Rubi Fructus

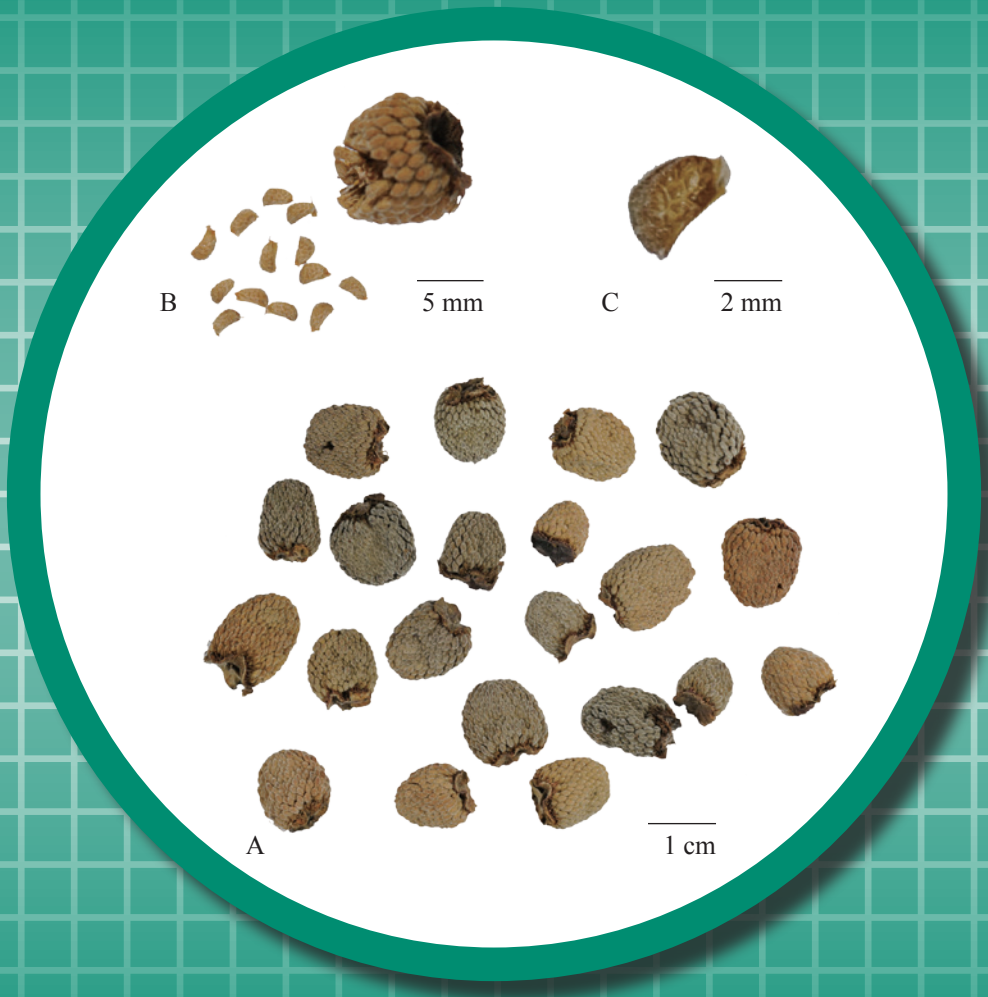

Figure 1 A photograph of Rubi Fructus

A. Rubi Fructus B. Magnified fruit and drupelets C. Magnified drupelet

1. NAMES

Official Name: Rubi Fructus

Chinese Name: 覆盆子

Chinese Phonetic Name: Fupenzi

2. SOURCE

Rubi Fructus is the dried fruit of *Rubus chingii* Hu (Rosaceae). The fruit is collected in early summer when it turns from green to greenish-yellow; fruit stalk and leaves removed; the fruit is immersed in boiling water for a moment or steamed briefly, then dried under the sun to obtain Rubi Fructus.

3. DESCRIPTION

An aggregate fruit, the shape conical or flattened-conical, 0.4-1.8 cm high, 4-14 mm in diameter. Externally yellowish-green to pale brown, apex obtuse, base depressed at the centre, consisting of numerous drupelets. Persistent calyx brown, bearing the mark of the scar of fruit stalk. Drupelets easily fallen off, crescent-shaped, the dorsal surface densely covered with greyish-white pubescence, with reticulate striations distinct on both sides, the ventral surface bear protuberant ridges. Texture hard and light in weight. Odour slight; taste slightly sour and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Aggregate Fruit: Receptacle rounded, surrounded with numerous drupelets. Vascular bundles collateral, arranged in a ring (Fig. 2).

Drupelet: Exocarp consists of 1 layer of cells, covered with sinuous cuticle at outer margin; with unicellular non-glandular hairs on dorsal surface. Mesocarp consists of 3-5 layers of cells, some containing clusters of calcium oxalate. Endocarp broad, outer part consists of several layers of parenchymatous cells, border with fusiform protuberance, wall lignified, inner part consists of several layers of fibres, crisscross or obliquely alternate. Testa consists of 1 layer of cells, tangentially elongated, containing brown pigments. Endosperm and cotyledon cells contain aleurone grains (Fig. 2).

Powder

Colour brownish-yellow. Non-glandular hairs numerous, unicellular, 35-514 μm long, 3-22 μm in diameter, walls greatly thickened, lignified, some with double-spiral striations. Pericarp epidermal cells sometimes with scars of non-glandular hairs, scars embed in epidermis, rounded-polygonal or oblong in surface view, lumens branched, similar to stone cells in shape, 7-38 μm in diameter. Clusters of calcium oxalate frequently visible, 6-45 μm in diameter; polychromatic under the polarized microscope. Fibres of endocarp in bundles, yellow, arrange in crisscross pattern or obliquely alternate; white to brownish-yellow under the polarized microscope (Fig. 3).

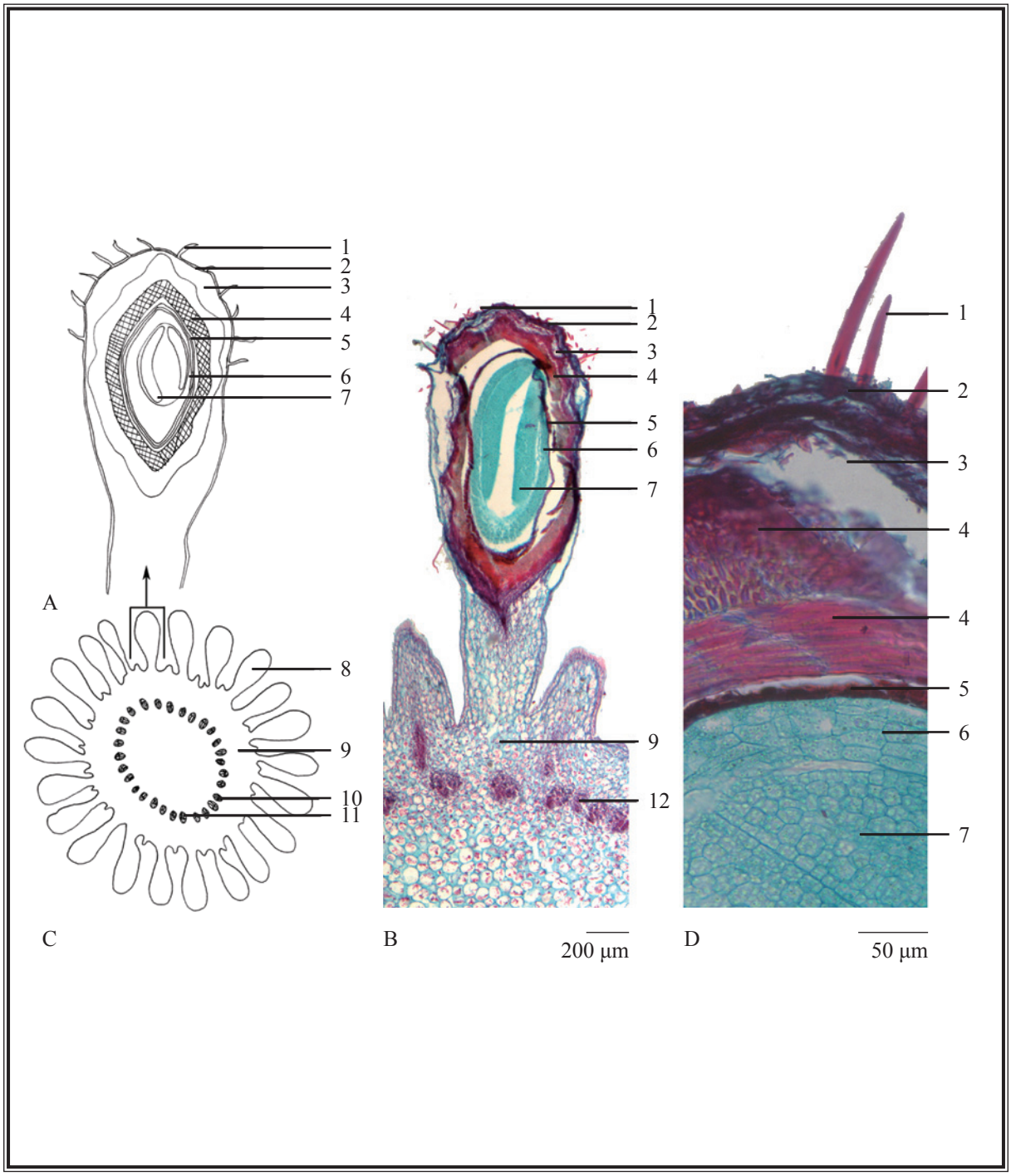

Figure 2 Microscopic features of transverse section of Rubi Fructus

A. Sketch of drupelet B. Section illustration
C. Sketch of aggregate fruit D. Magnified section illustration of drupelet

1. Non-glandular hair 2. Exocarp 3. Mesocarp 4. Endocarp 5. Testa
6. Endosperm 7. Cotyledon 8. Drupelet 9. Receptacle 10. Phloem
11. Xylem 12. Collateral vascular bundle

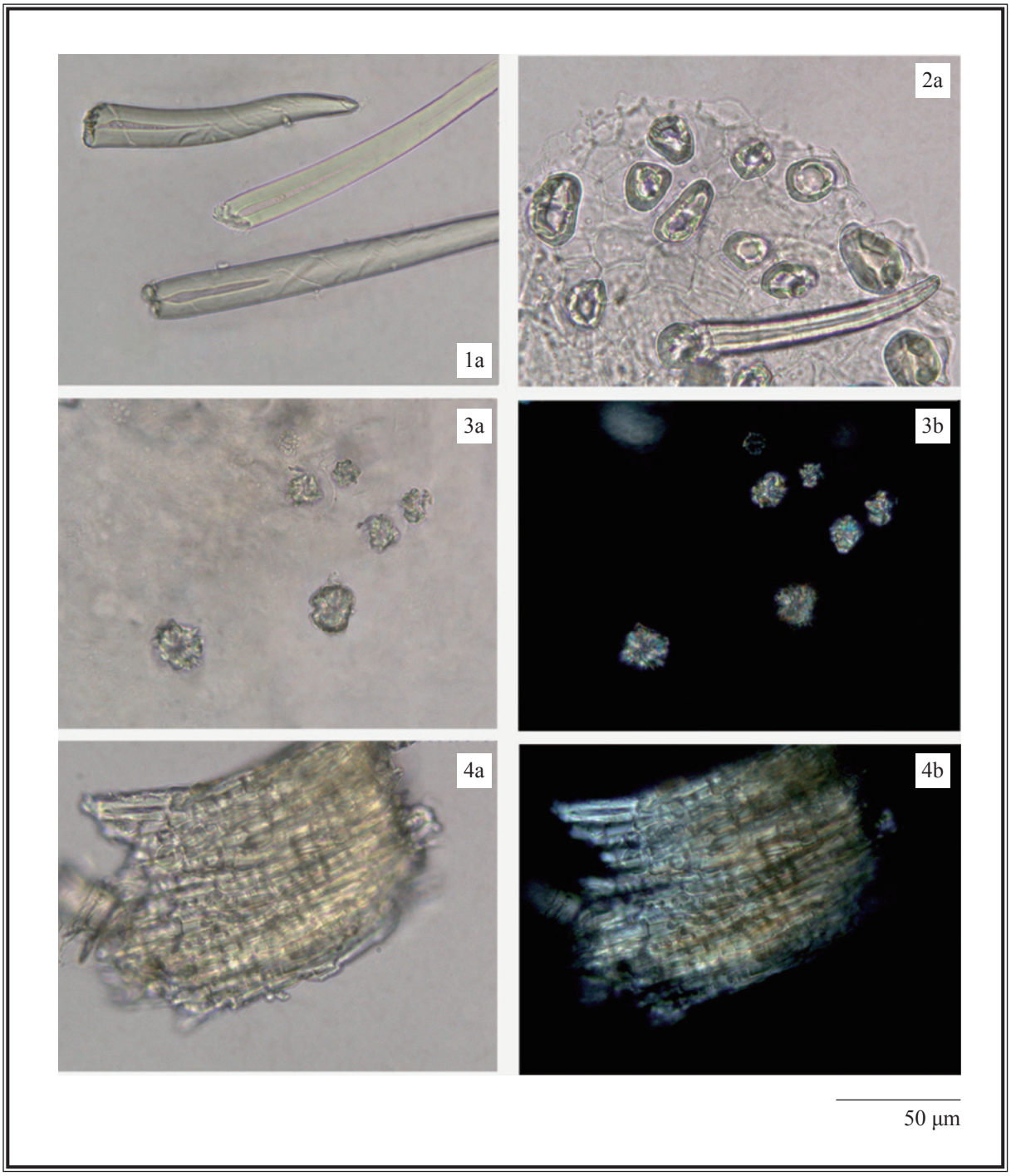

Figure 3 Microscopic features of powder of Rubi Fructus

1. Non-glandular hairs
2. Pericarp epidermal cells with scars and remains of non-glandular hairs
3. Clusters of calcium oxalate 4. Fibres of endocarp

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

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

Standard solution

Ellagic acid standard solution

Weigh 4.5 mg of ellagic acid CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, formic acid, ethyl acetate and water (6:2.5:2:0.5, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of ethanol (70%). Sonicate (150 W) the mixture for 10 min. Centrifuge at about 3000 × *g* for 5 min. Filter the mixture.

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 ellagic acid standard solution (0.3 μL) and the test solution (4 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 105ºC until the spots or bands become visible (about 1-3 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

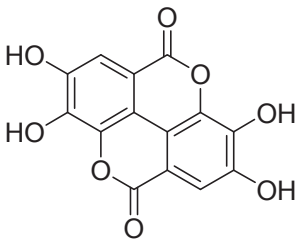

Figure 4 Chemical structure of ellagic acid

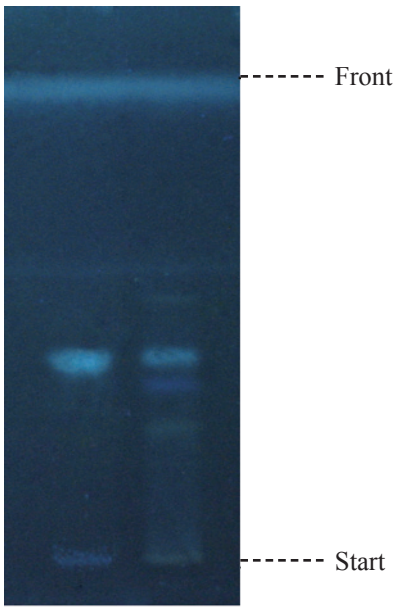

Figure 5 A reference HPTLC chromatogram of Rubi Fructus extract observed under UV light (366 nm) after staining

1. Ellagic acid 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 ellagic acid (Fig. 5).

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

Standard solution

Ellagic acid standard solution for fingerprinting, Std-FP (80 mg/L)

Weigh 0.8 mg of ellagic acid CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of ethanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 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)	Methanol (%, v/v)	0.5% Acetic acid (%, v/v)	Elution
0 – 15	20 → 35	80 → 65	linear gradient
15 – 30	35 → 45	65 → 55	linear gradient
30 – 50	45 → 65	55 → 35	linear gradient
50 – 60	65	35	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of ellagic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ellagic acid should not be more than 5.0%; the RSD of the retention time of ellagic acid peak should not be more than 2.0%; the column efficiency determined from ellagic acid peak should not be less than 25000 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 ellagic acid Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of ellagic acid peak in the chromatogram of ellagic acid Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify ellagic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ellagic acid Std-FP. The retention times of ellagic acid 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 Rubi Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Rubi Fructus extract

Peak No.	RRT	Acceptable Range
1	0.44	± 0.03
2 (marker, ellagic acid)	1.00	-
3 (tiliroside)	1.43	± 0.03
4	1.46	± 0.03

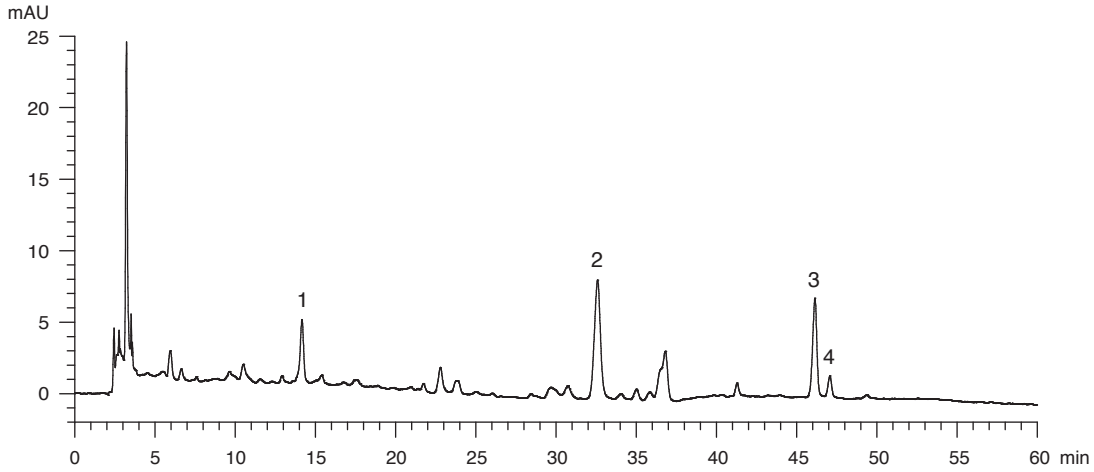

Figure 6 A reference fingerprint chromatogram of Rubi Fructus extract

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 – 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 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution

Ellagic acid standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of ellagic acid CRS and dissolve in 10 mL of methanol.

Ellagic acid standard solution for assay, Std-AS

Measure accurately the volume of the ellagic acid Std-Stock, dilute with methanol to produce a series of solutions of 2.5, 5, 10, 20, 40 mg/L for ellagic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of ethanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 3000 × *g* for 5 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 ethanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (258 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)	Methanol (%, v/v)	0.5% Acetic acid (%, v/v)	Elution
0 – 5	35	65	isocratic
5 – 10	35 → 50	65 → 50	linear gradient
10 – 30	50	50	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 µL of ellagic acid Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of ellagic acid should not be more than 5.0%; the RSD of the retention time of ellagic acid peak should not be more than 2.0%; the column efficiency determined from ellagic acid peak should not be less than 25000 theoretical plates.

The *R* value between ellagic acid 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 ellagic acid Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of ellagic acid against the corresponding concentrations of ellagic acid Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify ellagic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ellagic acid Std-AS. The retention times of ellagic acid 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 ellagic acid in the test solution, and calculate the percentage content of ellagic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.17% of ellagic acid ($C_{14}H_6O_8$), calculated with reference to the dried substance.