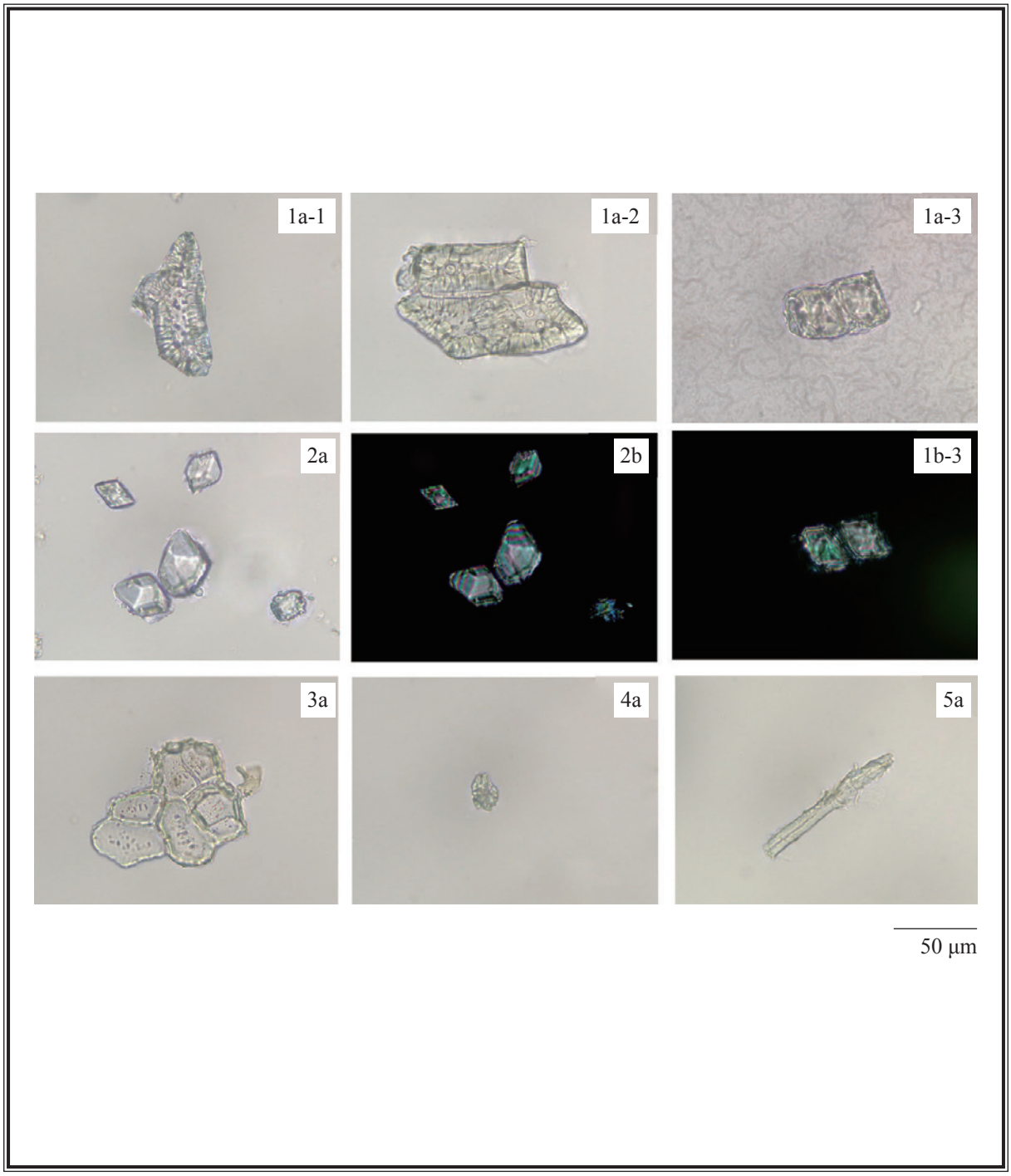


Figure 3 Microscopic features of powder of Ilicis Rotundae Cortex

1. Stone cells (1-1 scattered singly, 1-2 in group, 1-3 containing prisms of calcium oxalate)
2. Prisms of calcium oxalate 3. Cork cells 4. Cluster of calcium oxalate 5. Fibre

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

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

Standard solutions

Pedunculoside standard solution

Weigh 2.0 mg of pedunculoside CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Syringin (eleutheroside B) standard solution

Weigh 2.0 mg of syringin CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of dichloromethane, methanol, formic acid and water (8.5:1:1:0.1, v/v).

Spray reagent

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

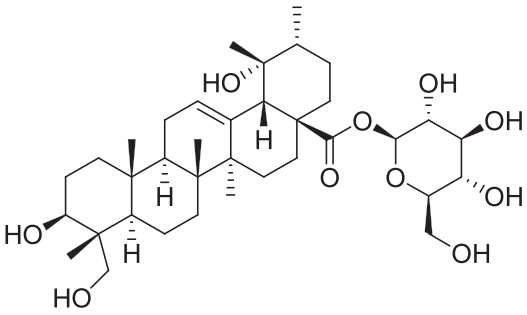
Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (150 W) the mixture for 1 h. 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 pedunculoside standard solution (4 µL), syringin standard solution (1 µL) and the test solution (2 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 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 105ºC (about 1-2 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

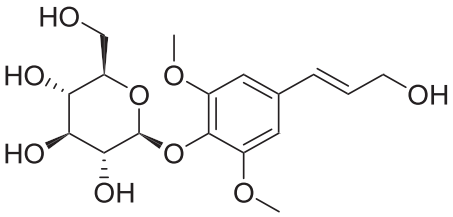


Figure 4 Chemical structures of (i) pedunculoside and (ii) syringin (eleutheroside B)

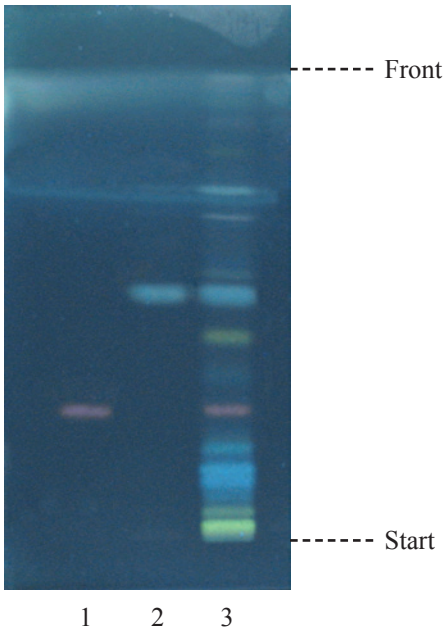


Figure 5 A reference HPTLC chromatogram of Ilicis Rotundae Cortex extract observed under UV light (366 nm) after staining

1. Syringin standard solution 2. Pedunculoside standard solution 3. Test solution

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 pedunculoside and syringin (Fig. 5).

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

Standard solutions

Pedunculoside standard solution for fingerprinting, Std-FP (340 mg/L)

Weigh 3.4 mg of pedunculoside CRS and dissolve in 10 mL of ethanol (50%).

Syringin (eleutheroside B) standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of syringin CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 40 mL of ethanol (50%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 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)	Acetonitrile (%, v/v)	0.05% Phosphoric acid (%, v/v)	Elution
0 – 5	5 → 10	95 → 90	linear gradient
5 – 20	10 → 20	90 → 80	linear gradient
20 – 30	20	80	isocratic
30 – 60	20 → 40	80 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of pedunculoside Std-FP and syringin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of pedunculoside and syringin should not be more than 5.0%; the RSD of the retention times of pedunculoside and syringin peaks should not be more than 2.0%; the column efficiencies determined from pedunculoside and syringin peaks should not be less than 400000 and 50000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and 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. 6).

Procedure

Separately inject pedunculoside Std-FP, syringin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of pedunculoside and syringin peaks in the chromatograms of pedunculoside Std-FP, syringin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify pedunculoside and syringin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of pedunculoside Std-FP and syringin Std-FP. The retention times of pedunculoside and syringin 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 five characteristic peaks of Ilicis Rotundae Cortex extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Ilicis Rotundae Cortex extract

Peak No.	RRT	Acceptable Range
1 (syringin)	0.23	± 0.03
2	0.25	± 0.03
3	0.53	± 0.04
4	0.61	± 0.05
5 (marker, pedunculoside)	1.00	-

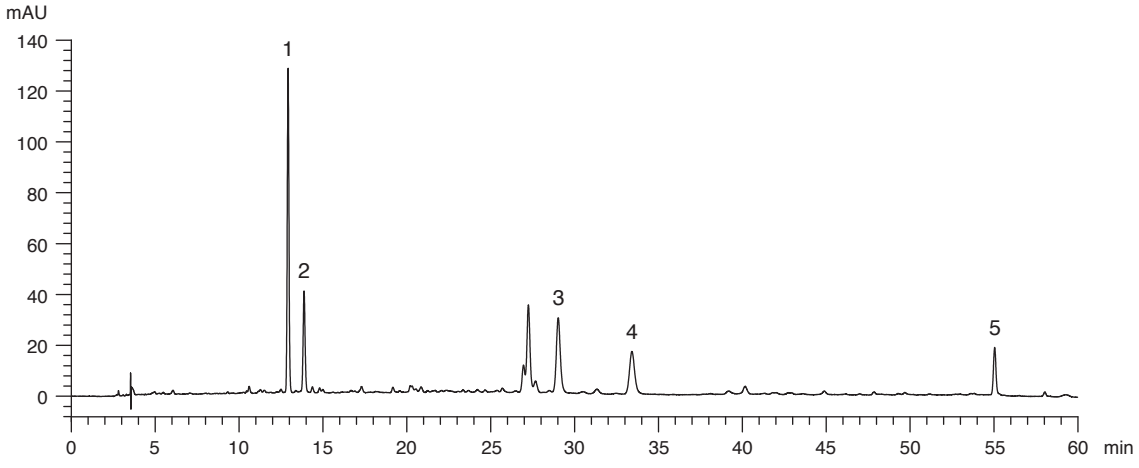


Figure 6 A reference fingerprint chromatogram of Ilicis Rotundae Cortex extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 6).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements (except cadmium should not be more than 5.5 mg/kg).

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 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 24.0%.
Ethanol-soluble extractives (cold extraction method): not less than 31.0%.

7. ASSAY

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

Standard solution

Mixed pedunculoside and syringin (eleutheroside B) standard stock solution, Std-Stock (800 mg/L for pedunculoside and 80 mg/L for syringin)

Weigh accurately 8.0 mg of pedunculoside CRS and 0.8 mg of syringin CRS, and dissolve in 10 mL of ethanol (50%).

Mixed pedunculoside and syringin standard solution for assay, Std-AS

Measure accurately the volume of the mixed pedunculoside and syringin Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 60, 80, 100, 200, 400 mg/L for pedunculoside and 8, 10, 20, 40, 60 mg/L for syringin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL round-bottomed flask, then add 20 mL of ethanol (50%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm for pedunculoside and 220 nm for syringin) 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)	Acetonitrile (%, v/v)	0.05% Phosphoric acid (%, v/v)	Elution
0 – 10	10	90	isocratic
10 – 20	10 → 31	90 → 69	linear gradient
20 – 40	31	69	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed pedunculoside and syringin Std-AS (100 mg/L for pedunculoside and 20 mg/L for syringin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of pedunculoside and syringin should not be more than 5.0%; the RSD of the retention times of pedunculoside and syringin peaks should not be more than 2.0%; the column efficiencies determined from pedunculoside and syringin peaks should not be less than 40000 and 7000 theoretical plates respectively.

The *R* value between pedunculoside peak and the closest peak; and the *R* value between syringin 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 pedunculoside and syringin Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of pedunculoside and syringin against the corresponding

concentrations of the mixed pedunculoside and syringin Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify pedunculoside and syringin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed pedunculoside and syringin Std-AS. The retention times of pedunculoside and syringin 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 pedunculoside and syringin in the test solution, and calculate the percentage contents of pedunculoside and syringin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 4.5% of pedunculoside ($C_{36}H_{58}O_{10}$) and not less than 1.0% of syringin ($C_{17}H_{24}O_9$), calculated with reference to the dried substance.

8. CAUTION

This CMM should be used after proper processing (such as decoction).