Ilicis Pubescentis Radix et Caulis

Figure 1 A photograph of Ilicis Pubescentis Radix et Caulis

A. Ilicis Pubescentis Radix et Caulis

B. Magnified image of stem in slice

C. Magnified image of root in slice

1. NAMES

Official Name: Ilicis Pubescentis Radix et Caulis

Chinese Name: 毛冬青

Chinese Phonetic Name: Maodongqing

2. SOURCE

Ilicis Pubescentis Radix et Caulis is the dried root and stem of *Ilex pubescens* Hook. & Arn. (Aquifoliaceae). The root and stem are collected all year round, washed clean, cut into sections or slices, then dried under the sun to obtain Ilicis Pubescentis Radix et Caulis.

3. DESCRIPTION

Cylindrical pieces, lumpy when cut, varying in size, 10-80 mm in diameter, 1-10 mm thick. Root externally greyish-brown to brown, slightly rough, with fine wrinkles and transverse lenticels; bark thin, sometimes sloughing off, slightly thickened in the old root; wood yellowish-white to pale yellowish-brown, striations fine and dense, fracture uneven with longitudinal striation; texture hard, uneasily broken; odour slight; taste bitter, astringent and followed by a sweet taste. Stem externally greyish-brown, slightly rough, with fine wrinkles and transverse lenticels; bark thin; wood broad, whitish to yellowish-white, xylem ray distinct, arranged radially; pith small, eccentric; texture hard, uneasily broken; odour slight; taste bitter, astringent and followed by a sweet taste (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of 5-10 layers of cells. Cortex narrow. Stone cells scattered singly or in groups in the cortex. Phloem narrow. Cambium indistinct. Xylem broad, occupying the major portion of the root, vessels scattered singly or 2-4 in a group. Xylem rays consist of 1-8 rows of cells, gradually widening outward. Annual ring distinct [Fig. 2 (i)].

Stem: Cork consists of several layers of cells, elongated tangentially, thin and flat. Cortex narrow, consisting of 4-8 layers of cells, with several stone cells scattered. Phloem relatively narrow. Cambium in a ring. Xylem broad. Xylem rays distinct, arranged radially. Pith small, eccentric. Crystals of calcium oxalate scattered; polychromatic under the polarized microscope [Fig. 2 (ii)].

Powder

Colour yellowish-white. Stone cells scattered singly or in groups, the cells subtriangular, subsquare, subrounded or irregular in shape, some lumens contain brown contents; bright yellow to brown under the polarized microscope. Xylem fibres abundant, 10-20 μm in diameter, sometimes with spiral striations; polychromatic under the polarized microscope. Xylem ray cells show distinct pit canals. Vessels mainly bordered-pitted. Simple starch granules subrounded, 5-10 μm in diameter, with pointed, cleft-like or star-like hilum; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-3 units. Prisms of calcium oxalate subrectangular or irregularly squared, 15-50 μm long; polychromatic under the polarized microscope (Fig. 3).

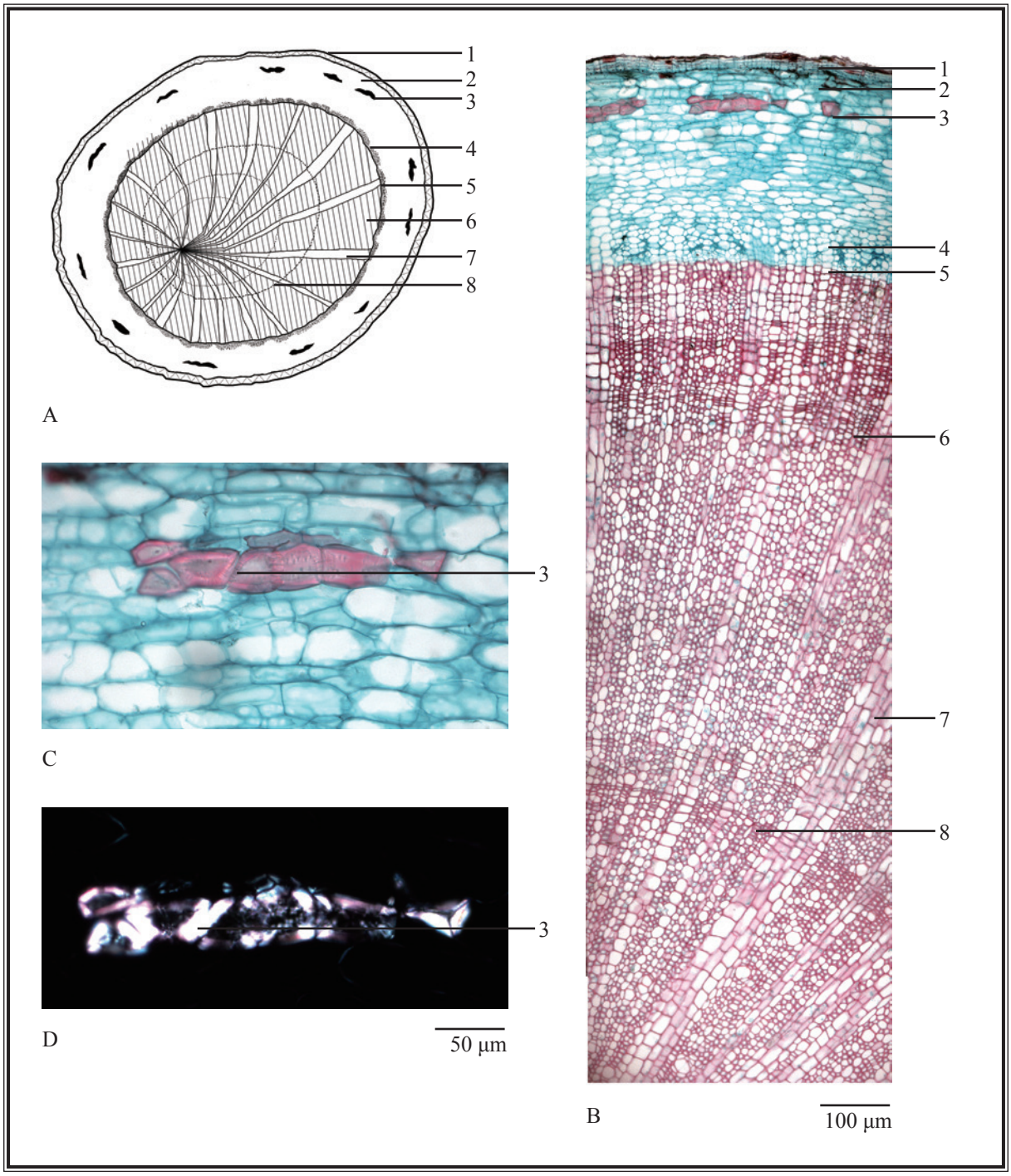

Figure 2 (i) Microscopic features of transverse section of root of Ilicis Pubescentis Radix et Caulis

A. Sketch B. Section illustration C. Stone cells (under the light microscope)
D. Stone cells (under the polarized microscope)

1. Cork 2. Cortex 3. Stone cell 4. Phloem 5. Cambium 6. Xylem 7. Xylem ray 8. Annual ring

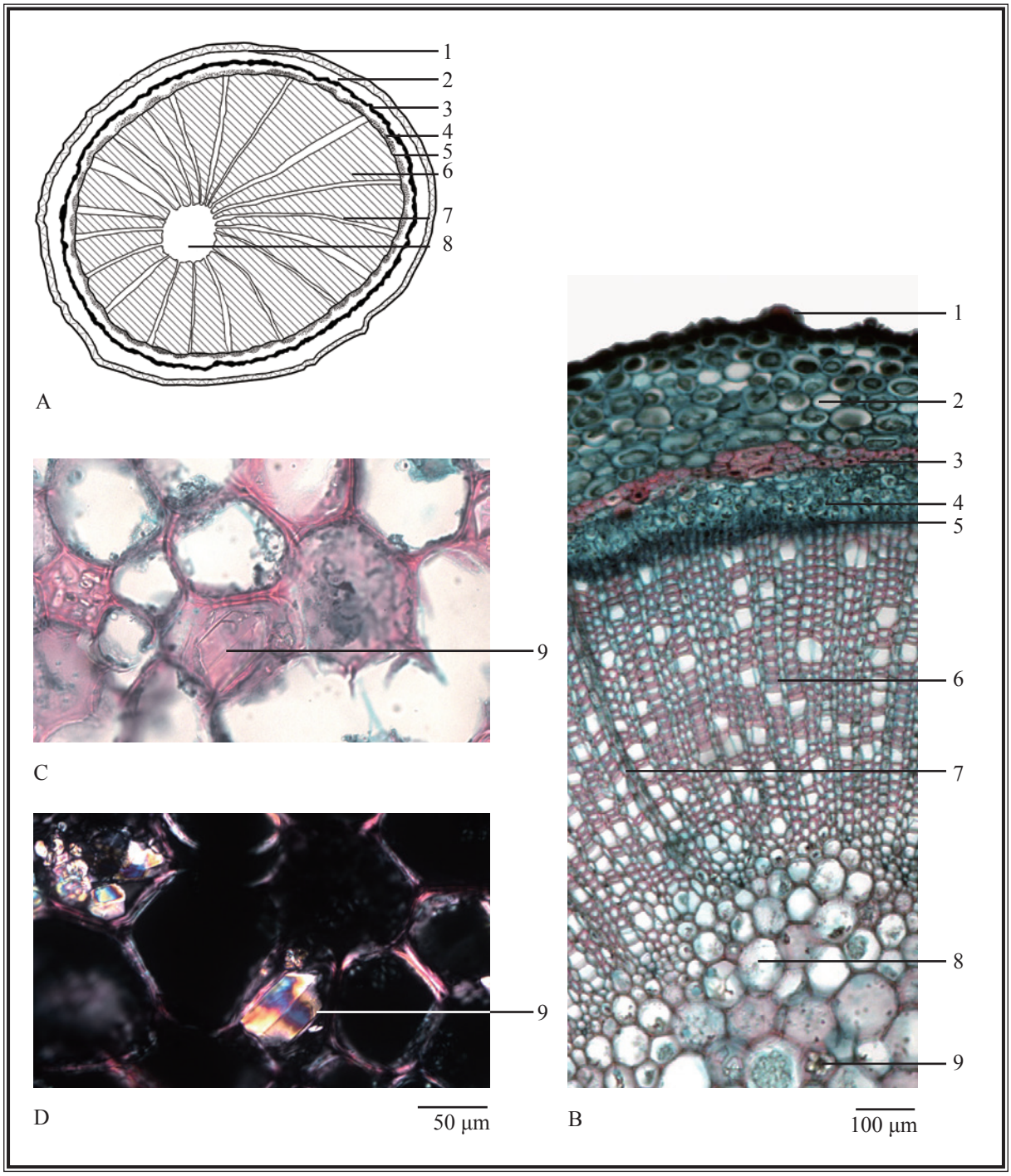

Figure 2 (ii) Microscopic features of transverse section of stem of Ilicis Pubescentis Radix et Caulis

A. Sketch B. Section illustration C. Crystals of calcium oxalate (under the light microscope)
D. Crystals of calcium oxalate (under the polarized microscope)

1. Cork 2. Cortex 3. Stone cell 4. Phloem 5. Cambium 6. Xylem 7. Xylem ray
8. Pith 9. Crystal of calcium oxalate

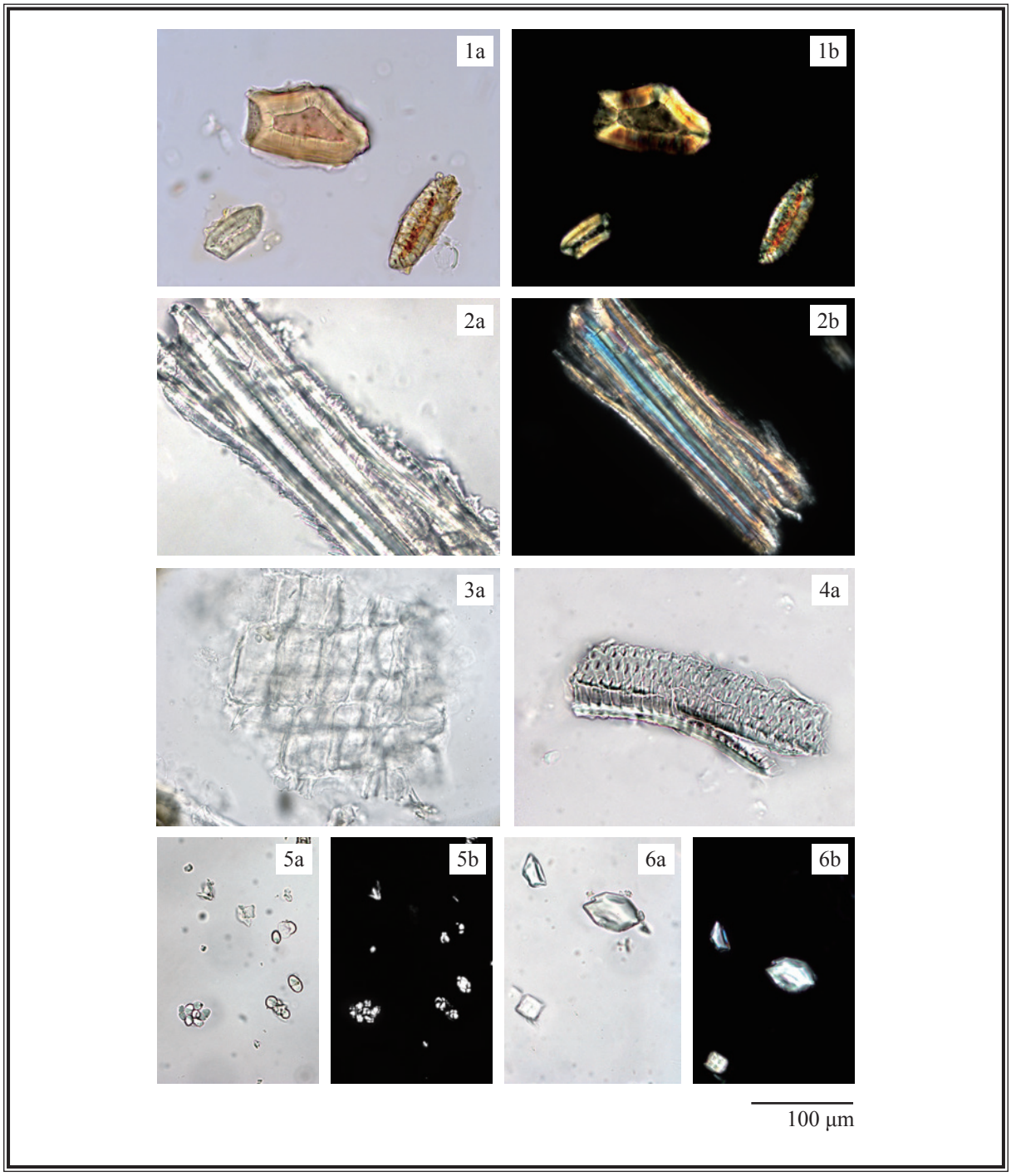

Figure 3 Microscopic features of powder of Ilicis Pubescentis Radix et Caulis

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

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

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

Standard solution

Ilexgenin A standard solution

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

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate, methanol, water and formic acid (20:5:2:1:0.3, v/v). Shake well and use the lower layer.

Spray reagent

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

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (320 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Collect the supernatant.

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 ilexgenin A standard solution (1 μL) and the test solution (5 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 105°C (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).

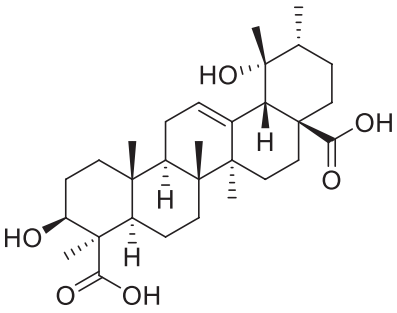

Figure 4 Chemical structure of ilexgenin A

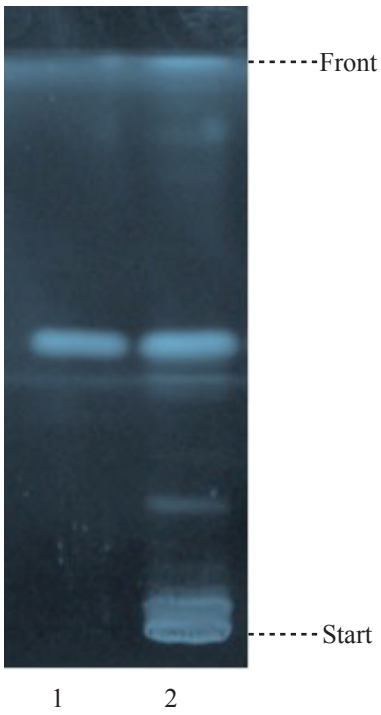

Figure 5 A reference HPTLC chromatogram of Ilicis Pubescentis Radix et Caulis extract observed under UV light (366 nm) after staining

1. Ilexgenin A standard solution 2. Test solution

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 ilexgenin A (Fig. 5).

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

Standard solution

Ilexgenin A standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of ilexgenin A CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (500 W) the mixture for 1 h. Centrifuge at about 3000 × *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 16	81 → 80	19 → 20	linear gradient
16 – 30	80 → 70	20 → 30	linear gradient
30 – 40	70 → 65	30 → 35	linear gradient
40 – 50	65 → 55	35 → 45	linear gradient
50 – 60	55 → 10	45 → 90	linear gradient
60 – 65	10	90	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ilexgenin A Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ilexgenin A should not be more than 5.0%; the RSD of the retention time of ilexgenin A peak should not be more than 2.0%; the column efficiency determined from ilexgenin A peak should not be less than 1200000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject ilexgenin A Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of ilexgenin A peak in the chromatogram of ilexgenin A Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify ilexgenin A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ilexgenin A Std-FP. The retention times of ilexgenin A 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 Ilicis Pubescentis Radix et Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Ilicis Pubescentis Radix et Caulis extract

Peak No.	RRT	Acceptable Range
1	0.28	± 0.03
2	0.61	± 0.03
3	0.82	± 0.03
4 (marker, ilexgenin A)	1.00	-

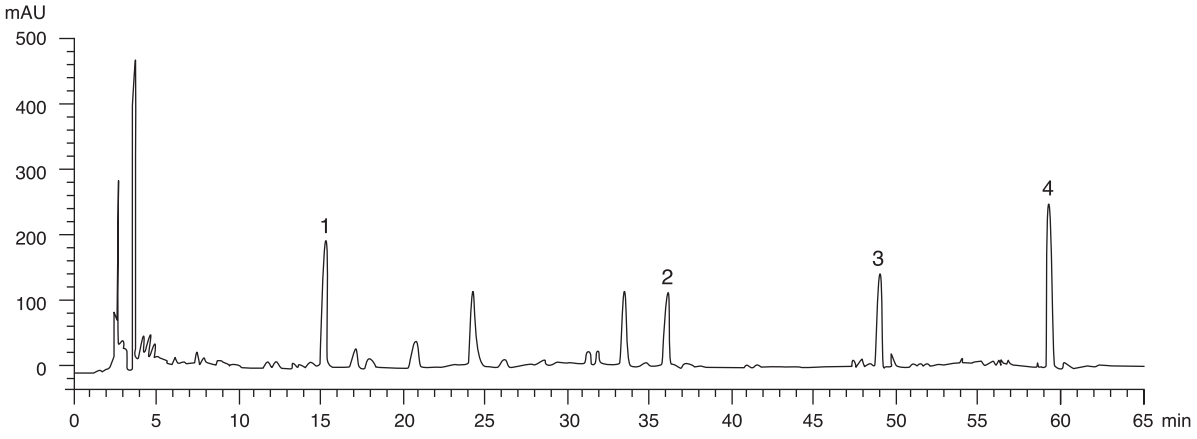

Figure 6 A reference fingerprint chromatogram of Ilicis Pubescentis Radix et Caulis extract

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. 6).

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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 2.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 8.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 3.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

Standard solution

Ilexgenin A standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of ilexgenin A CRS and dissolve in 5 mL of methanol.

Ilexgenin A standard solution for assay, Std-AS

Measure accurately the volume of the ilexgenin A Std-Stock, dilute with methanol to produce a series of solutions of 25, 50, 100, 200, 400 mg/L for ilexgenin A.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (500 W) the mixture for 1 h. Centrifuge at about 3000 × *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. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	70 → 20	30 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ilexgenin A Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of ilexgenin A should not be more than 5.0%; the RSD of the retention time of ilexgenin A peak should not be more than 2.0%; the column efficiency determined from ilexgenin A peak should not be less than 60000 theoretical plates.

The *R* value between ilexgenin A peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of ilexgenin A Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of ilexgenin A against the corresponding concentrations of ilexgenin A 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 ilexgenin A peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ilexgenin A Std-AS. The retention times of ilexgenin A 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 ilexgenin A in the test solution, and calculate the percentage content of ilexgenin A in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.26% of ilexgenin A ($C_{30}H_{46}O_6$), calculated with reference to the dried substance.

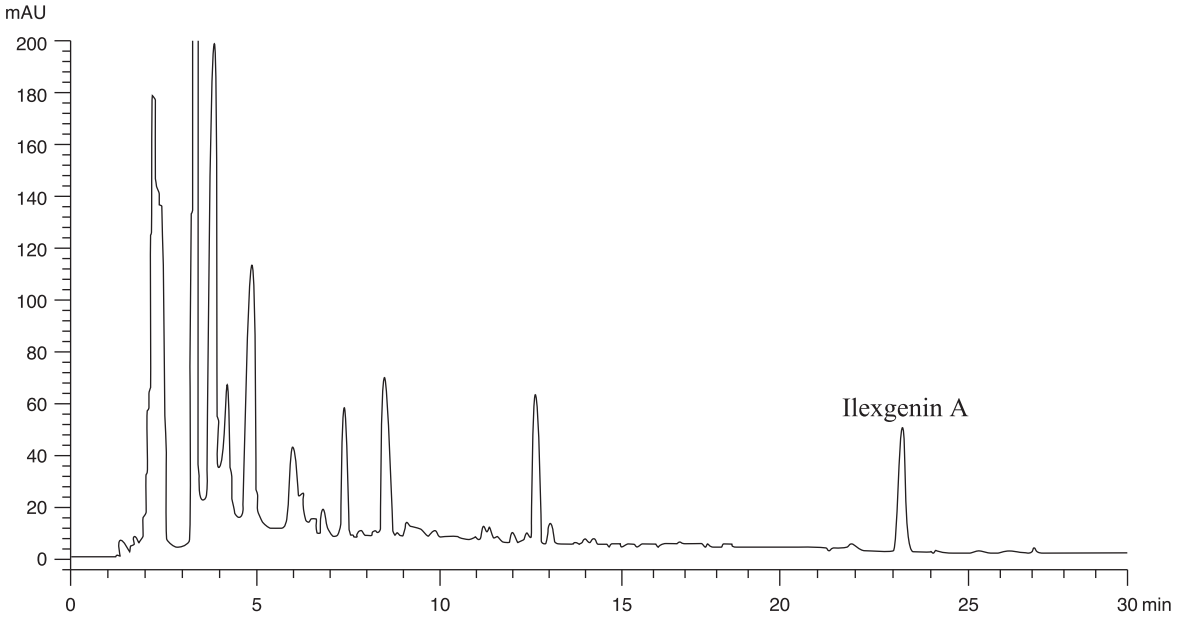

Figure 7 A reference assay chromatogram of Ilicis Pubescentis Radix et Caulis extract