

# Fructus Forsythiae



Figure 1 A photograph of Fructus Forsythiae (A: Qingqiao B: Laoqiao)

1. Fruit 2. Fruit splits open, showing pericarp and seeds 3. Seeds

## 1. NAMES

Official Name: Fructus Forsythiae

Chinese Name: 連翹

Chinese Phonetic Name: Lianqiao

## 2. SOURCE

Fructus Forsythiae is the dried fruit of *Forsythia suspensa* (Thunb.) Vahl (Oleaceae). The fruit is collected in autumn when nearly ripe, but still greenish, foreign matter removed, steamed thoroughly and dried under the sun (known as “Qingqiao”), or the fruit is collected when fully ripe, foreign matter removed, dried under the sun (known as “Laoqiao”) to obtain Fructus Forsythiae.

## 3. DESCRIPTION

Long-ovoid to ovoid, slightly compressed, 1.5-2.5 cm long, 5-10.3 mm in diameter. Externally with irregular longitudinal wrinkles, numerous small protuberant maculae, and a distinct longitudinal furrow on each of the two surfaces. Apex acute, bearing a small fruit stalk or its scar at the base. “Qingqiao” is mostly indehiscent, externally greenish-brown to brown, texture hard; seeds numerous, yellowish-green to brown, slender, winged on one side. “Laoqiao” is dehiscent starting from apex or into two segments, the outer surface yellowish-brown to reddish-brown, the inner surface mostly pale yellowish-brown, smooth, with a longitudinal septum, its texture brittle; seeds brown, mostly fallen off. Odour slightly aromatic; taste bitter (Fig. 1).

## 4. IDENTIFICATION

### 4.1 Microscopic Identification (Appendix III)

#### Transverse section of pericarp

Exocarp consists of 1 row of elongated tangentially oriented epidermal cells, and covered by a cuticle. Mesocarp parenchyma irregularly arranged, vascular bundles are scattered in the parenchyma on the outer portion, stone cells or fibres are sometimes also visible while many layers of stone cells with inlaid fibres are found on the inner portion, their shape oblong to subrounded, wall thickness variable, mostly tangentially parqueted. Endocarp consists of 1 layer of flat and tiny parenchyma cells (Fig. 2).

### Powder

Colour yellowish-brown to brown. Fibres mostly in bundles, the wall thickened. Stone cells oblong to suborbicular. Mesocarp parenchyma cells suborbicular, subsquare, polygonal or irregular in shape, some part of the walls slightly beaded-thickened. Vessels mostly spiral (Fig. 3).

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

### Standard solution

*Phillyrin standard solution*

Weigh 1.0 mg of phillyrin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

### Developing solvent system

Prepare a mixture of dichloromethane, ethanol and glacial acetic acid (8:1:0.5, v/v).

### Spray reagent

Add slowly 20 mL of sulphuric acid to 80 mL of ethanol.

### Test solution

Weigh 3.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (50%). Sonicate (560 W) the mixture for 30 min. Centrifuge at about  $3000 \times g$  for 5 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of water. Transfer the aqueous solution to a separatory funnel. Extract for three times each with 10 mL of hexane-ethyl acetate (1:1, v/v) and discard the upper layer. Extract the aqueous layer for three times each with 15 mL of ethyl acetate. Combine the ethyl acetate extracts and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

### Procedure

Carry out the method by using a HPTLC silica gel  $F_{254}$  plate and a freshly prepared developing solvent system as described above. Apply separately phillyrin standard solution and the test solution (5  $\mu$ L each) to the plate. 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 120°C until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. Calculate the  $R_f$  value by using the equation as indicated in Appendix IV (A).

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 phillyrin.

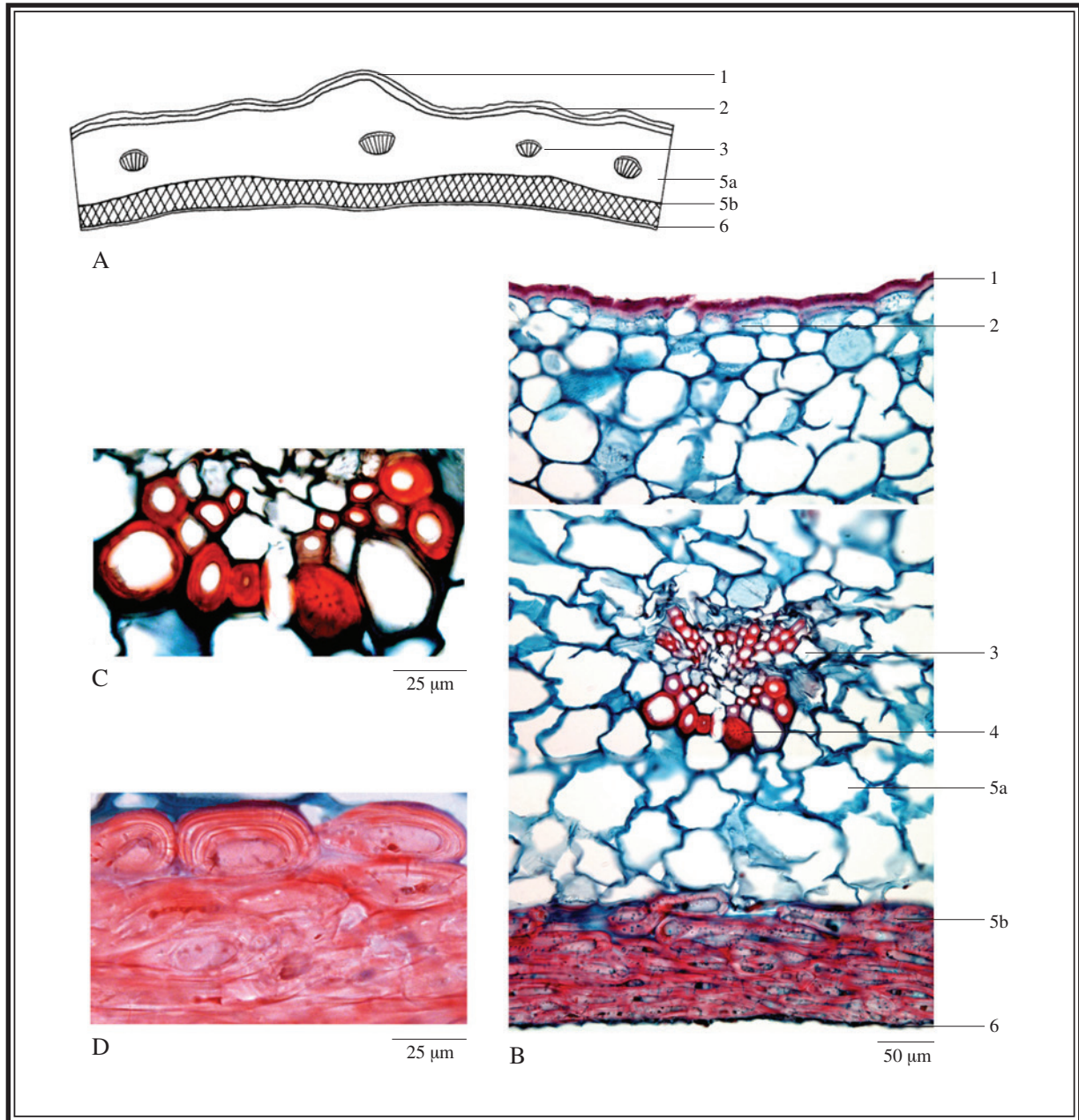


Figure 2 Microscopic features of transverse section of pericarp of *Fructus Forsythiae*

A. Sketch B. Section illustration C. Stone cells in xylem

D. Stone cells with inlaid fibres in the innermost layers of mesocarp

1. Cuticle 2. Exocarp 3. Vascular bundle 4. Stone cells

5a. Outer layers of mesocarp 5b. Inner layers of mesocarp with many layers of stone cells and inlaid fibres

6. Endocarp

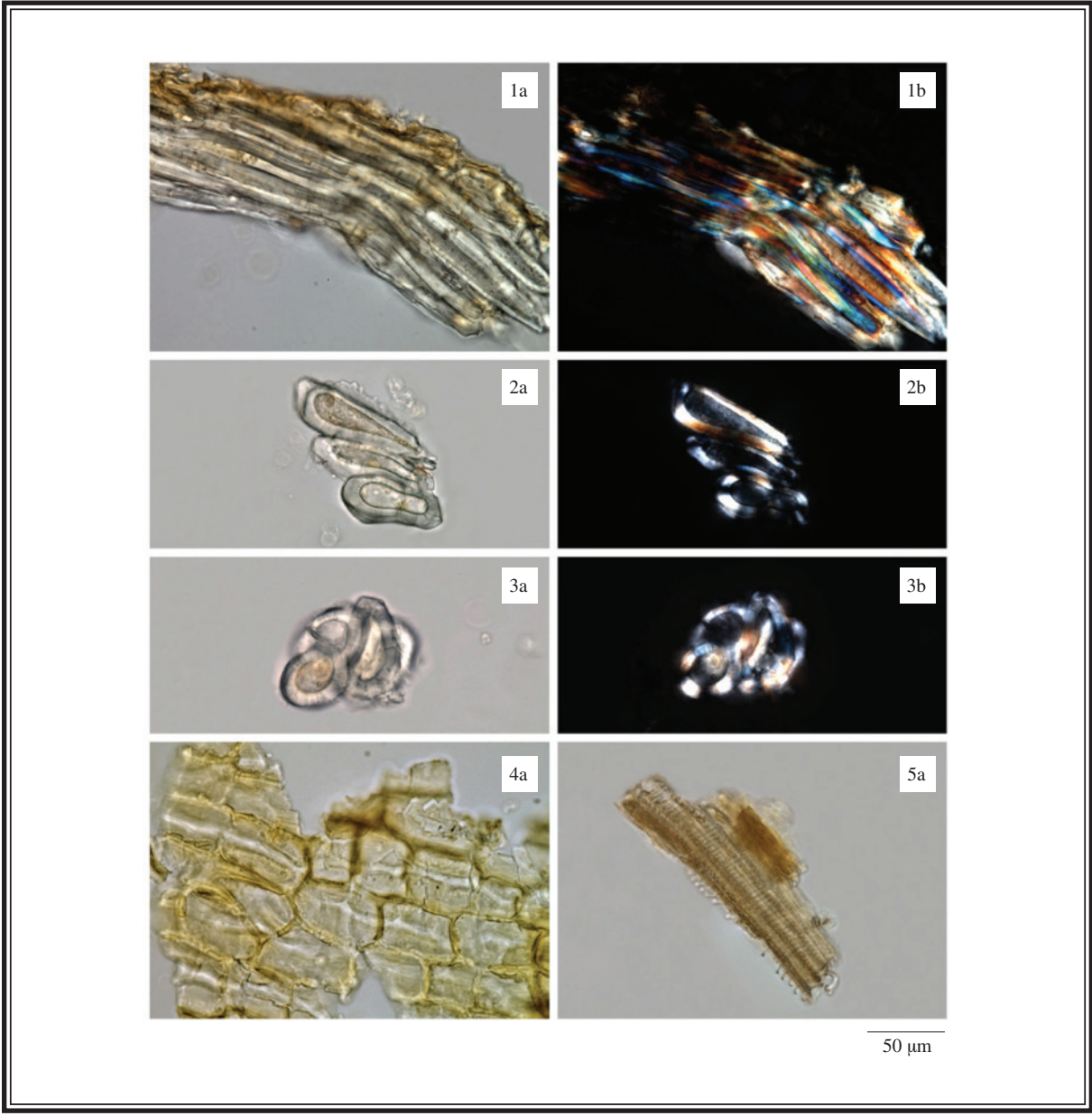


Figure 3 Microscopic features of powder of Fructus Forsythiae

- 1. Fibres 2. Stone cells oblong 3. Stone cells suborbicular
- 4. Mesocarp parenchyma cells 5. Vessels
- a. Features under the light microscope b. Features under the polarized microscope

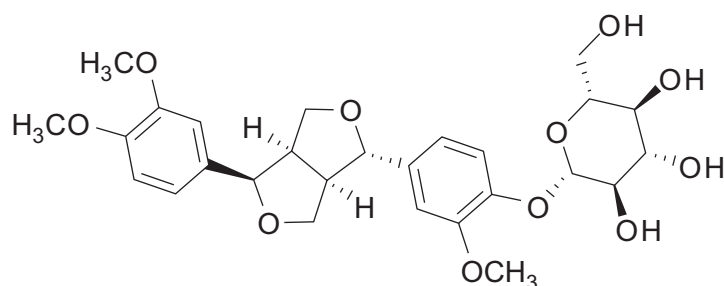


Figure 4 Chemical structure of phillyrin

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

#### Standard solution

*Phillyrin standard solution for fingerprinting, Std-FP (10 mg/L)*

Weigh 1.0 mg of phillyrin CRS and dissolve in 100 mL of ethanol (50%).

#### Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL test tube, then add 10 mL of ethanol (50%). Sonicate (560 W) the mixture for 30 min. Filter through a 0.45- $\mu$ m RC filter.

#### Chromatographic system

The liquid chromatograph is equipped with a DAD (229 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu$ m particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (% v/v)	Acetonitrile (% v/v)	Elution
0–10	80	20	isocratic
10–35	80 $\rightarrow$ 55	20 $\rightarrow$ 45	linear gradient
35–50	55 $\rightarrow$ 0	45 $\rightarrow$ 100	linear gradient

#### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of phillyrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of phillyrin should not be more than 5.0%; the RSD of the retention time of phillyrin peak should not be more than 2.0%; the column efficiency determined from phillyrin peak should not be less than 100000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

### Procedure

Separately inject phillyrin Std-FP and the test solution (10  $\mu$ L each) into the HPLC system and record the chromatograms. Measure the retention time of phillyrin peak in the chromatogram of phillyrin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify phillyrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of phillyrin Std-FP. The retention times of phillyrin 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 Fructus Forsythiae extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.40	$\pm 0.05$
2	0.66	$\pm 0.04$
3 (marker, phillyrin)	1.00	-
4	1.80	$\pm 0.05$

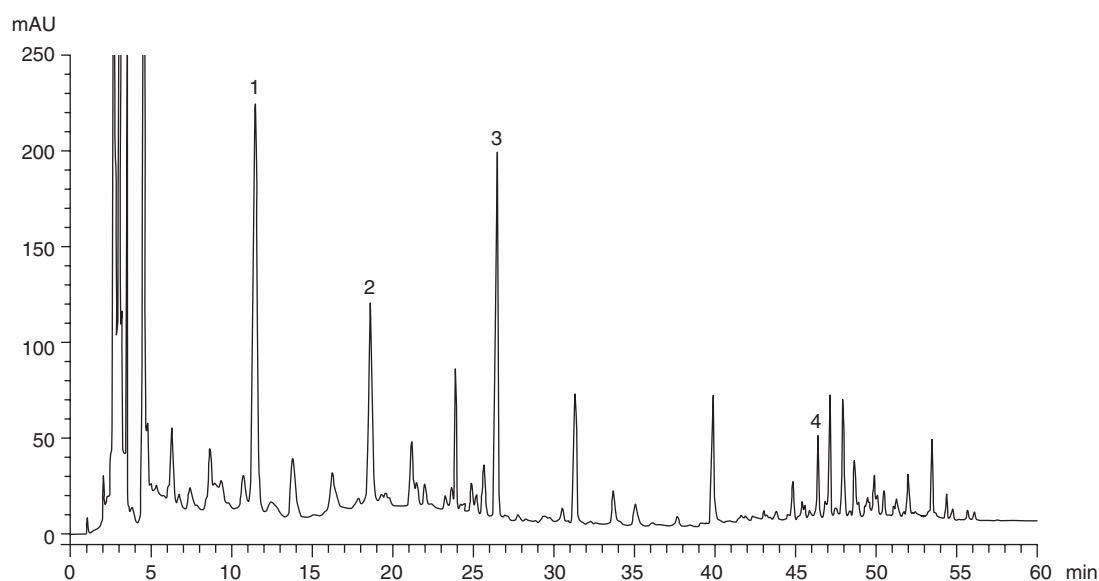


Figure 5 A reference fingerprint chromatogram of Fructus Forsythiae extract

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 XV*): meet the requirements.
- 5.5 Foreign Matter** (*Appendix VIII*): not more than 6.0%.
- 5.6 Ash** (*Appendix IX*)
- Total ash: not more than 4.5%.
- Acid-insoluble ash: not more than 1.0%.
- 5.7 Water Content** (*Appendix X*): not more than 10.0%.

## 6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (hot extraction method): not less than 12.0%.

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

## 7. ASSAY

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

### Standard solution

*Phillyrin standard stock solution, Std-Stock (1000 mg/L)*

Weigh accurately 10.0 mg of phillyrin CRS and dissolve in 10 mL of ethanol (50%).

*Phillyrin standard solution for assay, Std-AS*

Measure accurately the volume of the phillyrin Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 0.5, 1, 5, 10, 50 mg/L for phillyrin.

### Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (560 W) the mixture for 30 min. Centrifuge at about  $3000 \times g$  for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times each



with 5 mL of ethanol (50%). Combine the extracts and make up to the mark with ethanol (50%). Filter through a 0.45- $\mu$ m RC filter.

### Chromatographic system

The liquid chromatograph is equipped with a DAD (229 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu$ m particle size). The flow rate is about 0.8 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	75→45	25→55	linear gradient
25–30	45→0	55→100	linear gradient

### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of phillyrin Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of phillyrin should not be more than 5.0%; the RSD of the retention time of phillyrin peak should not be more than 2.0%; the column efficiency determined from phillyrin peak should not be less than 40000 theoretical plates.

The *R* value between phillyrin 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 phillyrin Std-AS (10  $\mu$ L each) into the HPLC system and record the chromatograms. Plot the peak areas of phillyrin against the corresponding concentrations of phillyrin Std-AS. Obtain the slope, y-intercept and the  $r^2$  value from the 5-point calibration curve.

### Procedure

Inject 10  $\mu$ L of the test solution into the HPLC system and record the chromatogram. Identify phillyrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of phillyrin Std-AS. The retention times of phillyrin 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 phillyrin in the test solution, and calculate the percentage content of phillyrin in the sample by using the equations indicated in Appendix IV(B).

### Limits

The sample contains not less than 0.015% of phillyrin (C<sub>27</sub>H<sub>34</sub>O<sub>11</sub>), calculated with reference to the dried substance.