Descurainiae Semen

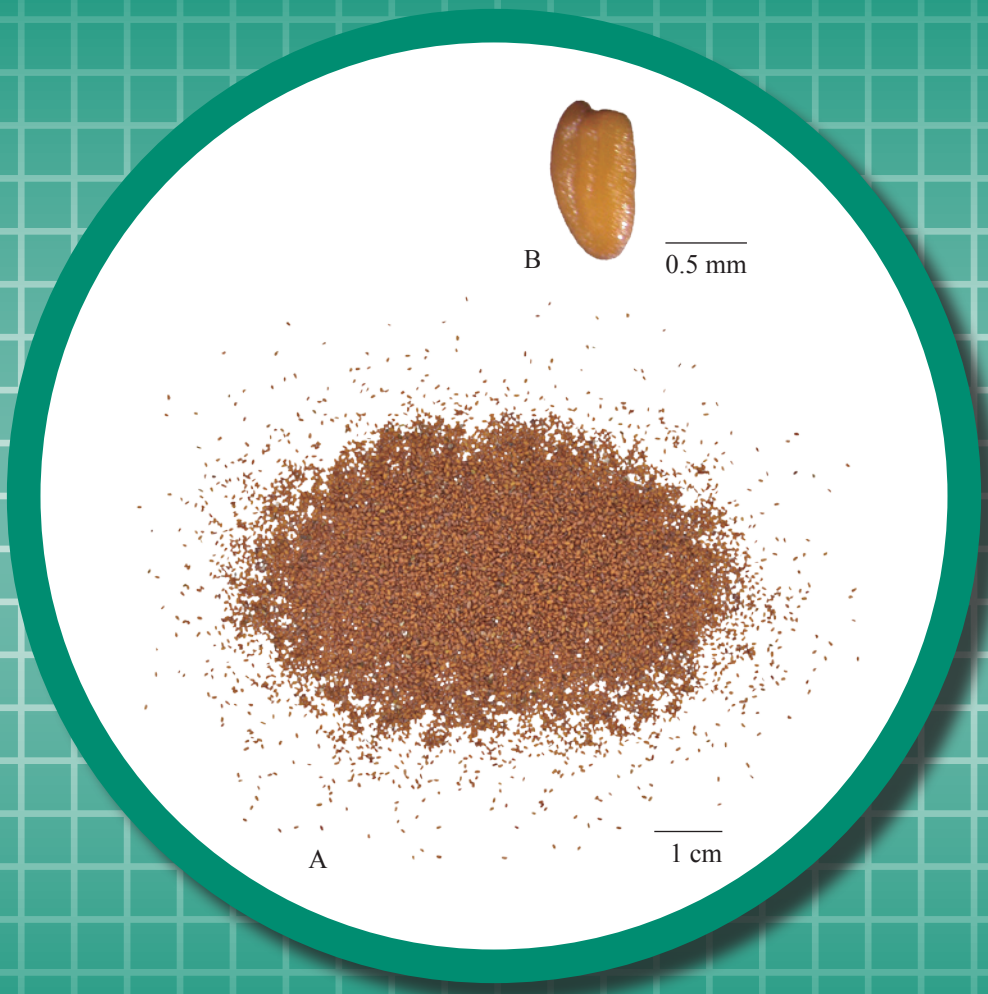

Figure 1 A photograph of Descurainiae Semen

A. Descurainiae Semen B. Magnified seed

1. NAMES

Official Name: Descurainiae Semen

Chinese Name: 南葶藶子

Chinese Phonetic Name: Nantinglizi

2. SOURCE

Descurainiae Semen is the dried ripe seed of *Descurainia sophia* (L.) Webb. ex Prantl. (Brassicaceae). The fruit is collected in summer when ripe, dried under the sun, then the seeds gathered and foreign matter removed to obtain Descurainiae Semen.

3. DESCRIPTION

Oblong-ellipsoid and slightly flattened, about 1 mm long, 0.5 mm wide. Externally yellowish-brown, marked with petty and dense reticulate texture, and 2 longitudinal shallow furrows. One end obtuse, the other slightly concave in the centre with a hilum or relatively truncate. Odour slight; taste slightly pungent and bitter, slightly viscous (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Outer epidermis of testa consists of 1 layer of mucilage cells, with thickened inner walls, extending outwards to cellulose columns, oblique, lengthened or truncate at apex; cellulose columns truncate at apex, relatively wide, with mucilage striations in periphery. Inner epidermis of testa consists of 1 layer of subsquare cells, with inner and lateral wall thickened and strongly lignified. Endosperm cells 1 layer, flat square, containing aleurone granules. Cotyledon composed of 2 slices, together with radicle occupies the major portion of the seed (Fig. 2).

Powder

Colour yellowish-brown. Outer epidermal cells of testa consist of 1 layer of mucilage cells, colourless to pale yellow, rectangular in lateral view, inner walls thickened, extending outwards to form cellulose column, cellulose column 8-17 μm long, apex truncate, mucilage striations visible in periphery; polygonal or subsquare in surface view, 16-40 μm in diameter, cellulose column visible at centre, mucilage striations visible in periphery. Inner epidermal cells of testa 1 layer in lateral view, pale yellow to reddish-brown, lateral walls unevenly thickened; polygonal, subsquare, sometimes long-polygonal in surface view, 12-43 μm in diameter, walls 2-3 μm thick, lumens subrounded to polygonal. Endosperm cells colourless, flat square in section view, polygonal in surface view, walls slightly thickened, containing aleurone grains and oil droplets. Cotyledon cells irregular polygonal (Fig. 3).

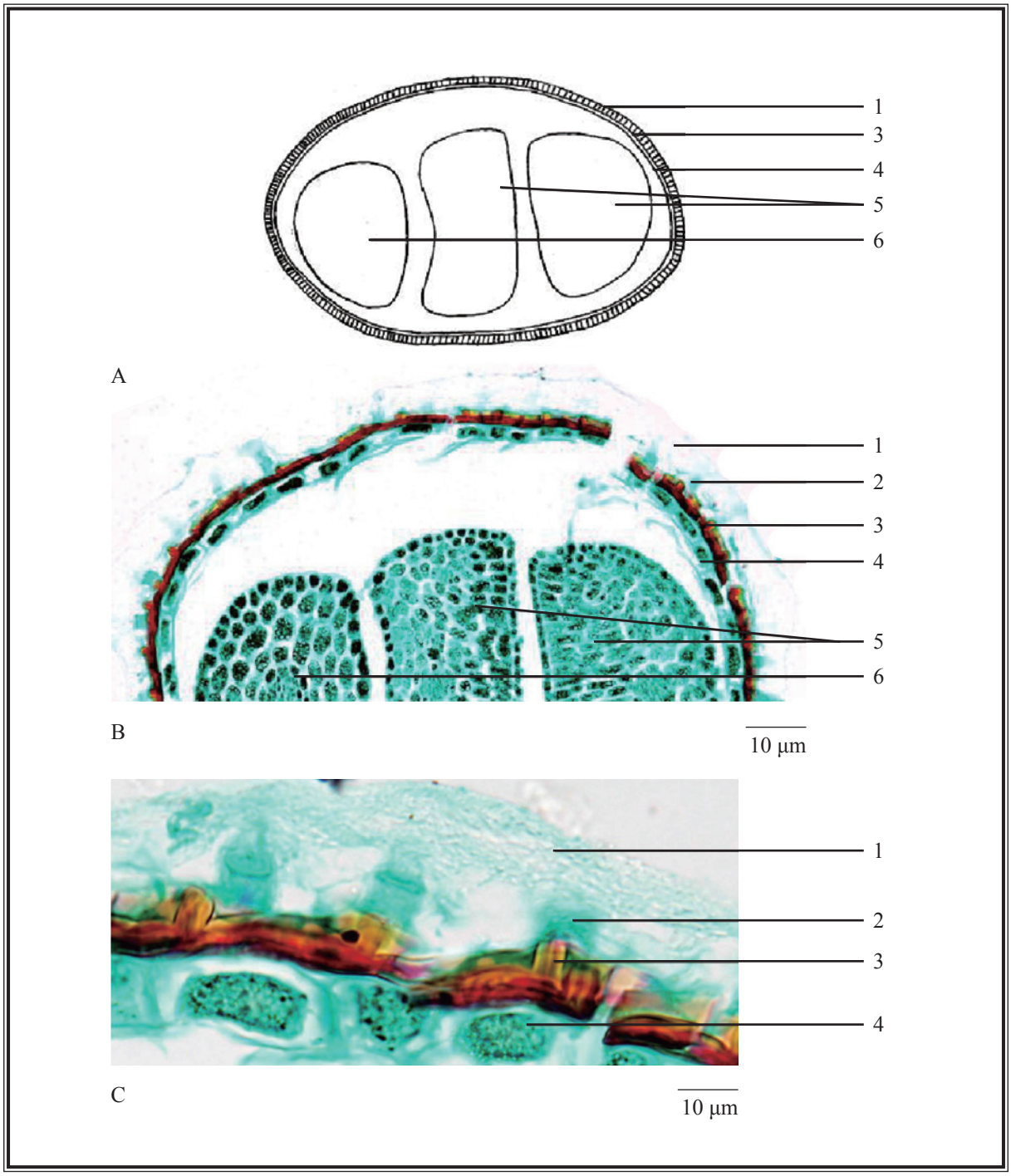

Figure 2 Microscopic features of transverse section of Descurainiae Semen

A. Sketch B. Section illustration C. Testa magnified

1. Outer epidermis of testa 2. Cellulose column
3. Inner epidermis of testa 4. Endosperm 5. Cotyledons 6. Radicle

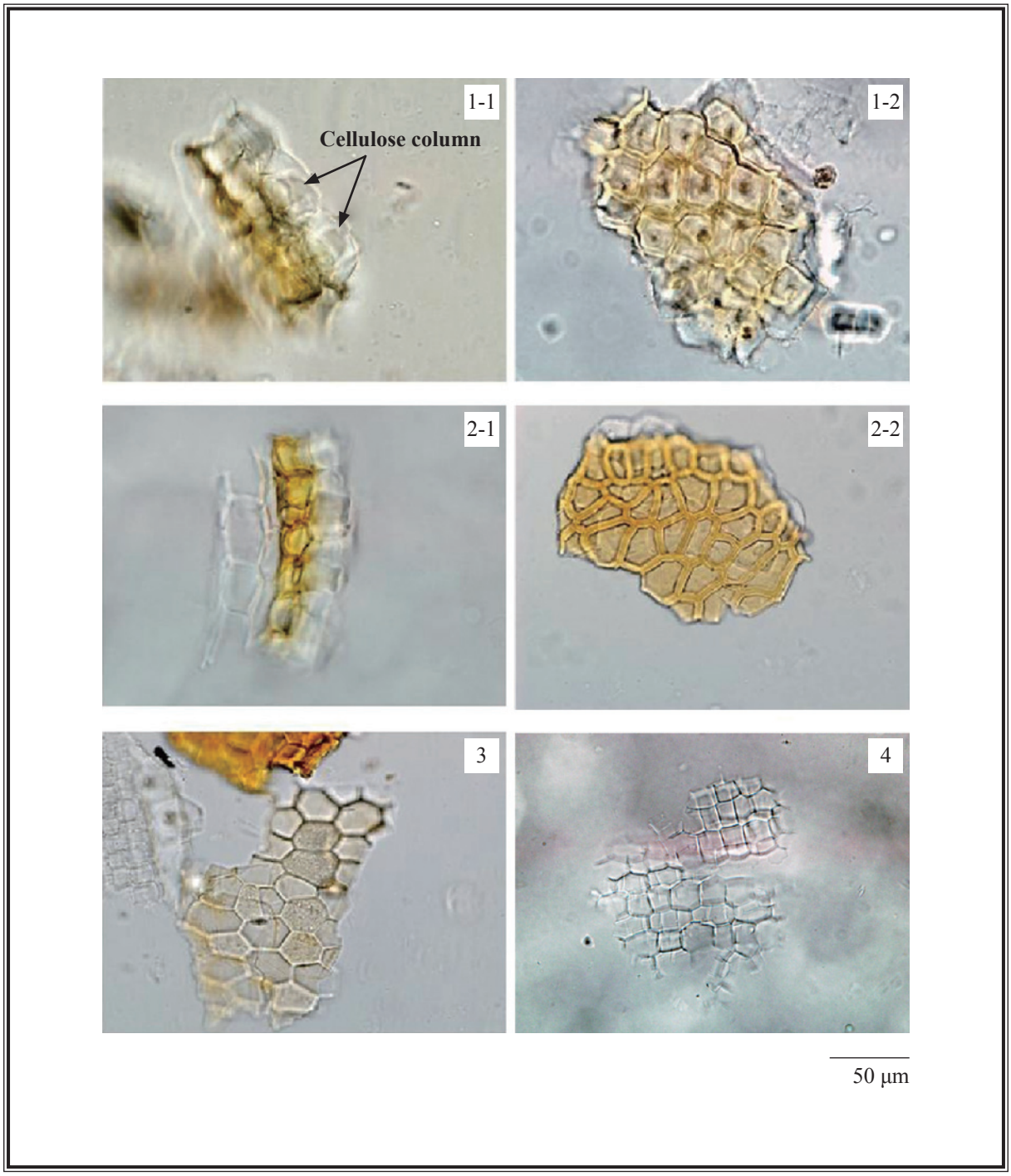

Figure 3 Microscopic features of powder of Descurainiae Semen (under the light microscope)

1. Outer epidermal cells of testa (1-1 in lateral view, 1-2 in surface view)
2. Inner epidermal cells of testa (2-1 in lateral view, 2-2 in surface view)
3. Endosperm cells 4. Cotyledon cells

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

Standard solution

Quercetin-3-O-β-D-glucopyranosyl-7-O-β-D-gentiobioside standard solution

Weigh 1.0 mg of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of *n*-butanol, water and formic acid (6:3:2, v/v).

Spray reagent

Weigh 2 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol (70%). Sonicate (220 W) the mixture for 30 min. Filter the mixture.

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 quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside standard solution and the test solution (2 µL each) to the plate. 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 dry in air. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

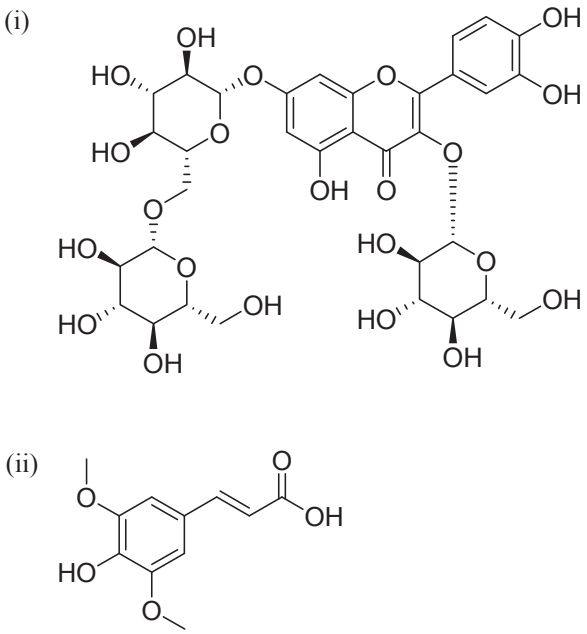

Figure 4 Chemical structures of (i) quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside and (ii) sinapic acid

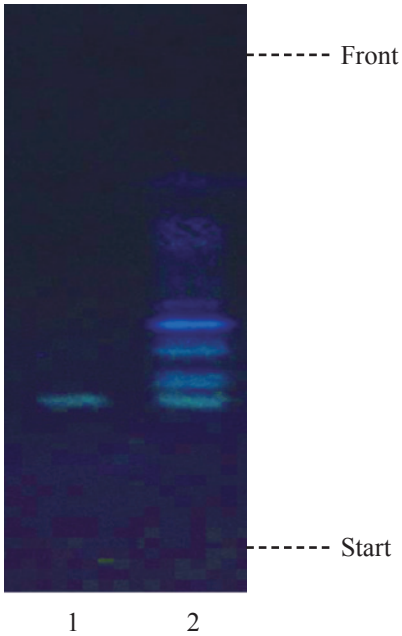

Figure 5 A reference HPTLC chromatogram of Descurainiae Semen extract observed under UV light (366 nm) after staining

1. Quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside 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 quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside (Fig. 5).

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

Standard solution

Sinapic acid standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of sinapic acid CRS (Fig. 4) and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol (70%). Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Make up to the mark with methanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 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% Acetic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 60	65 → 50	35 → 50	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of sinapic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of sinapic acid should not be more than 5.0%; the RSD of the retention time of sinapic acid peak should not be more than 2.0%; the column efficiency determined from sinapic acid peak should not be less than 10000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject sinapic acid Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of sinapic acid peak in the chromatogram of sinapic acid Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify sinapic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sinapic acid Std-FP. The retention times of sinapic acid 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 five characteristic peaks of Descurainiae Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Descurainiae Semen extract

Peak No.	RRT	Acceptable Range
1 (marker, sinapic acid)	1.00	-
2	1.82	± 0.04
3	1.93	± 0.03
4	2.29	± 0.03
5	2.53	± 0.03

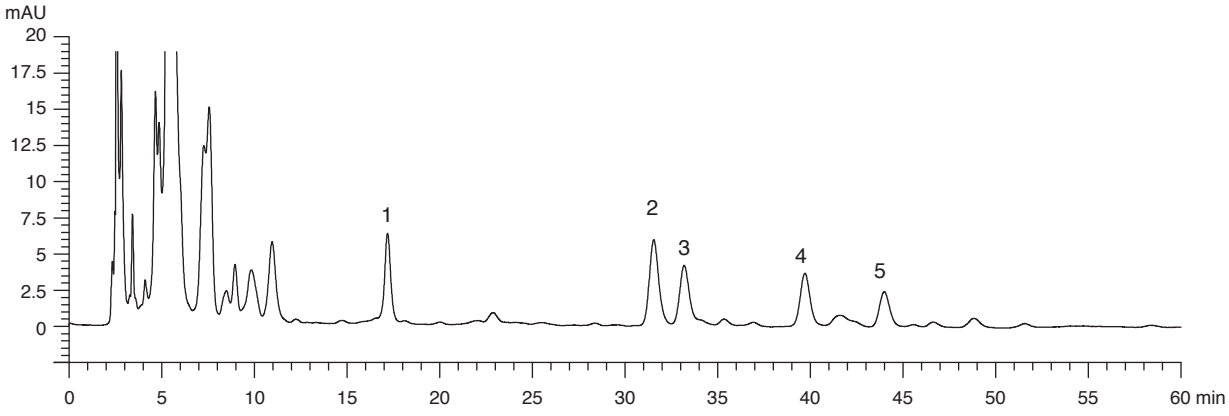

Figure 6 A reference fingerprint chromatogram of Descurainiae Semen extract

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.

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 5.0%.

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)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Quercetin-3-O-β-D-glucopyranosyl-7-O-β-D-gentiobioside standard stock solution, Std-Stock (100 mg/L)
Weigh accurately 0.5 mg of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside CRS and dissolve in 5 mL of methanol (70%).
Quercetin-3-O-β-D-glucopyranosyl-7-O-β-D-gentiobioside standard solution for assay, Std-AS
Measure accurately the volume of the quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside Std-Stock, dilute with methanol (70%) to produce a series of solutions of 1, 10, 15, 20, 40 mg/L for quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of methanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with 10 mL of methanol (70%). Filter and combine the filtrates. Make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 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 0.1% acetic acid and acetonitrile (89:11, v/v). The elution time is about 20 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside Std-AS (15 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside should not be more than 5.0%; the RSD of the retention time of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside peak should not be more than 2.0%; the column efficiency determined from quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside peak should not be less than 5000 theoretical plates.

The *R* value between quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside against the corresponding concentrations of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside 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 quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside Std-AS. The retention times of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside 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 quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside in the test solution, and calculate the percentage content of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.076% of quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-gentiobioside ($C_{33}H_{40}O_{22}$), calculated with reference to the dried substance.