Phyllanthi Fructus

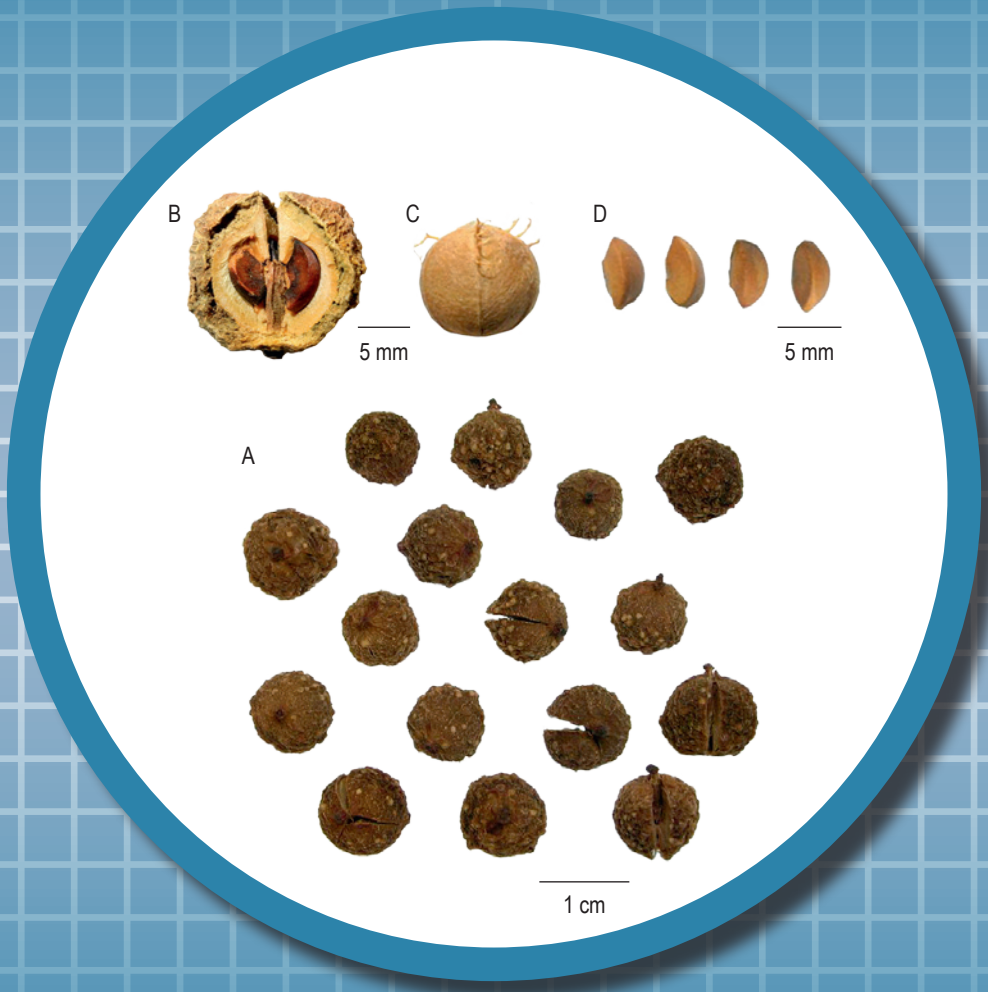


Figure 1 A photograph of Phyllanthi Fructus

A. Phyllanthi Fructus B. Longitudinal section C. Outer surface of endocarp
D. Seeds

1. NAMES

Official Name: Phyllanthi Fructus

Chinese Name: 餘甘子

Chinese Phonetic Name: Yuganzi

2. SOURCE

Phyllanthi Fructus is the dried ripe fruit of *Phyllanthus emblica* L. (Euphorbiaceae). The ripe fruit is collected in October to December, foreign matter removed, dried under the sun to obtain Phyllanthi Fructus.

3. DESCRIPTION

Spheroidal or flattish-spheroidal, some cracked, 12-20 mm in diameter. Externally brown to dark green, with pale yellow granular protuberance, wrinkles, and 6 indistinct ribs. Fruit stalk about 1 mm long. Exocarp fused with mesocarp, 1-4 mm thick, texture hard and fragile. Endocarp yellowish-white, hard kernel-like, showing 6 indistinct ribs on the surface and several veins striations (vascular bundles) at the upper part of dorsal suture, cracking to 6 valves after dried. Seeds 6, subtriquetrous, brown. Odour slight; taste sour, astringent and followed by sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Transverse section subrounded, externally with 6 raised ribs, and divided into 3 loculi by the septum which radiates from the centre. Exocarp consists of 1 layer subrounded epidermal cell, arranged regularly, covered with thick cuticle. Septum consists of several rows of flattened cells, cells relatively small, arranged densely. The 2-7 layers of sclerenchymatous cells located underneath the epidermis, subrounded, elongated-rounded or subpolygonal. Mesocarp relatively thick, consisting of parenchymatous cells, the cells often containing crystals of calcium oxalate, small vascular bundles scattered throughout the mesocarp. Endocarp consists

of sclerenchymatous cells; 4-6 layers of stone cells arranged in a dense layer on the outer side, the cells relatively small, with extremely thickened wall and indistinct lumen; next 5-7 layers of slender, slat-shaped lignified fibres arranged in the middle part, palisade-like; 4-6 layers of subpolygonal sclerenchymatous cells situated in the innermost side, with relatively thickened wall, and with a distinct lumen. Each locule contains 2 subtriangular seeds. Each seed bordered by a testa 2-3 layers of palisade-like cell located between testa and endotesta, arranged densely, with a relatively thickened wall and dense pit canals. Pigment layer very thin, located immediately inside the endotesta; the cells subpolygonal or subrounded, the lumen containing reddish-brown contents. Endosperm cells subpolygonal, containing numerous clusters of calcium oxalate and some starch granules. Cotyledon cells relatively small, subpolygonal, with distinct nuclei (Fig. 2).

Powder

Colour brown. Epidermal cells of exocarp yellow to nearly colourless, subpolygonal or subsquare, 25-40 μm in diameter, wall slightly thickened. Fibres of endocarp abundant, scattered singly or in groups, nearly colourless, slat-shaped, 10-30 μm in diameter, wall with sparse pit canals. Pigment cells scattered singly or in groups, subpolygonal or subrounded, lumen containing reddish-brown contents. Sclerenchymatous cells scattered singly or in groups, pale yellow or nearly colourless, subpolygonal or subrounded, 100-163 μm long, 50-88 μm wide, wall relatively thickened, 13-23 μm thick, pit canals distinct. Stone cells scattered singly or in groups, pale yellow to nearly colourless, subpolygonal or subrounded, 63-100 μm long, 50-75 μm wide, wall extremely thickened, 25-38 μm thick, lumen indistinct, pit canals fine and dense. Palisade cells of testa pale yellow to nearly colourless, polygonal in surface view, cylindrical or subpolygonal in the section view, arranged densely, 125-175 μm long, 50-100 μm wide, wall thickened, pit canals extremely fine and dense, lumen large. Crystals of calcium oxalate relatively abundant, polyhedral, rhombic, subsquare or irregular, some broken; polychromatic under the polarized microscope. Clusters of calcium oxalate found in endosperm cells, approximately equal in size, 5-9 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

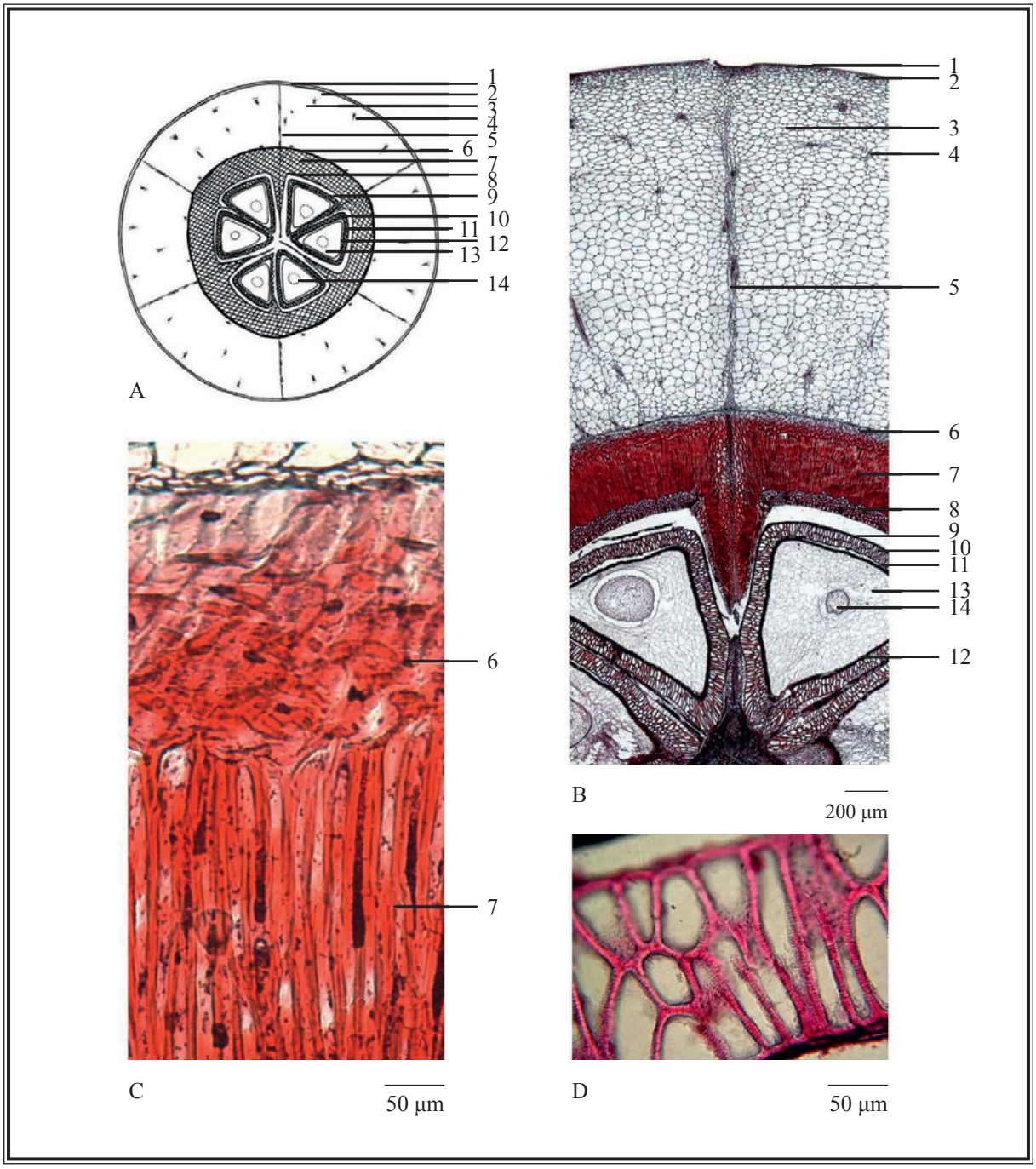


Figure 2 Microscopic features of transverse section of Phyllanthi Fructus

A. Sketch B. Section illustration C. Fibre of endocarp
D. Palisade tissue of testa

1. Epidermis 2. Sclerenchyma of exocarp 3. Mesocarp 4. Vascular bundle
5. Septum 6. Stone cells of endocarp 7. Fibres of endocarp
8. Sclerenchyma of endocarp 9. Testa 10. Palisade tissue of testa
11. Endotesta 12. Pigment layer 13. Endosperm 14. Cotyledon

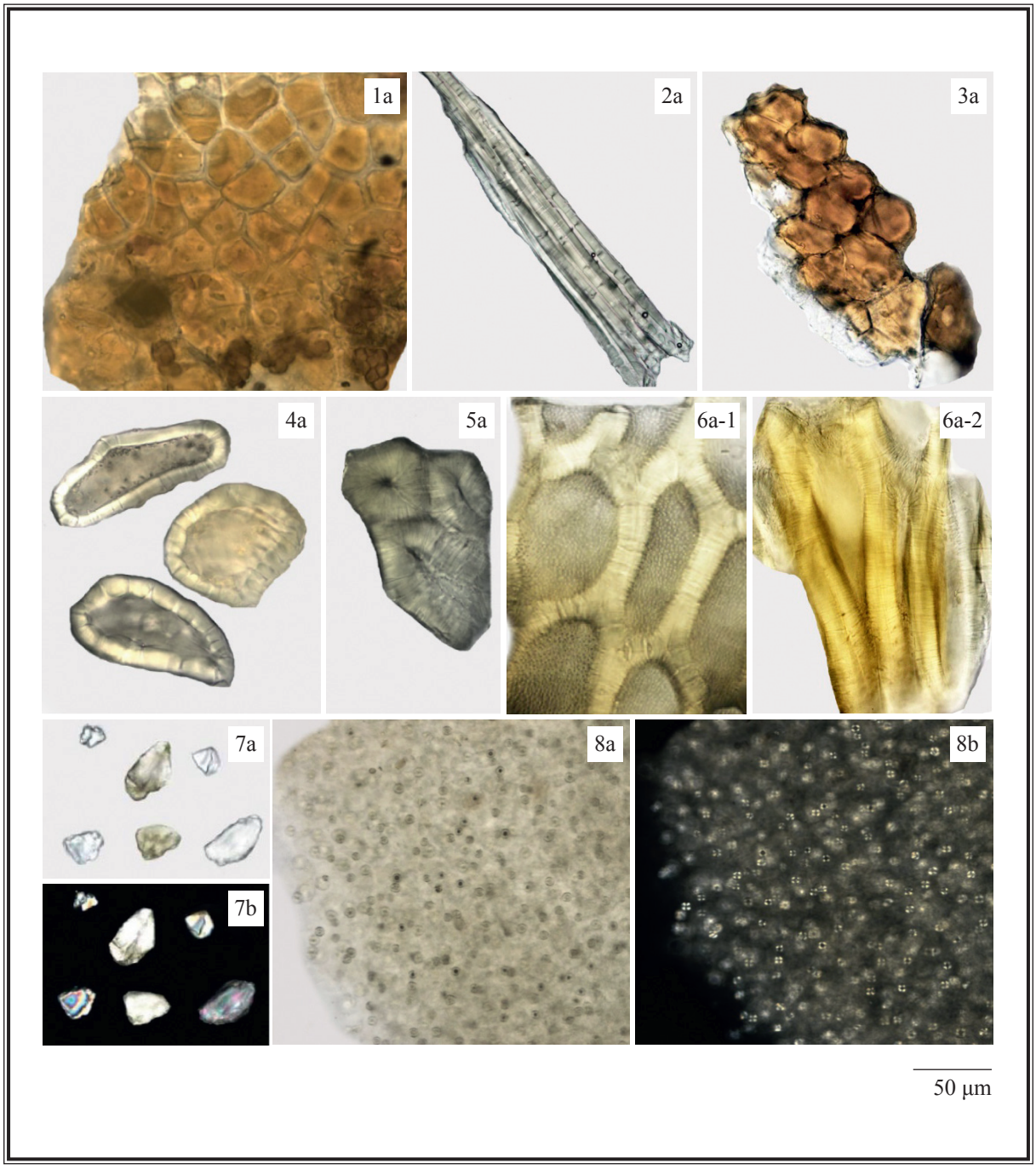


Figure 3 Microscopic features of powder of Phyllanthi Fructus

1. Epidermal cells of exocarp 2. Fibres of endocarp 3. Pigment cells
4. Sclerenchymatous cells 5. Stone cells 6. Palisade cells of testa (6-1 in surface view, 6-2 in section view)
7. Crystals of calcium oxalate 8. Clusters 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 solutions

Gallic acid standard solution

Weigh 0.5 mg of gallic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Ellagic acid standard solution

Weigh 0.5 mg of ellagic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of dichloromethane, formic acid, ethyl acetate and water (7:2.5:2:0.5, v/v).

Spray reagent

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

Test solution

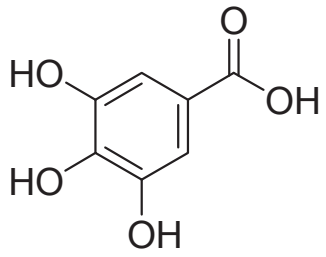
Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of ethanol (70%). Sonicate (100 W) the mixture for 10 min. Filter the mixture.

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 gallic acid standard solution (4 µL), ellagic acid standard solution (2 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 7 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 ºC until the spots or bands become visible (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

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 gallic acid and ellagic acid.

(i)



(ii)

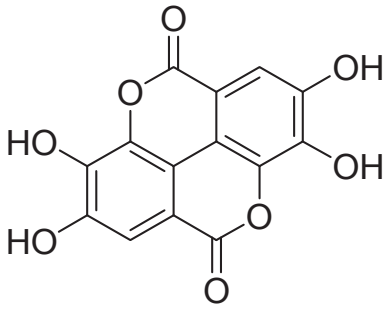


Figure 4 Chemical structures of (i) gallic acid and (ii) ellagic acid

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

Standard solutions

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

Weigh 0.5 mg of gallic acid CRS and dissolve in 20 mL of methanol.

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

Weigh 0.5 mg of ellagic acid CRS and dissolve in 20 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (100 W) the mixture for 30 min. 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 (273 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)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 30	5	95	isocratic
30 – 35	5 → 50	95 → 50	linear gradient
35 – 60	50	50	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 µL of gallic acid Std-FP and ellagic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of gallic acid and ellagic acid should not be more than 5.0%; the RSD of the retention times of gallic acid and ellagic acid peaks should not be more than 2.0%; the column efficiencies determined from gallic acid and ellagic acid peaks should not be less than 4000 and 90000 theoretical plates respectively.

The *R* value between peak 1 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 1.0 (Fig. 5).

Procedure

Separately inject gallic acid Std-FP, ellagic acid Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention times of gallic acid and ellagic acid peaks in the chromatograms of gallic acid Std-FP, ellagic acid Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify gallic acid and ellagic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of gallic acid Std-FP and ellagic acid Std-FP. The retention times of gallic acid and ellagic acid 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 Phyllanthi Fructus extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (gallic acid)	0.36	± 0.03
2	0.50	± 0.03
3	0.56	± 0.03
4 (marker, ellagic acid)	1.00	-

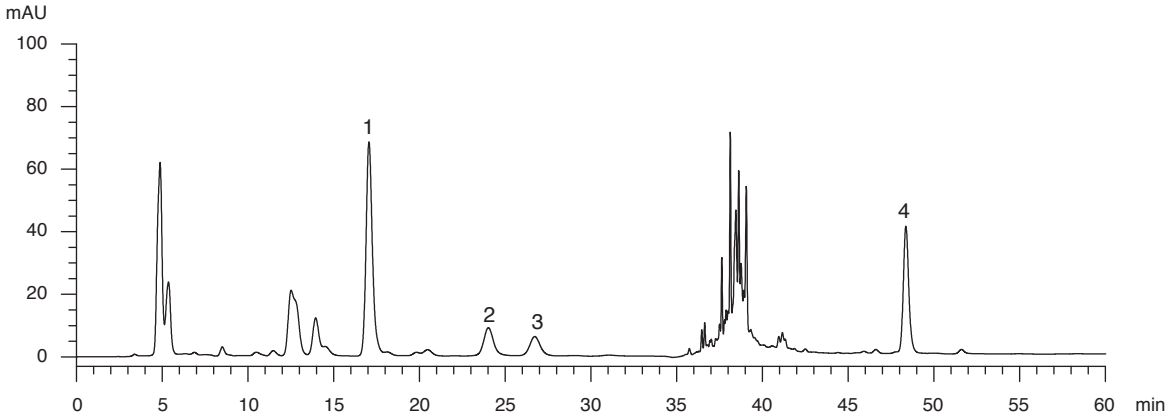


Figure 5 A reference fingerprint chromatogram of Phyllanthi Fructus extract

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. 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 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 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 33.0%.
Ethanol-soluble extractives (hot extraction method): not less than 16.0%.

7. ASSAY

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

Standard solution

Mixed gallic acid and ellagic acid standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of gallic acid CRS and 2.0 mg of ellagic acid CRS, and dissolve in 10 mL of methanol.

Mixed gallic acid and ellagic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed gallic acid and ellagic acid Std-Stock, dilute with methanol to produce a series of solutions of 5, 10, 25, 50, 80 mg/L for both gallic acid and ellagic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction for three more times. Combine the supernatants and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (273 nm for gallic acid and 254 nm for ellagic acid) 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)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 18	5	95	isocratic
18 – 22	5 → 50	95 → 50	linear gradient
22 – 60	50	50	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed gallic acid and ellagic acid Std-AS (25 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of gallic acid and ellagic acid should not be more than 5.0%; the RSD of the retention times of gallic acid and ellagic acid peaks should not be more than 2.0%; the column efficiencies determined from gallic acid and ellagic acid peaks should not be less than 7000 and 50000 theoretical plates respectively.

The *R* value between gallic acid peak and the closest peak; and 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 curves

Inject a series of the mixed gallic acid and ellagic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of gallic acid and ellagic acid against the corresponding concentrations of the mixed gallic acid and ellagic acid 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 gallic acid and ellagic acid peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed gallic acid and ellagic acid Std-AS. The retention times of gallic acid and ellagic acid 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 gallic acid and ellagic acid in the test solution, and calculate the percentage contents of gallic acid and ellagic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 2.9% of the total content of gallic acid ($C_7H_6O_5$) and ellagic acid ($C_{14}H_6O_8$), calculated with reference to the dried substance.