

Dictamni Cortex

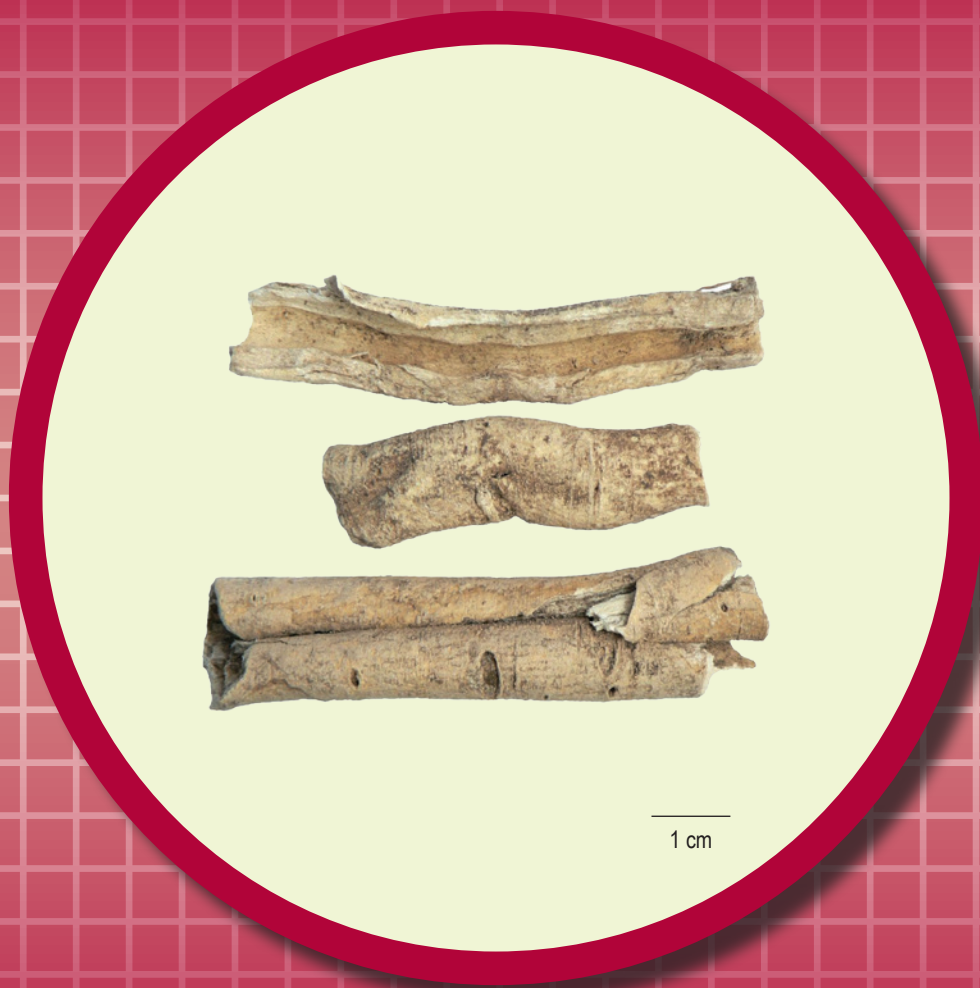


Figure 1 A photograph of Dictamni Cortex

1. NAMES

Official Name: Dictamni Cortex

Chinese Name: 白鮮皮

Chinese Phonetic Name: Baixianpi

2. SOURCE

Dictamni Cortex is the dried root bark of *Dictamnus dasycarpus* Turcz. (Rutaceae). The root is collected in spring and autumn, the soil and fibrous roots removed, washed clean, then incised vertically. Afterward, the root bark is stripped off, then dried immediately to obtain Dictamni Cortex.

3. DESCRIPTION

Quilled or semi-quilled shape, both edges of the cortex usually curved inwards, 3.1-17.5 cm long, 4-20 mm in diameter, 1-5 mm thick. Outer surface greyish-white or pale yellow, marked with fine longitudinal wrinkles, transverse lenticels and rootlet scars, frequently with small protruding granular dots; inner surface almost pale yellow, with fine longitudinal wrinkles. Texture fragile, easily broken, dusting on breaking, fracture uneven and somewhat lamellar. After cork is removed, showing numerous glittering small spots on exposing to light. Odour muttoney; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Cork contains 3-10 layers of cells, lengthened tangential, thin and flat. Cortex narrow, parenchymatous cells elongated round, tangentially arranged, with large clefts scattered. Phloem broad, parenchymatous cells arranged dispersely, with clefts. Phloem ray consists of 1-3 rows of parenchymatous cells wide. Fibres mostly scattered singly in the cortex and phloem, subsquare or elongated-polygonal, walls extremely thickened, obviously striated and lignified. Parenchymatous cells contain abundant clusters of calcium oxalate and starch granules (Fig. 2).

Powder

Colour greyish-white. Starch granules mostly simple, subrounded, 3-11 μm in diameter, with dotted or cleft-shaped hilum in large one and striation indistinct, compound granules composed

2 units or more; black and cruciate in shape under the polarized microscope. Clusters of calcium oxalates abundant, 6-33 μm in diameter, frequently arranged as a strip; polychromatic under the polarized microscope. Fibres are more frequently observed, usually singly scattered, mostly fusiform, or slender fusiform with narrow lumen, 81-775 μm long, 10-128 μm in diameter, walls extremely thickened, lignified, striations meticulous and distinct; polychromatic under the polarized microscope. Cork cell polygonal or square in surface view (Fig. 3).

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

Standard solutions

Dictamnine standard solution

Weigh 3.0 mg of dictamnine CRS (Fig. 4) and dissolve in 10 mL of methanol.

Fraxinellone standard solution

Weigh 0.5 mg of fraxinellone CRS (Fig. 4) and dissolve in 1 mL of methanol.

Obacunone standard solution

Weigh 0.5 mg of obacunone CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of toluene, methanol and glacial acetic acid (10:0.5:0.1, v/v).

Spray reagent

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

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (90 W) the mixture for 15 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately dictamnine standard solution (2 μL), fraxinellone standard solution (5 μL), obacunone standard solution (10 μL) and the test solution (8 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 5 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 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 3 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

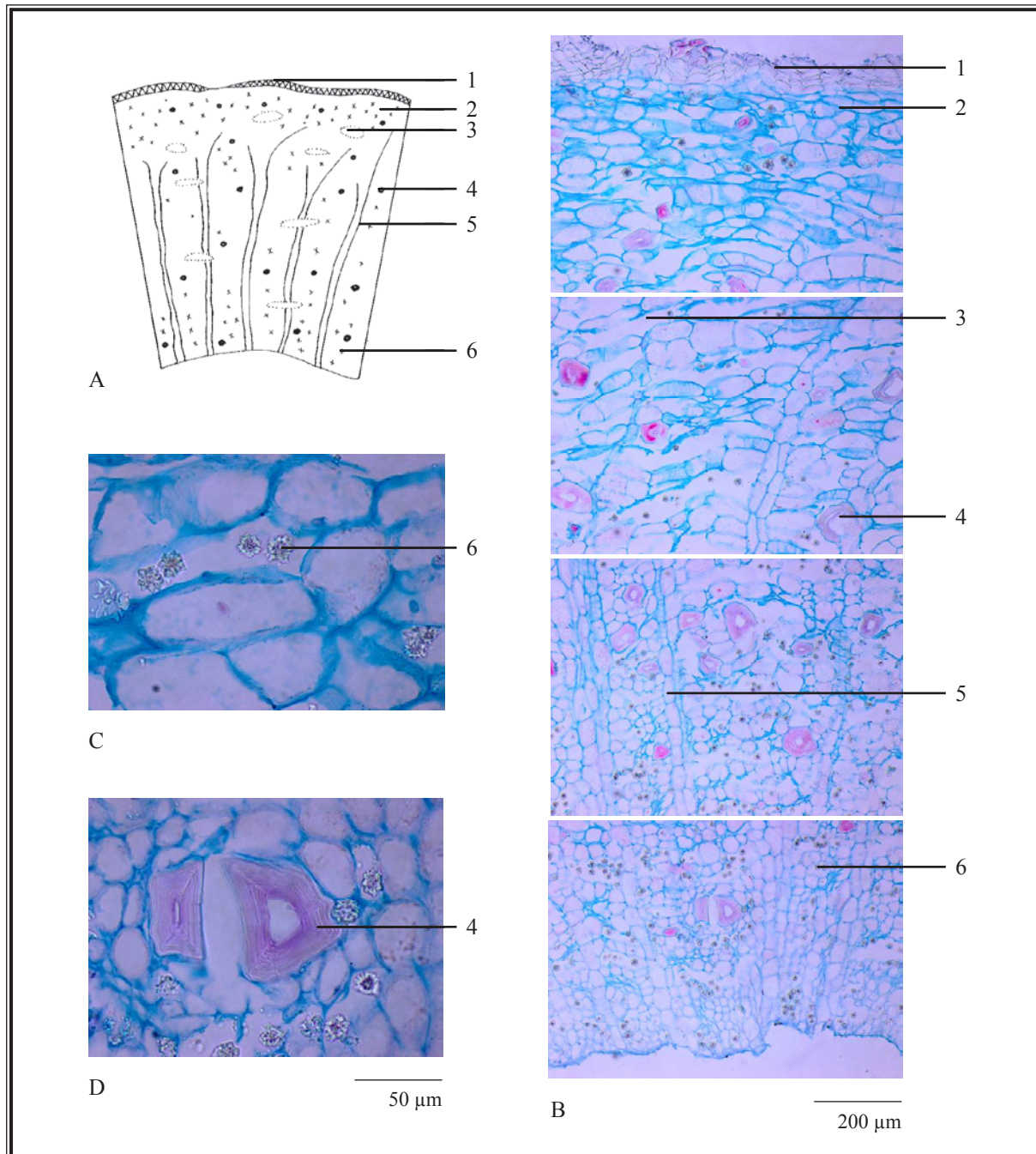


Figure 2 Microscopic features of transverse section of *Dictamnii Cortex*

A. Sketch B. Section illustration C. Clusters of calcium oxalate D. Fibre

1. Cork 2. Cortex 3. Cleft 4. Fibres 5. Phloem ray 6. Clusters of calcium oxalate

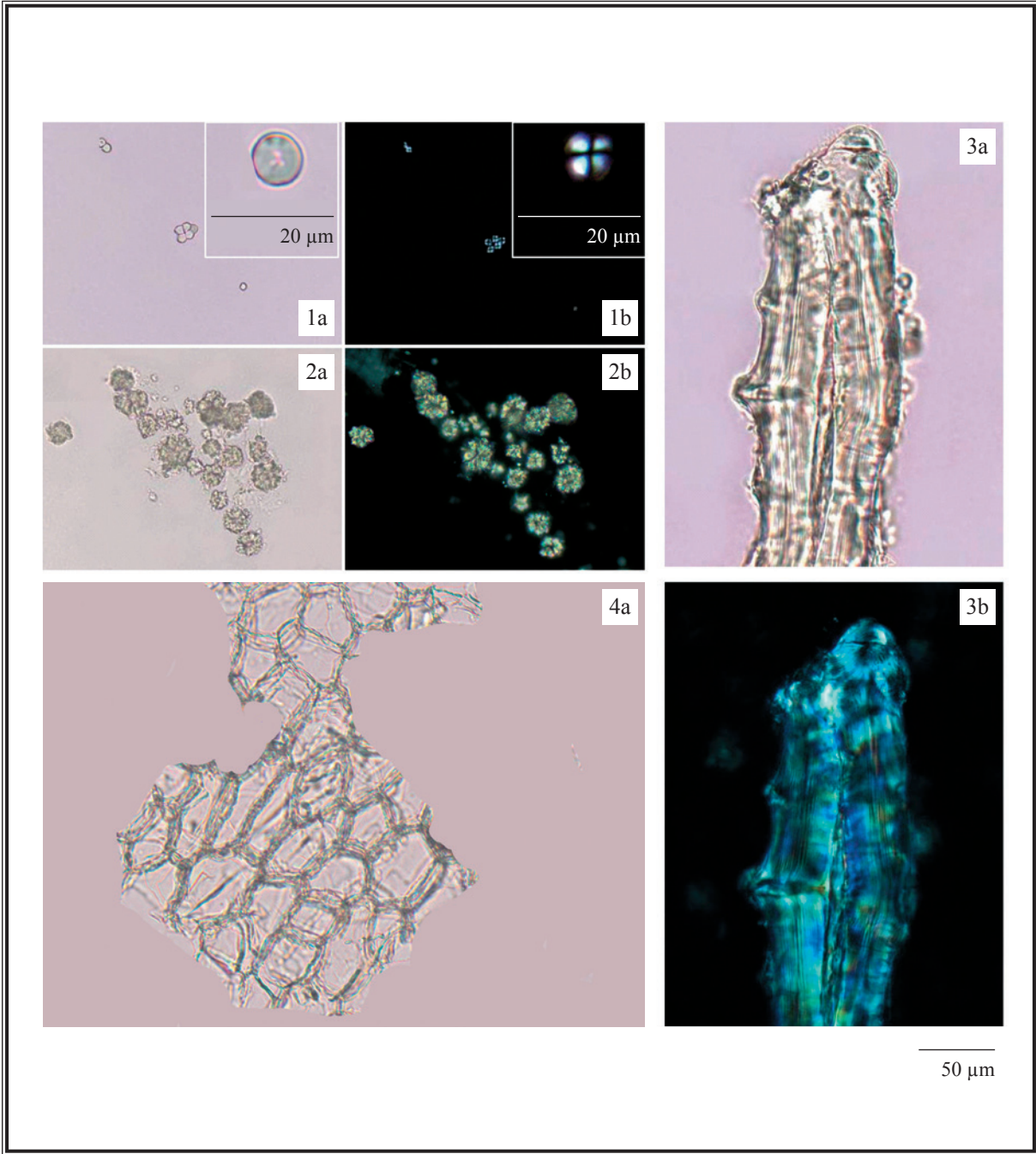


Figure 3 Microscopic features of powder of Dictamni Cortex

- 1. Starch granules 2. Clusters of calcium oxalate 3. Fibre 4. Cork cells
- a. Features under the light microscope b. Features under the polarized microscope

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 dictamnine, fraxinellone and obacunone.

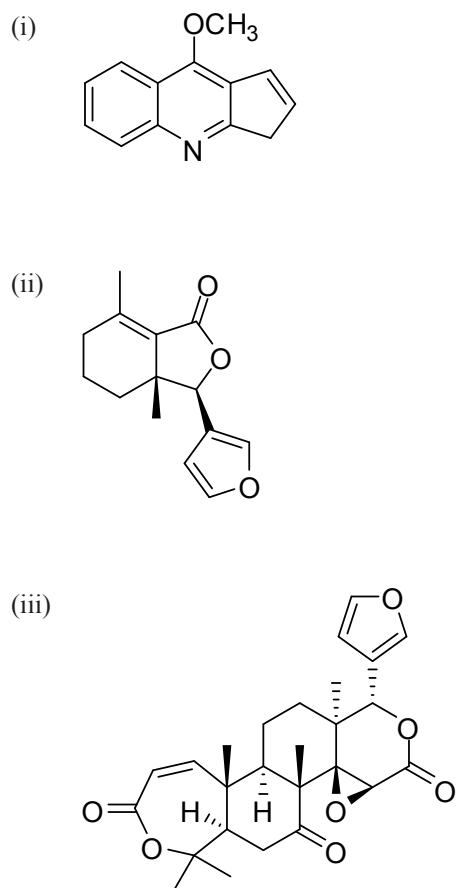


Figure 4 Chemical structures of (i) dictamnine (ii) fraxinellone and (iii) obacunone

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

Standard solutions

Dictamnine standard solution for fingerprinting, Std-FP (16 mg/L)

Weigh 0.16 mg of dictamnine CRS and dissolve in 10 mL of ethanol (70%).

Fraxinellone standard solution for fingerprinting, Std-FP (64 mg/L)

Weigh 0.64 mg of fraxinellone CRS and dissolve in 10 mL of ethanol (70%).

Obacunone standard solution for fingerprinting, Std-FP (400 mg/L)

Weigh 4.0 mg of obacunone CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 15 min. Filter through a 0.45- μ m nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ 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)	0.1% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 30	85 \rightarrow 47	15 \rightarrow 53	linear gradient
30 – 35	47 \rightarrow 37	53 \rightarrow 63	linear gradient
35 – 60	37 \rightarrow 10	63 \rightarrow 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μ L of dictamnine Std-FP, fraxinellone Std-FP and obacunone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of dictamnine, fraxinellone and obacunone should not be more than 5.0%; the RSD of the retention times of dictamnine, fraxinellone and obacunone peaks should not be more than 2.0%; the column efficiencies determined from dictamnine, fraxinellone and obacunone peaks should not be less than 60000, 200000 and 200000 theoretical plates respectively.

The *R* value between peak 1 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. 5).

Procedure

Separately inject dictamnine Std-FP, fraxinellone Std-FP, obacunone Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention times of dictamnine, fraxinellone and obacunone peaks in the chromatograms of dictamnine Std-FP, fraxinellone Std-FP, obacunone Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify dictamnine, fraxinellone and obacunone peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of dictamnine Std-FP, fraxinellone Std-FP and obacunone Std-FP. The

retention times of dictamnine, fraxinellone and obacunone 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 Dictamni Cortex extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Dictamni Cortex extract

Peak No.	RRT	Acceptable Range
1 (dictamnine)	0.80	± 0.04
2	0.85	± 0.03
3 (marker, obacunone)	1.00	-
4 (fraxinellone)	1.05	± 0.03

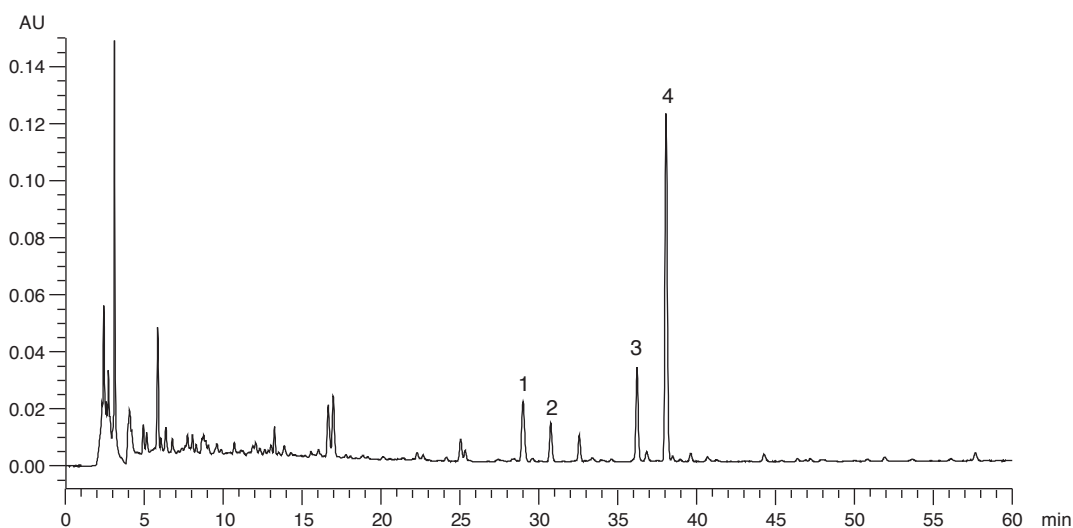


Figure 5 A reference fingerprint chromatogram of Dictamni Cortex extract

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 (*Appendix VII*): meet the requirements.

5.4 Sulphur Dioxide Residues (*Appendix XVIII*): meet the requirements.

5.5 Foreign Matter (*Appendix VIII*): not more than 1.0%.

5.6 Ash (*Appendix IX*)

Total ash: not more than 13.0%.

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 (cold extraction method): not less than 21.0%.

Ethanol-soluble extractives (hot extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

Mixed dictamnine, fraxinellone and obacunone standard stock solution, Std-Stock (16 mg/L for dictamnine, 64 mg/L for fraxinellone and 400 mg/L for obacunone)

Weigh accurately 0.16 mg of dictamnine CRS, 0.64 mg of fraxinellone CRS and 4.0 mg of obacunone CRS, and dissolve in 10 mL of ethanol (70%).

Mixed dictamnine, fraxinellone and obacunone standard solution for assay, Std-AS

Measure accurately the volume of the mixed dictamnine, fraxinellone and obacunone Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 0.4, 0.8, 1.6, 4, 6.4 mg/L for dictamnine, 1.6, 3.2, 6.4, 16, 25.6 mg/L for fraxinellone and 10, 20, 40, 100, 160 mg/L for obacunone.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 15 min. Centrifuge at about $2000 \times g$ for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times by using 10 mL and 5 mL of ethanol (70%) respectively. Combine the supernatants and make up to the mark with ethanol (70%). Filter through a 0.45- μ m nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (238 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 – 15	60 → 57	40 → 43	linear gradient
15 – 20	57 → 50	43 → 50	linear gradient
20 – 35	50 → 46	50 → 54	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed dictamnine, fraxinellone and obacunone Std-AS (1.6 mg/L for dictamnine, 6.4 mg/L for fraxinellone and 40 mg/L for obacunone). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of dictamnine, fraxinellone and obacunone should not be more than 5.0%; the RSD of the retention times of dictamnine, fraxinellone and obacunone peaks should not be more than 2.0%; the column efficiencies determined from dictamnine, fraxinellone and obacunone peaks should not be less than 15000, 40000 and 50000 theoretical plates respectively.

The *R* value between dictamnine peak and the closest peak; the *R* value between fraxinellone peak and the closest peak; and the *R* value between obacunone 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 dictamnine, fraxinellone and obacunone Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of dictamnine, fraxinellone and obacunone against the corresponding concentrations of the mixed dictamnine, fraxinellone and obacunone Std-AS. Obtain the slopes, y-intercepts and the *r*² 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 dictamnine, fraxinellone and obacunone peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed dictamnine, fraxinellone and obacunone Std-AS. The retention times of dictamnine, fraxinellone and obacunone peaks in the

chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of dictamnine, fraxinellone and obacunone in the test solution, and calculate the percentage contents of dictamnine, fraxinellone and obacunone in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.019% of dictamnine ($C_{12}H_9NO_2$); not less than 0.12% of fraxinellone ($C_{14}H_{16}O_3$); and not less than 0.24% of obacunone ($C_{26}H_{30}O_7$), calculated with reference to the dried substance.