Toosendan Fructus

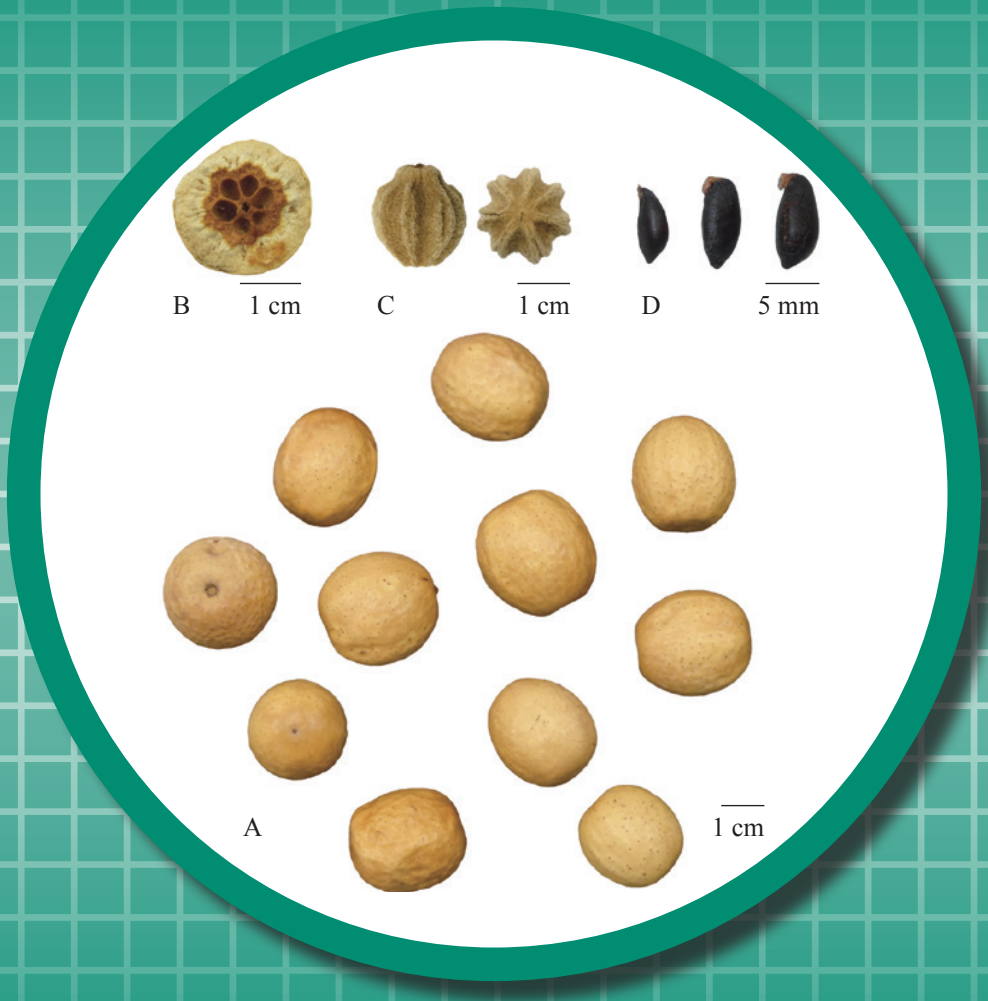

Figure 1 A photograph of Toosendan Fructus

A. Toosendan Fructus B. Transverse section of fruit C. Kernels
D. Magnified seeds

1. NAMES

Official Name: Toosendan Fructus

Chinese Name: 川楝子

Chinese Phonetic Name: Chuanlianzi

2. SOURCE

Toosendan Fructus is the dried ripe fruit of *Melia toosendan* Sieb. et Zucc. (Meliaceae). The fruit is collected in winter when ripe, foreign matter removed, then dried under the sun to obtain Toosendan Fructus.

3. DESCRIPTION

Subspherical to ellipsoid, 16-30 mm in diameter. Externally yellow to brownish-yellow, slightly lustrous, dented or shrunken, with brown dots. Apex with a dotted style scar, base dented and with a fruit stalk scar. Exocarp leathery, often separated from the sarcocarp; sarcocarp lax and soft, yellowish-white to pale brownish-yellow, viscous when moistened. Kernels spherical to ovoid, yellowish-brown, texture hard, both ends truncate, with 6-8 longitudinal ribs, 6-8 loculed, each locule contains 1 ellipsoid, brownish-black seed. Odour characteristic; taste sour and bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pericarp:

Exocarp consists of 1 layer of cells, covered with cuticle. Mesocarp broad, scattered with secretory cells; parenchymatous cells contain starch granules, some containing clusters of calcium oxalate, prisms of calcium oxalate occasionally found. Endocarp consists of fibres and stone cells; prisms of calcium oxalate occasionally found, forming crystal fibres [Fig. 2 (i)].

Seed:

Epidermis of testa consists of 1 layer of subsquare cells, with fine longitudinal striations. Hypodermis consists of 1-2 layers of brown cells. Parenchymatous cell layer consists of 1 layer of subrectangular cells. Pigment layer consists of several layers of pigment cells. Endotesta consists of 1 layer of subsquare or rounded crystal cells, walls thickened, containing small prisms of calcium oxalate. Endosperm cells and cotyledon cells filled with aleurone granules [Fig. 2 (ii)].

Powder

Colour brownish-yellow. Fibres 6-46 μm in diameter, walls extremely thickened, lumens sometimes filled with yellowish-brown contents; some surrounded by cells contain prisms of calcium oxalate of 4-20 μm in diameter, forming crystal fibres; orange-yellow or polychromatic under the polarized microscope. Stone cells subelliptical, elongated or irregular, some with warty protrusions or short obtuse branches, 23-147 μm long, 19-66 μm in diameter, walls extremely thickened, lumens sometimes filled with yellowish-brown contents; yellowish-white or orange-yellow under the polarized microscope. Epidermal cells of testa bright yellow or yellowish-orange, polygonal in surface view, with fine and dense granular striations. Crystal cells of endotesta rounded or oblong, mostly stone cell-like, 8-33 μm in diameter, walls vary in thickness, containing small prisms of calcium oxalate; orange-yellow under the polarized microscope. Exocarp cells subrounded or subpolygonal in surface view. Pigment cells reddish-brown, with indistinct boundaries. Clusters of calcium oxalate small, occasionally found; polychromatic under the polarized microscope (Fig. 3).

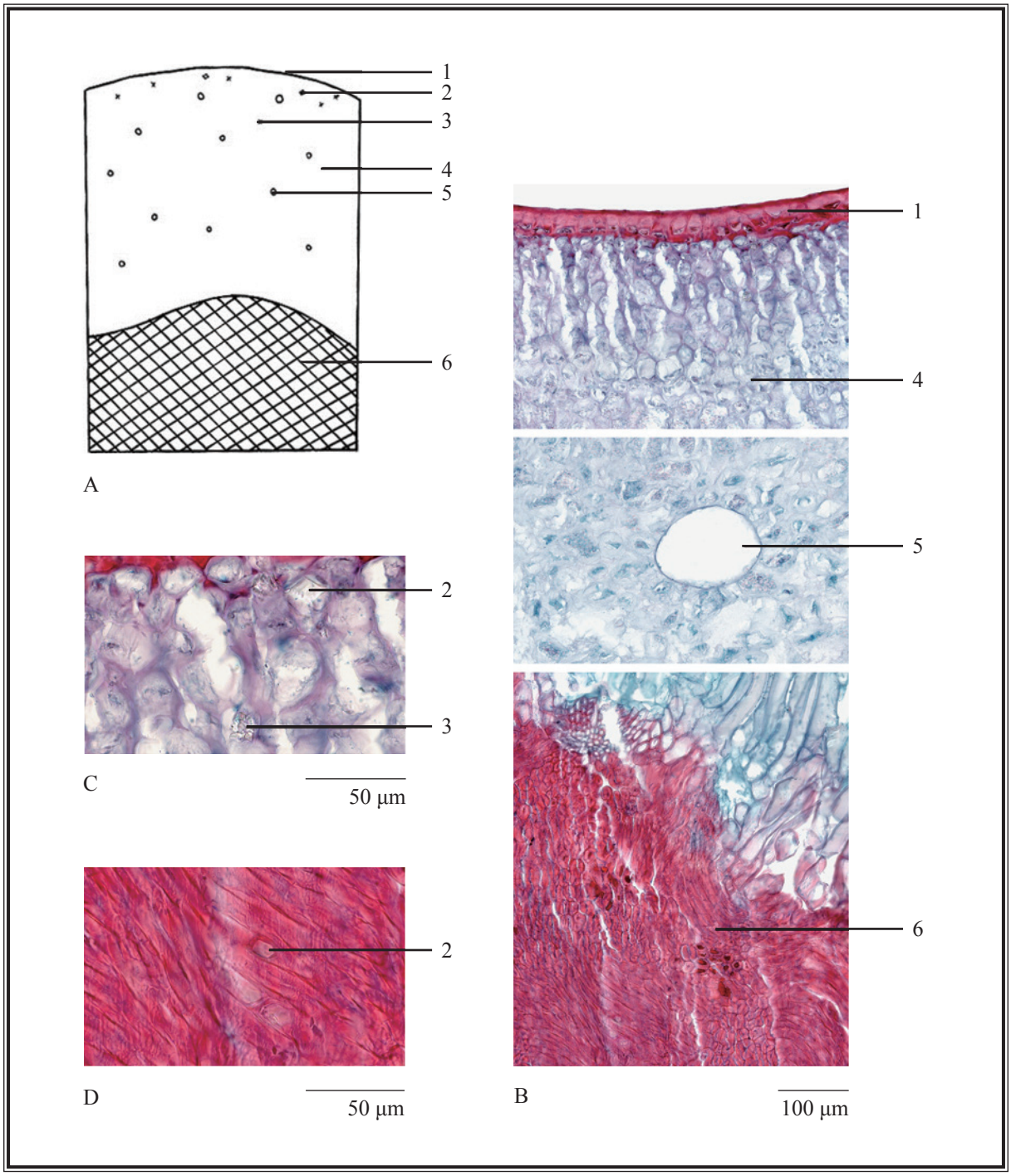

Figure 2 (i) Microscopic features of transverse section of pericarp of Toosendan Fructus

A. Sketch B. Section illustration C. Prisms of calcium oxalate and clusters of calcium oxalate in mesocarp D. Prisms of calcium oxalate in endocarp

1. Exocarp 2. Prisms of calcium oxalate 3. Cluster of calcium oxalate
4. Mesocarp 5. Secretory cell 6. Endocarp

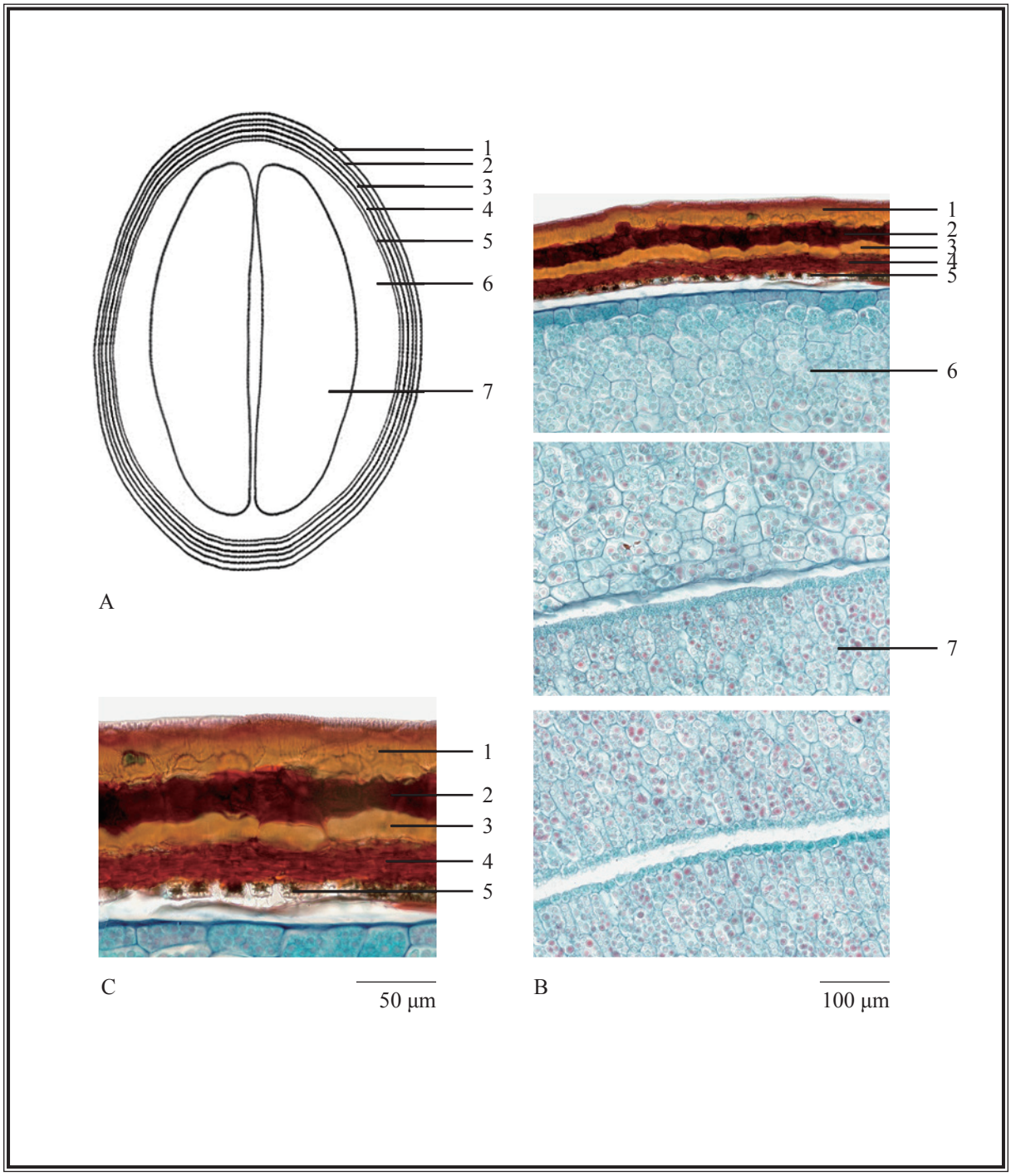

Figure 2 (ii) Microscopic features of transverse section of seed of Toosendan Fructus

A. Sketch B. Section illustration C. Testa

1. Epidermis of testa 2. Hypodermis 3. Parenchymatous cell layer
4. Pigment layer 5. Endotesta 6. Endosperm 7. Cotyledon

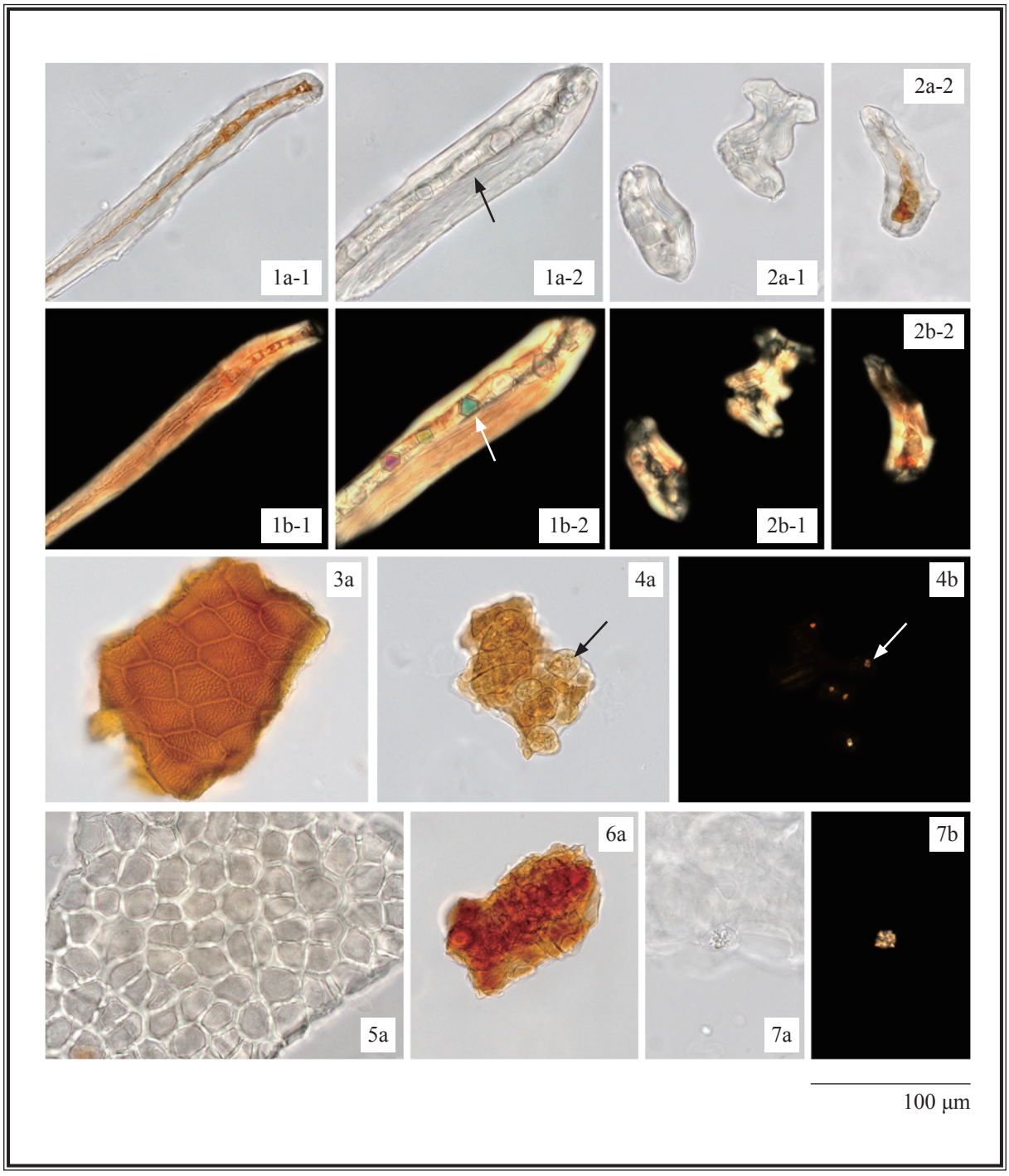

Figure 3 Microscopic features of powder of Toosendan Fructus

1. Fibres (1-1 fibre, 1-2 crystal fibres, prism of calcium oxalate ⟶) 2. Stone cells
3. Epidermal cells of testa 4. Crystal cells of endotesta (prism of calcium oxalate ⟶)
5. Exocarp cells 6. Pigment cells 7. Cluster 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

Toosendanin standard solution

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

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC) and acetone (6:5, 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 centrifuge tube, then add 20 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Transfer the supernatant to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol. Filter through a 0.45-μm nylon filter.

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 toosendanin standard solution and the test solution (2 μL each) 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 6 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 until the spots or bands become visible (about 3-5 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

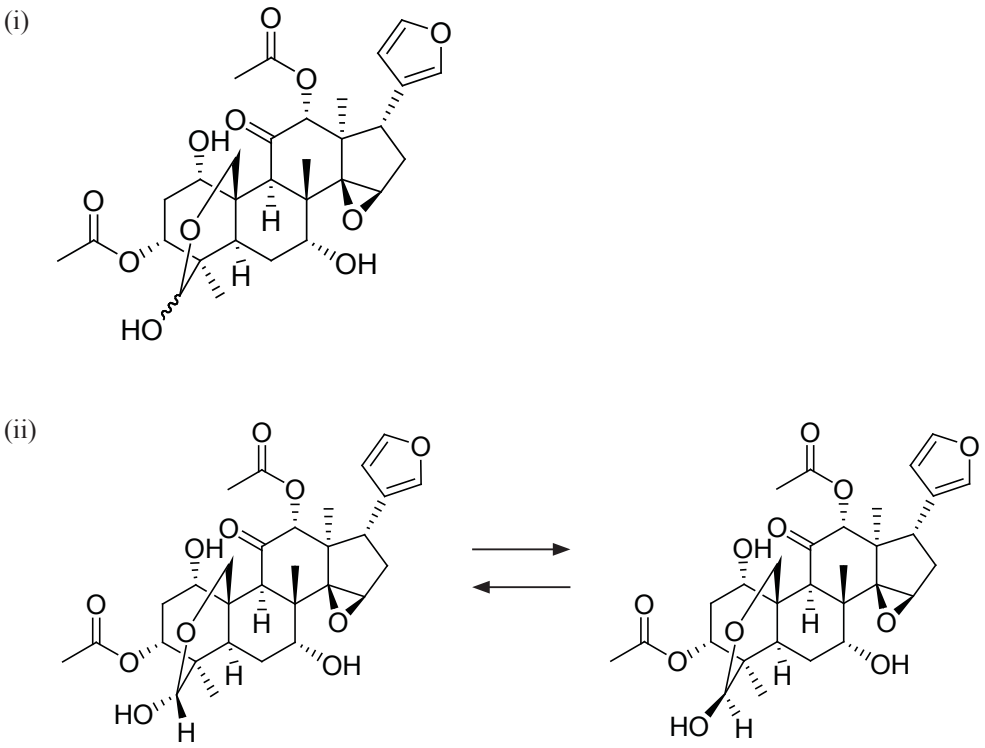

Figure 4 Chemical structures of (i) toosendanin and (ii) its interchangeable isomers

Remarks: Toosendanin naturally exists as two interchangeable isomers.

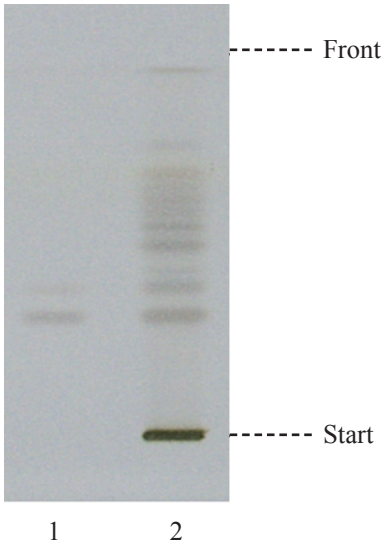

Figure 5 A reference HPTLC chromatogram of Toosendan Fructus extract observed under visible light after staining

1. Toosendanin standard solution 2. Test solution

For positive identification, the sample must give two spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of toosendanin (Fig. 5).

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

Standard solution

Toosendanin standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.25 mg of toosendanin CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Combine the solutions and 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 (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 25	80 → 65	20 → 35	linear gradient
25 – 40	65	35	isocratic
40 – 60	65 → 25	35 → 75	linear gradient

System suitability requirements

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

The *R* value between peak 6 and the closest peak; and the *R* value between peak 7 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject toosendanin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of each toosendanin isomeric peak in the chromatogram of toosendanin Std-FP and the retention times of the seven characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify each toosendanin isomeric peak in the chromatogram of the test solution by comparing their retention times with that in the chromatogram of toosendanin Std-FP. The retention times of toosendanin isomeric 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 seven characteristic peaks of Toosendan Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the seven characteristic peaks of Toosendan Fructus extract

Peak No.	RRT	Acceptable Range
1	0.33 (vs peak 6)	± 0.03
2	0.39 (vs peak 6)	± 0.03
3	0.41 (vs peak 6)	± 0.03
4	0.72 (vs peak 6)	± 0.03
5	0.74 (vs peak 6)	± 0.03
6 (marker, toosendanin)	1.00	-
7 (marker, toosendanin)	1.16 (vs peak 6)	± 0.03

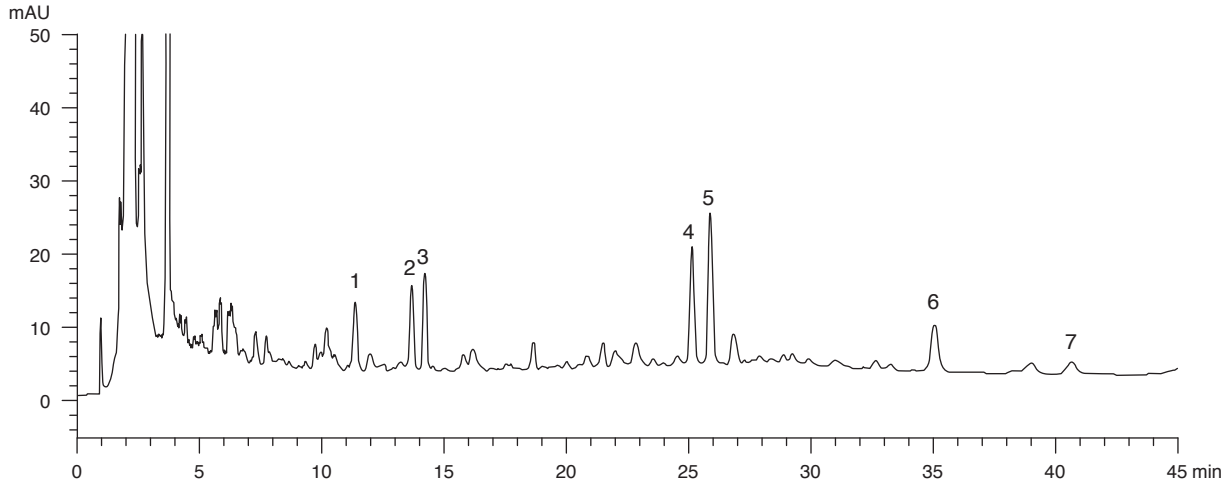

Figure 6 A reference fingerprint chromatogram of Toosendan Fructus extract

For positive identification, the sample must give the above seven 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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 0.5%.

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 30.0%.
Ethanol-soluble extractives (cold extraction method): not less than 17.0%.

7. ASSAY

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

Standard solution

Toosendanin standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of toosendanin CRS and dissolve in 10 mL of methanol (70%).

Toosendanin standard solution for assay, Std-AS

Measure accurately the volume of the toosendanin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 5, 10, 25, 50, 100 mg/L for toosendanin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (70%). Combine the solutions and 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 (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 25	80 → 65	20 → 35	linear gradient
25 – 40	65	35	isocratic
40 – 60	65 → 25	35 → 75	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of toosendanin Std-AS (25 mg/L). Toosendanin elutes as two isomeric peaks in the chromatogram of toosendanin Std-AS. The requirements of the system suitability parameters are as follows: the RSD of the peak area of each toosendanin isomeric peak should not be more than 5.0%; the RSD of the retention time of each toosendanin isomeric peak should not be more than 2.0%; the column efficiency determined from each toosendanin isomeric peak should not be less than 10000 theoretical plates.

The *R* value between each toosendanin isomeric peak and their corresponding closest peaks in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of toosendanin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the sum of peak area of toosendanin isomers against the corresponding concentrations of toosendanin 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 each toosendanin isomeric peak in the chromatogram of the test solution by comparing their retention times with that in the chromatogram of toosendanin Std-AS. The retention times of toosendanin isomeric peaks from the two chromatograms should not differ by more than 5.0%. Measure the sum of peak area of toosendanin isomers and calculate the concentration (in milligram per litre) of toosendanin in the test solution, and calculate the percentage content of toosendanin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains 0.060% to 0.20% of toosendanin ($C_{30}H_{38}O_{11}$), calculated with reference to the dried substance.