Tribuli Fructus

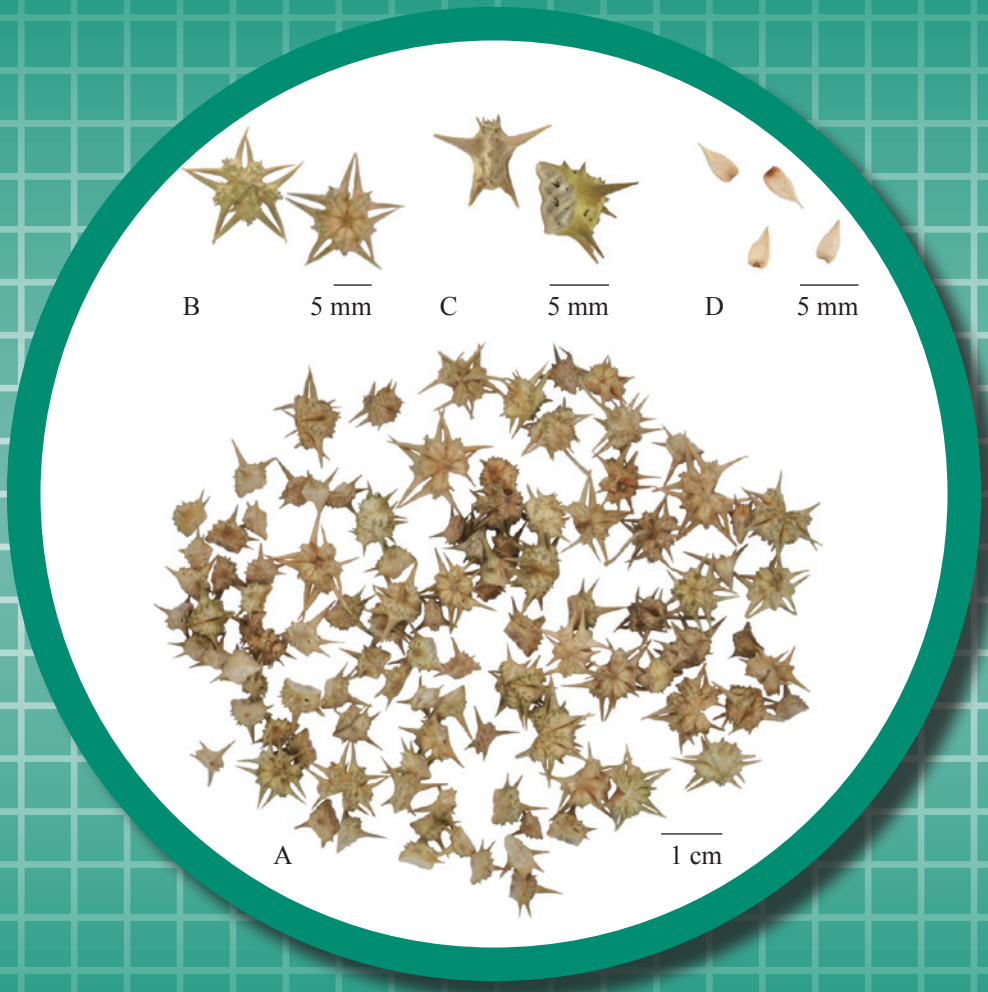

Figure 1 A photograph of Tribuli Fructus

A. Tribuli Fructus B. Magnified fruits (left: top view, right: bottom view)
C. Magnified mericarps D. Magnified seeds

1. NAMES

Official Name: Tribuli Fructus

Chinese Name: 蒺藜

Chinese Phonetic Name: Jili

2. SOURCE

Tribuli Fructus is the dried ripe fruit of *Tribulus terrestris* L. (Zygophyllaceae). The plant is collected in autumn when the fruit is ripe, dried under the sun, then the fruit is tapped out and foreign matter removed to obtain Tribuli Fructus.

3. DESCRIPTION

Intact fruit consists of 5 radically arranged mericarps, pentagonal or star-like, 9-18 mm in diameter, externally yellowish-green to yellowish-brown, often splitting into single mericarps. Mericarp dolabriform, 3-9 mm long, the dorsal side prominent, with longitudinal ridges and numerous spinules, bearing symmetrically splaying pairs of long and short spines each; the lateral side relatively thin, white to yellowish-white, with several oblique ridges. Each mericarp contains 3-4 seeds. Seeds long ovate, flat, white to yellowish-white, oily. Texture hard. Odour slight; taste bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of flat cells. Mesocarp consists of several layers of parenchymatous cells, with small vascular bundles scattered; conical fibre bundles located at the spine part, with group of stone cells at the base; and 1-2 layers of cells contain prisms of calcium oxalate located near the endocarp. Endocarp consists of fibres arranged in a crisscross fashion. Testa consists of 1 layer of closely packed cells. Cotyledon cells contain oil droplets (Fig. 2).

Powder

Colour yellowish-green to pale brownish-yellow. Prisms of calcium oxalate numerous, rhombus, subsquare or subpolygonal, 8-72 μm long, 6-58 μm in diameter; polychromatic under the polarized microscope. Stone cells scattered or in groups, pale yellow, subrounded, subelliptical or irregular, 16-140 μm long, 11-58 μm in diameter, with distinct pits, pit canals and striations, those with thick walls show narrow lumens; yellowish-white under the polarized microscope. Epidermal cells of testa yellow to pale yellowish-brown, polygonal or subsquare in surface view, walls reticulately thickened. Exocarp cells polygonal or subsquare in surface view, sometimes with annular scars of non-glandular hair. Fibres scattered, in bundles or arranged in crisscross pattern, 6-55 μm in diameter, pits sparse; yellowish-white to orange-yellow under the polarized microscope. Vessels mainly spiral and reticulate, 4-26 μm in diameter (Fig. 3).

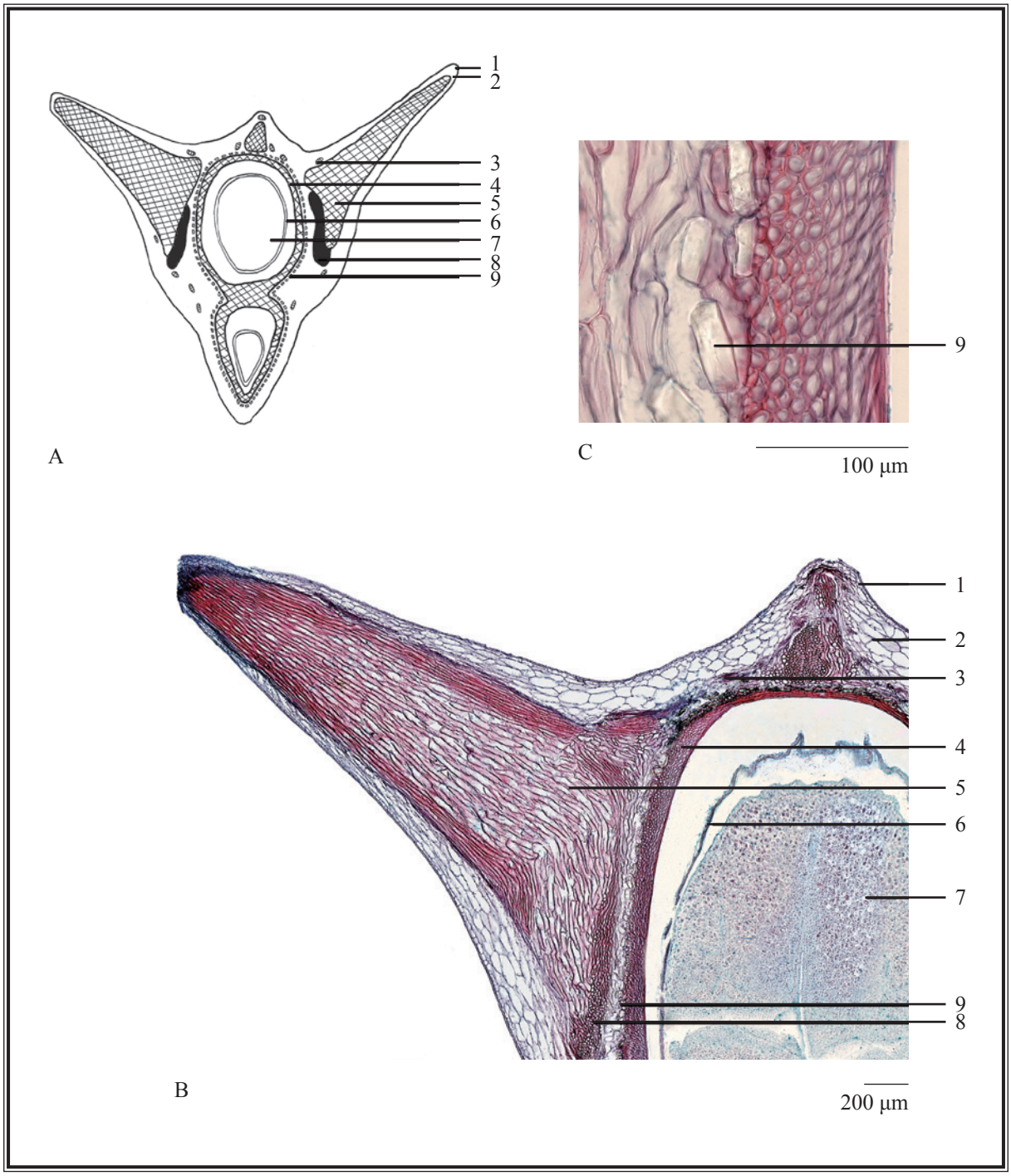

Figure 2 Microscopic features of transverse section of Tribuli Fructus

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

1. Exocarp 2. Mesocarp 3. Vascular bundle 4. Endocarp 5. Fibre bundle
6. Testa 7. Cotyledon 8. Group of stone cells 9. Prisms of calcium oxalate

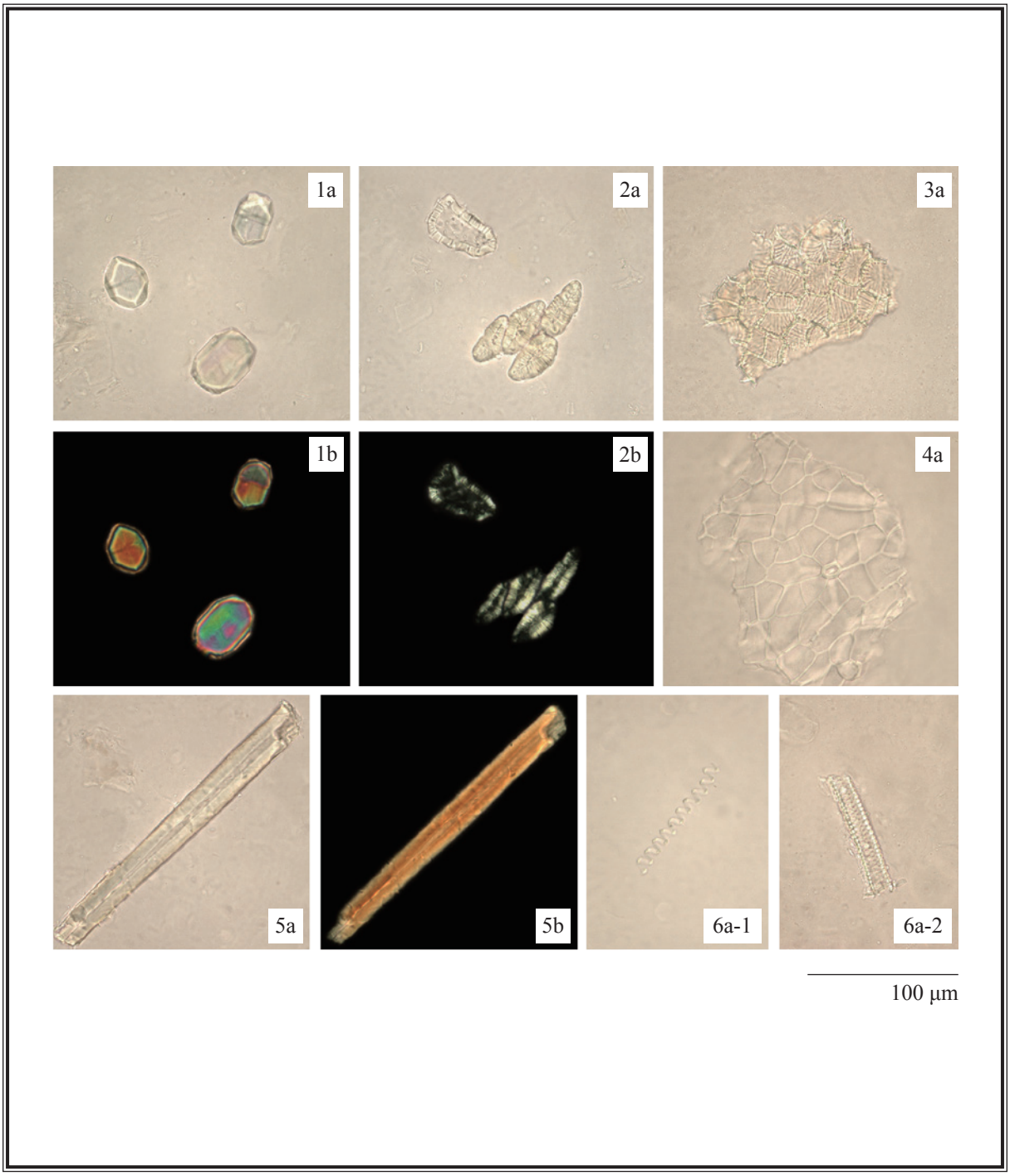

Figure 3 Microscopic features of powder of Tribuli Fructus

1. Prisms of calcium oxalate 2. Stone cells 3. Epidermal cells of testa
4. Exocarp cells 5. Fibre 6. Vessels (6-1 spiral vessel, 6-2 reticulate vessel)

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

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

Standard solution

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

Weigh 0.5 mg of quercetin-3-*O*-β-D-gentiobioside CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, methanol, water and formic acid (5:1:1:0.5, v/v).

Spray reagent

Weigh 2.5 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. 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 quercetin-3-*O*-β-D-gentiobioside standard solution (1.5 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 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 85ºC until the spots or bands become visible (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

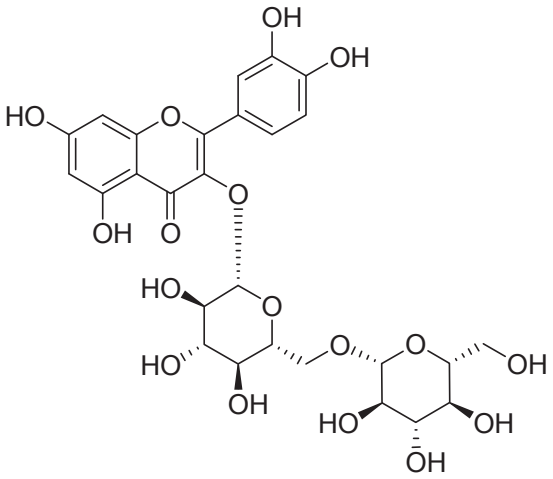

Figure 4 Chemical structure of quercetin-3-*O*-β-D-gentiobioside

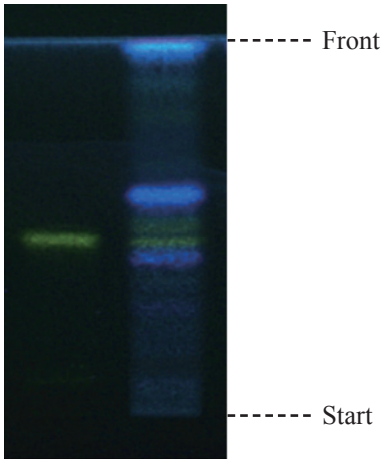

Figure 5 A reference HPTLC chromatogram of Tribuli Fructus extract observed under UV light (366 nm) after staining

1. Quercetin-3-*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-gentiobioside (Fig. 5).

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

Standard solution

Quercetin-3-O-β-D-gentiobioside standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.5 mg of quercetin-3-*O*-β-D-gentiobioside CRS and dissolve in 100 mL of methanol (70%).

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 (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Wash the residue with methanol (70%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants 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 (254 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 45	88 → 80	12 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercetin-3-*O*-β-D-gentiobioside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercetin-3-*O*-β-D-gentiobioside should not be more than 5.0%; the RSD of the retention time of quercetin-3-*O*-β-D-gentiobioside peak should not be more than 2.0%; the column efficiency determined from quercetin-3-*O*-β-D-gentiobioside peak should not be less than 10000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject quercetin-3-*O*-β-D-gentiobioside Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of quercetin-3-*O*-β-D-gentiobioside peak in the chromatogram of quercetin-3-*O*-β-D-gentiobioside Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify quercetin-3-*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-gentiobioside Std-FP. The retention times of quercetin-3-*O*-β-D-gentiobioside 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 three characteristic peaks of Tribuli Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Tribuli Fructus extract

Peak No.	RRT	Acceptable Range
1	0.34	± 0.03
2 (marker, quercetin-3-*O*-β-D-gentiobioside)	1.00	-
3	1.45	± 0.03

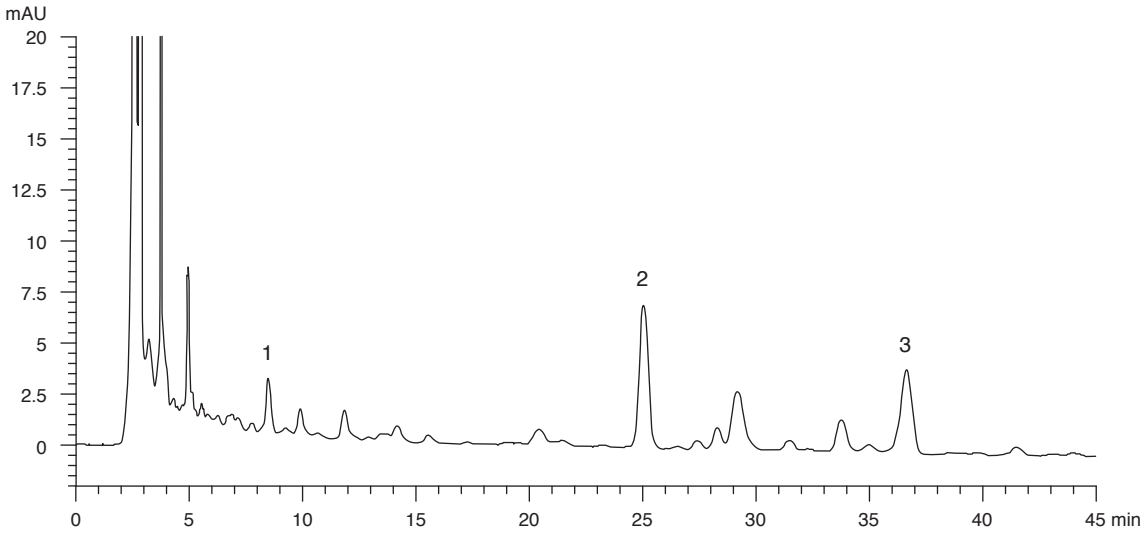

Figure 6 A reference fingerprint chromatogram of Tribuli Fructus extract

For positive identification, the sample must give the above three 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 12.0%.
Acid-insoluble ash: not more than 1.0%.

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 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

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

Standard solution

Quercetin-3-O-β-D-gentiobioside standard stock solution, Std-Stock (600 mg/L)

Weigh accurately 6.0 mg of quercetin-3-*O*-β-D-gentiobioside CRS and dissolve in 10 mL of methanol (70%).

Quercetin-3-O-β-D-gentiobioside standard solution for assay, Std-AS

Measure accurately the volume of the quercetin-3-*O*-β-D-gentiobioside Std-Stock, dilute with methanol (70%) to produce a series of solutions of 1.2, 3, 6, 30, 60 mg/L for quercetin-3-*O*-β-D-gentiobioside.

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 (70%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Wash the residue with methanol (70%). Centrifuge at about 3500 × *g* for 10 min. Combine the supernatants 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 (254 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 45	88 → 80	12 → 20	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercetin-3-*O*-β-D-gentiobioside Std-AS (6 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercetin-3-*O*-β-D-gentiobioside should not be more than 5.0%; the RSD of the retention time of quercetin-3-*O*-β-D-gentiobioside peak should not be more than 2.0%; the column efficiency determined from quercetin-3-*O*-β-D-gentiobioside peak should not be less than 10000 theoretical plates.

The *R* value between quercetin-3-*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-gentiobioside Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of quercetin-3-*O*-β-D-gentiobioside against the corresponding concentrations of quercetin-3-*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-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-gentiobioside Std-AS. The retention times of quercetin-3-*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-gentiobioside in the test solution, and calculate the percentage content of quercetin-3-*O*-β-D-gentiobioside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.022% of quercetin-3-*O*-β-D-gentiobioside ($C_{27}H_{30}O_{17}$), calculated with reference to the dried substance.