# Polygoni Chinensis Herba


**Figure 1** A photograph of Polygoni Chinensis Herba

A. Polygoni Chinensis Herba

B. Magnified image of upper surface of leaf

C. Magnified image of lower surface of leaf

D. Magnified image of stem

E. Magnified image of transverse section of stem

## 1. NAMES

Official Name: Polygoni Chinensis Herba

Chinese Name: 火炭母

Chinese Phonetic Name: Huotanmu

## 2. SOURCE

Polygoni Chinensis Herba is the dried aerial part of *Polygonum chinense* L. (Polygonaceae). The aerial part is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Polygoni Chinensis Herba.

## 3. DESCRIPTION

Stem compressed-cylindrical, branched, 20-40 cm long, nodes slightly swollen, the lower part of nodes with rootlets; externally pale green to purplish-brown, glabrous, with ridges; texture fragile, easily broken, fracture greyish-yellow, usually hollowed. Leaves simple, alternate, mostly crumpled and broken, when whole, ovate-oblong in shape, 5-10 cm long, 2-5 cm wide; Apex short-acute, base truncate or slightly rounded, margins entire; the upper surface dark green with a purplish-black or greyish-white V shaped pattern on it and the lower surface lighter in colour; membranaceous ochrea tubular, apex oblique. Flowers small, orange-yellow in colour. Odour slight; taste sour, slightly astringent (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification *(Appendix III)*

**Transverse Section**

**Stem:** Epidermis consists of 1 layer of cells, cells tangentially elongated, some containing reddish-brown pigment, covered with thin cuticle. Collenchyma consists of 1-2 layers of cells, underneath the epidermis. Cortex consists of several layers of parenchymatous cells. Pericycle fibres consist of 1-5 layers of cells, arranged in an interrupted ring. Phloem relatively broad. Xylem vessels 2-6 arranged. Pith large, the cells polygonal or irregular in shape. Some parenchymatous cells contain clusters of calcium oxalate [Fig 2 (i)].

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**Leaf:** Upper and lower epidermis consists of 1 layer of rectangular cells. Mesophyll consists of 1 layer of palisade tissue and several layers of spongy tissue. Collenchyma consists of several layers of cells, located underneath the upper and lower epidermis of the midrib. Fibres in group, lignified. Vascular bundle of midrib 2-6, collateral, arranged in an interrupted ring. Clusters of calcium oxalate scattered in the parenchymatous cells. Non-glandular hairs raised from upper and lower epidermis [Fig 2 (ii)].

**Powder**

Colour yellowish-green. Epidermal cells of leaf subpolygonal, some with cuticle striation; anomocytic stomata relatively dense. Epidermal cells of stem subrectangular, some with cuticle striation. Non-glandular hairs multicellular and multiseriate, arranged imbricately. Glandular hairs unicellular or multicellular. Clusters of calcium oxalate abundant, 20-80 μm in diameter, with acute angles; pale white or polychromatic under the polarized microscope. Pollen grains subspherical, with distinct reticulate-polygonal sculptures, 26-40 μm in diameter. Vessels mainly reticulate, 36-70 μm in diameter (Fig. 3).

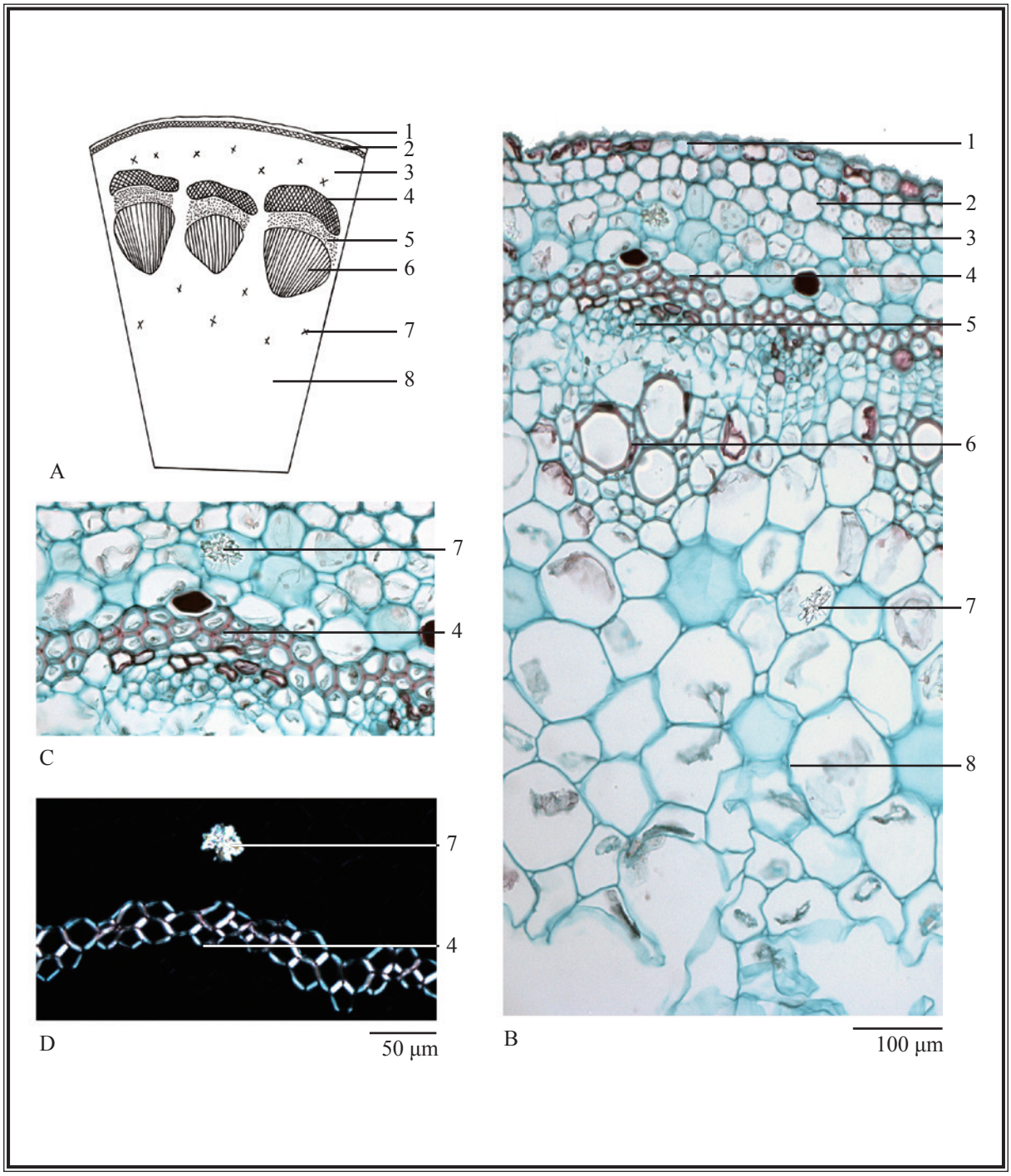


**Figure 2 (i)** Microscopic features of transverse section of stem of Polygoni Chinensis Herba

A. Sketch B. Section illustration

C. Cluster of calcium oxalate (under the light microscope)

D. Cluster of calcium oxalate (under the polarized microscope)

1. Epidermis 2. Collenchyma 3. Cortex 4. Pericycle fibre

5. Phloem 6. Xylem 7. Cluster of calcium oxalate 8. Pith

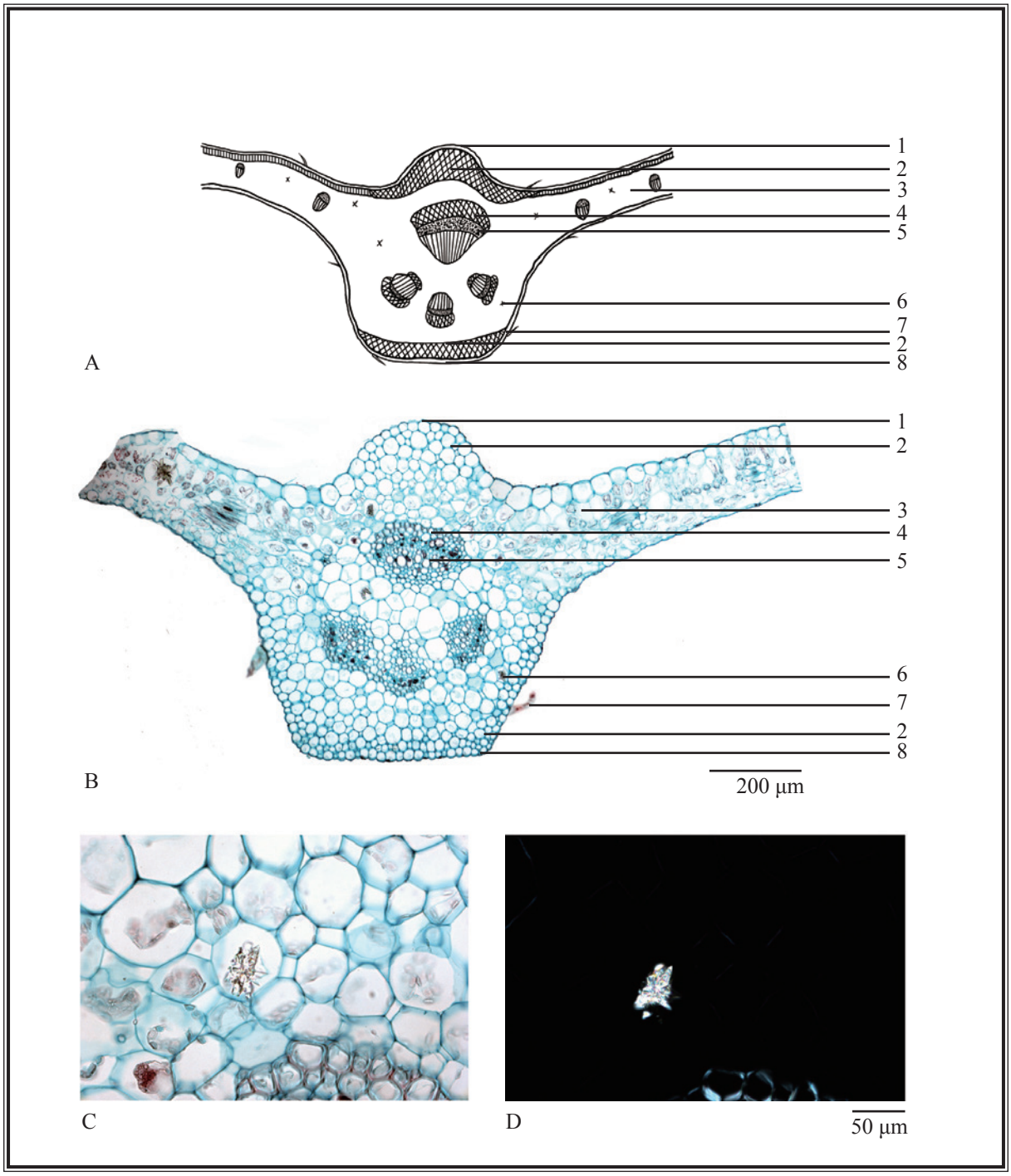


**Figure 2 (ii)** Microscopic features of transverse section of leaf of Polygoni Chinensis Herba

A. Sketch B. Section illustration

C. Cluster of calcium oxalate (under the light microscope)

D. Cluster of calcium oxalate (under the polarized microscope)

1. Upper epidermis 2. Collenchyma 3. Mesophyll 4. Fibre 5. Vascular bundle

6. Cluster of calcium oxalate 7. Non-glandular hair 8. Lower epidermis

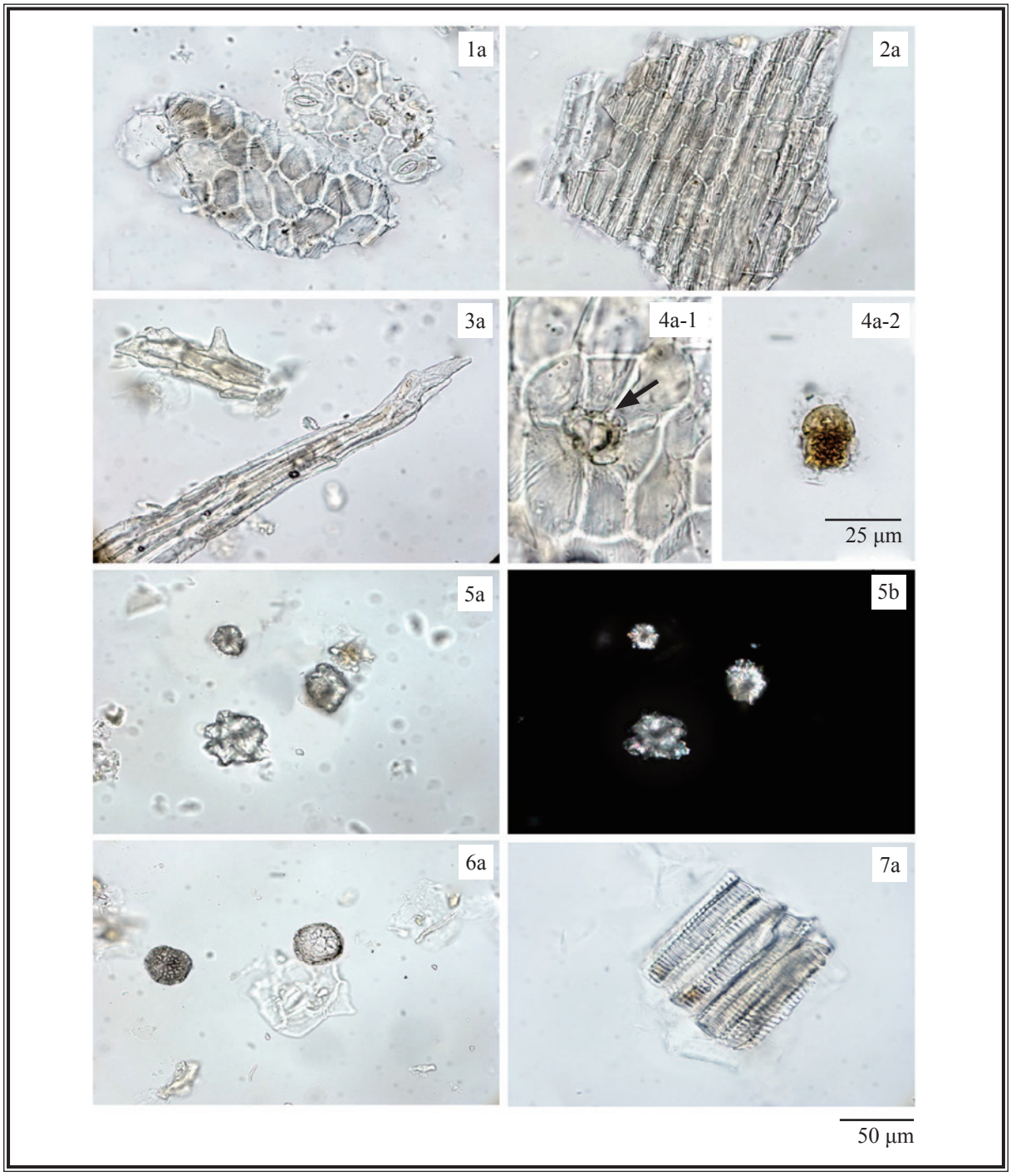


**Figure 3** Microscopic features of powder of Polygoni Chinensis Herba

1. Epidermal cells of leaf 2. Epidermal cells of stem 3. Non-glandular hairs
4. Glandular hairs (4-1 surface view →, 4-2 lateral view) 5. Clusters of calcium oxalate
6. Pollen grains 7. Vessels

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 0.2 mg of ellagic acid CRS (Fig. 4) and dissolve in 2 mL of methanol.

**Developing solvent system**

Prepare a mixture of dichloromethane, ethyl acetate, formic acid and water (4:4:2:0.5, 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 methanol. Sonicate (100 W) the mixture for 25 min. Centrifuge at about 2000 × *g* for 5 min. Collect the supernatant.

**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 (2 μL) and the test solution (7 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 7.5 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 (about 5 min). Examine the plate under UV light (366 nm). Calculate the $R_f$ value by using the equation as indicated in Appendix IV (A).

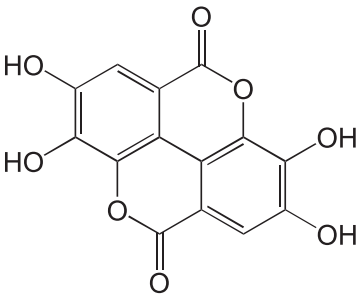


**Figure 4** Chemical structure of ellagic acid

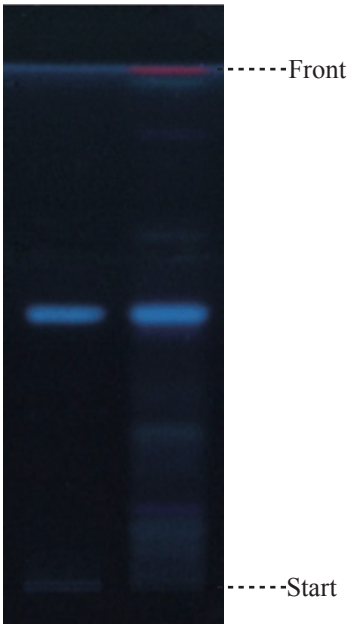


**Figure 5** A reference HPTLC chromatogram of Polygoni Chinensis Herba extract observed under UV light (366 nm) after staining

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 (50 mg/L)*

Weigh 0.5 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 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (100 W) the mixture for 1 h. Centrifuge at about 1000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (365 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 | 0.2% Formic acid (%, v/v) | Acetonitrile (%, v/v) | Elution |
|---|---|---|---|
| 0 – 20 | 85 | 15 | isocratic |
| 20 – 40 | 85 → 70 | 15 → 30 | linear gradient |
| 40 – 60 | 70 → 50 | 30 → 50 | linear gradient |

**System suitability requirements**

Perform at least five replicate injections, each using 10 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 30000 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 (10 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 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 four characteristic peaks of Polygoni Chinensis Herba extract are listed in Table 2.

**Table 2** The RRTs and acceptable ranges of the four characteristic peaks of Polygoni Chinensis Herba extract

| Peak No. | RRT | Acceptable Range |
|---|---|---|
| 1 | 0.28 | ± 0.03 |
| 2 (marker, ellagic acid) | 1.00 | - |
| 3 | 1.19 | ± 0.03 |
| 4 | 1.59 | ± 0.03 |

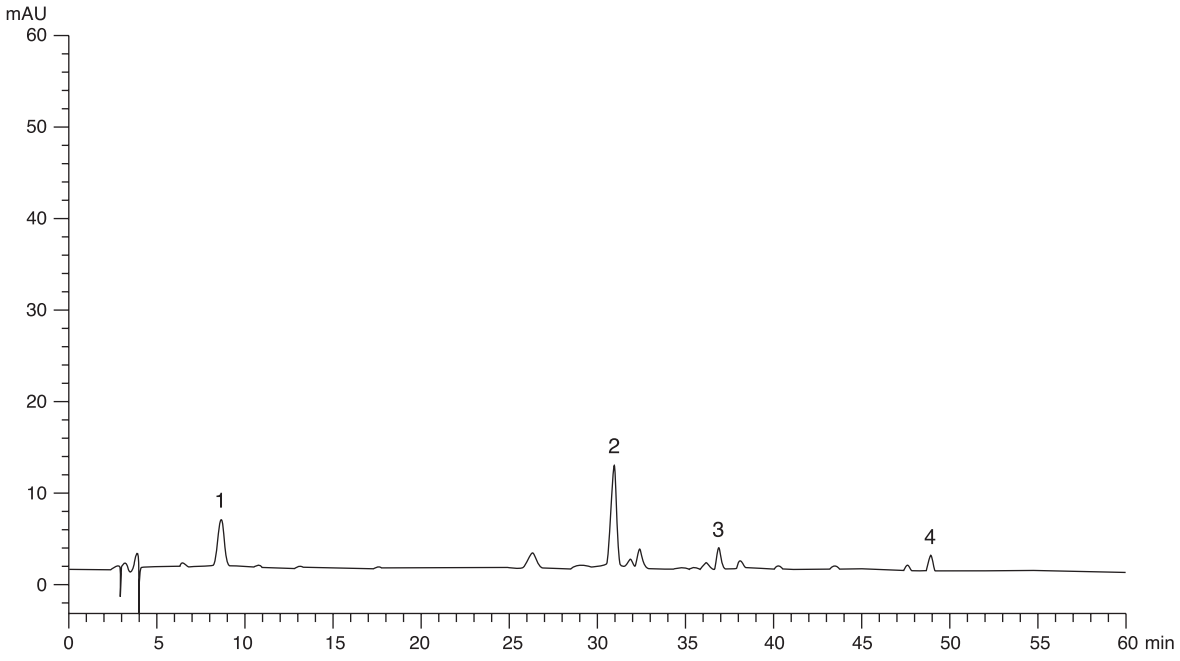


**Figure 6** A reference fingerprint chromatogram of Polygoni Chinensis Herba extract

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** *(Appendix VII)*: meet the requirements.

**5.4 Sulphur Dioxide Residues** *(Appendix XVI)*: meet the requirements.

**5.5 Foreign Matter** *(Appendix VIII)*: not more than 2.0%.

**5.6 Ash** *(Appendix IX)*

Total ash: not more than 9.0%.
Acid-insoluble ash: not more than 1.5%.

**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 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**
*Ellagic acid standard stock solution, Std-Stock (50 mg/L)*
Weigh accurately 1.0 mg of ellagic acid CRS and dissolve in 20 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 1, 2.5, 5, 10, 20 mg/L for ellagic acid.

**Test solution**
Weigh accurately 0.1 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 25 mL of methanol. Reflux the mixture at about 80ºC for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 25-mL volumetric flask. 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 (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 0.2% phosphoric acid and methanol (55:45, v/v). The elution time is about 30 min.

**System suitability requirements**

Perform at least five replicate injections, each using 10 μL of ellagic acid Std-AS (5 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 5000 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 (10 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 10 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 0.13% of ellagic acid ($C_{14}H_6O_8$), calculated with reference to the dried substance.