

Fructus Ligustri Lucidi



Figure 1 A photograph of Fructus Ligustri Lucidi

1. NAMES

Official Name: Fructus Ligustri Lucidi

Chinese Name: 女貞子

Chinese Phonetic Name: Nüzhenzi

2. SOURCE

Fructus Ligustri Lucidi is the dried ripe fruit of *Ligustrum lucidum* Ait. (Oleaceae). The ripe fruit is collected in winter, by removing from leafy branches, steamed or treated with boiling water for a few minutes, then dried; or dried directly without treatment to obtain Fructus Ligustri Lucidi.

3. DESCRIPTION

Reniform, sometimes ovoid or ellipsoid, 5-8.5 mm long, 3-6.5 mm in diameter. Externally blackish-purple or greyish-black, shrunk and uneven, with a fruit stalk scar or persistent calyx and a short fruit stalk at the base. Texture light. Pericarp thin, mesocarp relatively lax and soft, easily stripped off, endocarp woody, yellowish-brown, with longitudinal ridges. Seed 1, reniform, purplish-black, oily. Odour slight; taste sweet, with slight bitterness and somewhat astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Exocarp consists of 1 layer of subpolygonal epidermis cells, containing oil droplets, the cuticle covering the outer and lateral walls thickened. Mesocarp consists of 10-20 layers of parenchyma cells, scattered with vascular bundles near the bulge of the endocarp. Endocarp consists of 4-8 layers of lignified fibres, its surface rough and uneven. Epidermal cells of testa composed of elongated round or oblong parenchyma cells, often with secretory cells on the ridges. Parenchyma of testa consisting of several rows of parenchyma cells, mostly shrunk. Endosperm and cotyledons consist of parenchyma cells. When fruit is ripening, only one seed is developed from 2 celled ovary (Fig. 2).

Powder

Colour greyish-brown or blackish-grey. Fibres of endocarp in bundles or single, colourless or pale yellow, cross-overlapping in the bundles; the single fibre has a band or a strip-shape, mostly curved, straight or twisted, tapering, blunt or branched at one end, some fibres are

enlarged, boot-shaped, 9-54 μm in diameter. Epidermal cells of the pericarp sub-oblate in surface view, with the cuticle of the outer wall thickened, divided into 4-10 irregular small lumina by several arris, 36-80 μm in diameter, containing yellowish-brown or purplish-brown masses. Epidermal cells of testa scattered with secretory cells, pale-brown or brown in colour. Secretory cells rounded or oblong, 45-96 μm in diameter, containing yellowish-brown secretion and oil droplet (Fig. 3).

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

Standard solution

Salidroside standard solution

Weigh 1.0 mg of salidroside CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of water, methanol and acetic acid (90:10:2, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 10.0 g of the powdered sample and place it in a 500-mL round-bottomed flask, then add 200 mL of dichloromethane. Sonicate (220 W) the mixture for 30 min. Filter the mixture. Discard the filtrate. Repeat the above procedure for one more time. Add 200 mL of methanol to the residue. Sonicate (220 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol. Transfer 1 mL of solution to a centrifuge tube. Centrifuge at about $4000 \times g$ for 10 min. Transfer 200 μL of supernatant to the Diol clean-up column (4 mL, 500 mg) pre-conditioned with 2 mL of ethyl acetate. Add 1.5 mL of ethyl acetate to the clean-up column and discard this portion of eluant. Add 2.5 mL of ethyl acetate to the clean-up column and collect the eluant in a 10-mL round-bottomed flask. Repeat the elution for one more time with 2.5 mL of ethyl acetate. Combine the eluants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 200 μL of methanol.

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 salidroside standard solution (1 μL) and the test solution (10 μL) to the plate. 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, then heat at 105°C until the spots or bands become visible (about 15 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV(A).

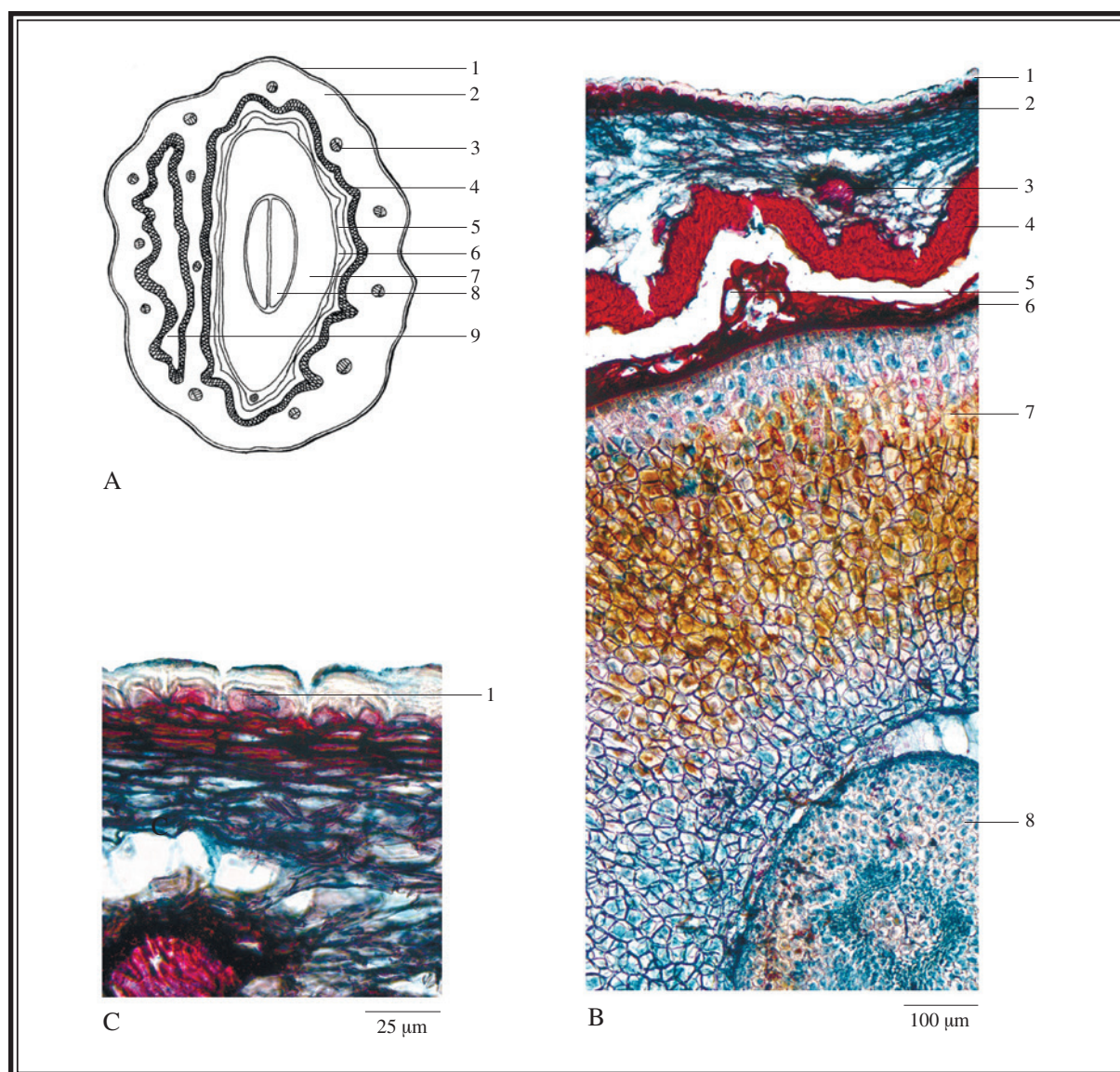


Figure 2 Microscopic features of transverse section of Fructus Ligustri Lucidi

A. Sketch B. Section illustration C. Exocarp

1. Exocarp 2. Mesocarp 3. Vascular bundles 4. Endocarp 5. Epidermal cells of testa
6. Parenchyma of testa 7. Endosperm 8. Cotyledon 9. Aborted seed

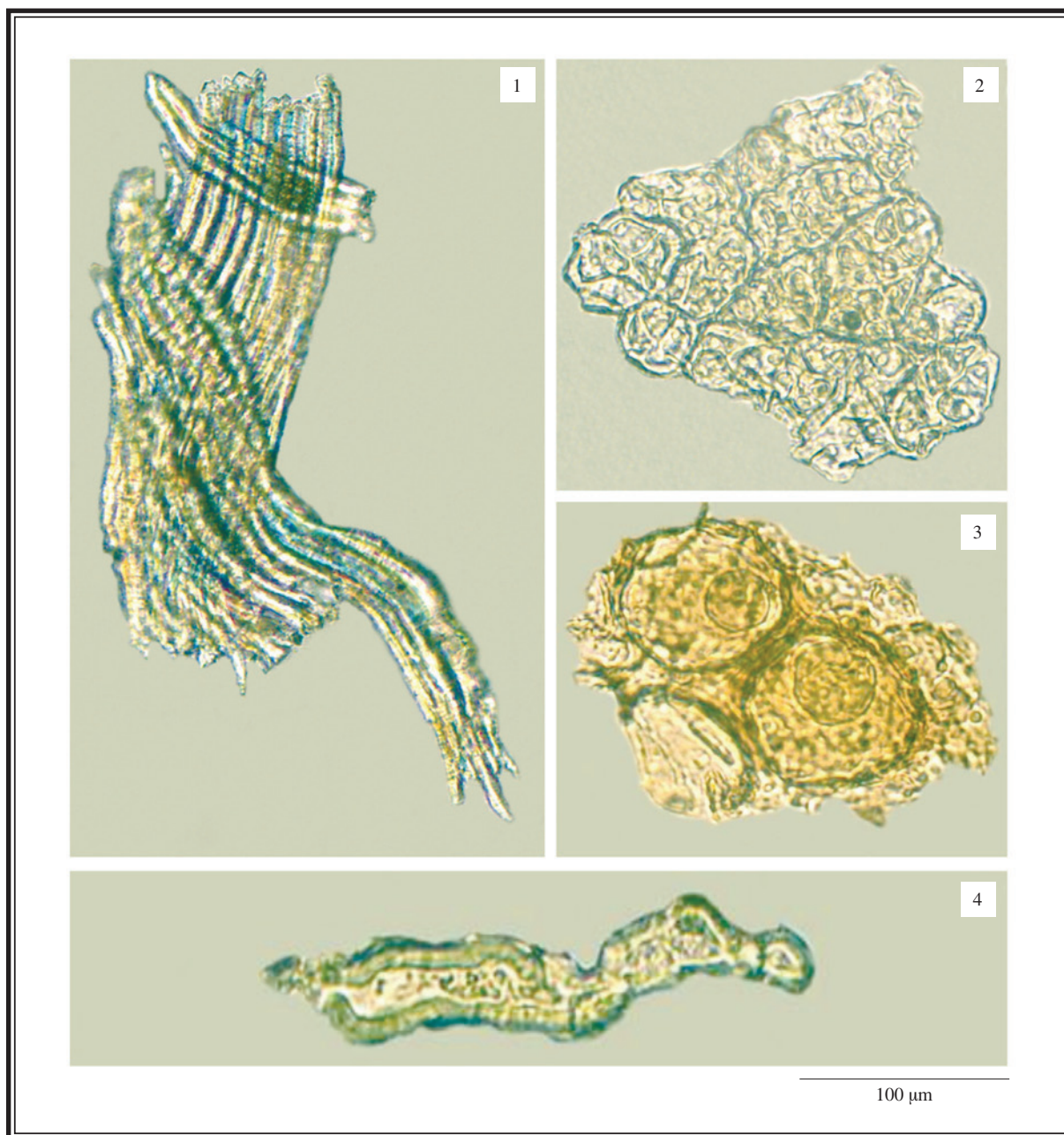


Figure 3 Microscopic features of powder of Fructus Ligustri Lucidi (under the light microscope)

1. Fibres of endocarp 2. Pericarp epidermis 3. Epidermal cells of testa
4. A single fibre cell from the endocarp

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 salidroside.

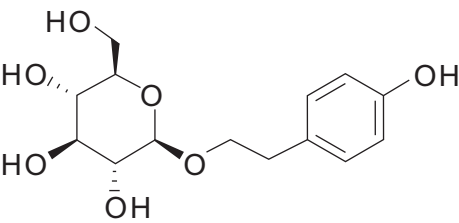


Figure 4 Chemical structure of salidroside

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Salidroside standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 1.0 mg of salidroside and dissolve in 5 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL conical flask, then add 100 mL of methanol. Sonicate (220 W) the mixture for 30 min. Filter and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (70%). Transfer the solution to a 10-mL volumetric flask 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 (230 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)	Acetonitrile (% , v/v)	0.1 % Phosphoric acid (% , v/v)	Elution
0 – 15	4	96	isocratic
15 – 35	4→6	96→94	linear gradient
35 – 60	6→15	94→85	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μ L of salidroside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of salidroside should not be more than 3.0%; the RSD of the retention time of salidroside peak should not be more than 2.0%; the column efficiency determined from salidroside peak should not be less than 14000 theoretical plates.

The *R* value between peak 2 and peak 3 in the chromatogram of the test solution should not be less than 1.5 and the *R* value between peak 3 and peak 4 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Fructus Ligustri Lucidi extract

Peak No.	RRT	Acceptable Range
1	0.84 (vs peak 3)	± 0.03
2 (tyrosol)	0.93 (vs peak 3)	± 0.03
3 (marker, salidroside)	1.00	-
4	1.11 (vs peak 3)	± 0.03
5	1.64 (vs peak 4)	± 0.09

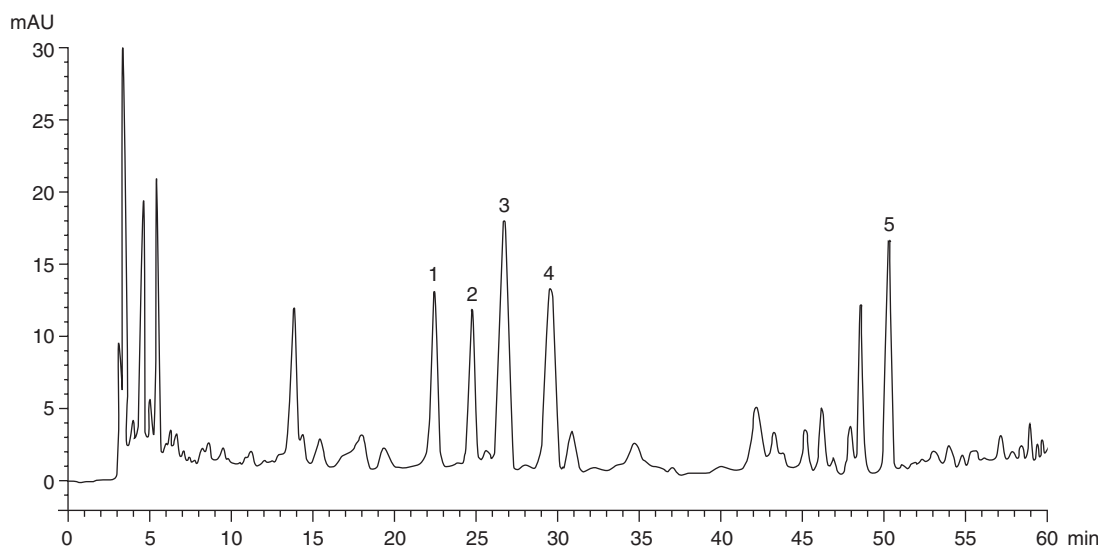


Figure 5 A reference fingerprint chromatogram of Fructus Ligustri Lucidi

For positive identification, the sample must give the above five 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 – Aflatoxins (*Appendix VII*): meet the requirements.

5.4 Sulphur Dioxide Residues (*Appendix XV*): 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 1.0%.

5.7 Water Content (*Appendix X*): not more than 9.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (hot extraction method): not less than 27.0%.

Ethanol-soluble extractives (hot extraction method): not less than 27.0%.

7. ASSAY

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

Standard solution

Salidroside standard stock solution, Std-Stock (400 mg/L)

Weigh accurately 4.0 mg of salidroside CRS and dissolve in 10 mL of methanol (70%).

Salidroside standard solution for assay, Std-AS

Measure accurately the volume of the salidroside Std-Stock, dilute with methanol (70%) to produce a series of solutions of 5, 10, 20, 40, 80 mg/L for salidroside.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 50 mL of methanol (70%). Sonicate (220 W) the mixture for 30 min. Centrifuge at about 3000 × g for 5 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Combine the filtrate. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (70%). Transfer the solution to a 10-mL volumetric flask 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 (230 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 190 Å pore size and 12% carbon loading). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (% v/v)	0.1 % Phosphoric acid (% v/v)	Elution
0–15	4	96	isocratic
15–45	4 → 0	96 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of salidroside Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of salidroside should not be more than 5.0%; the RSD of the retention time of salidroside peak should not be more than 2.0%; the column efficiency determined from salidroside peak should not be less than 3500 theoretical plates.

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

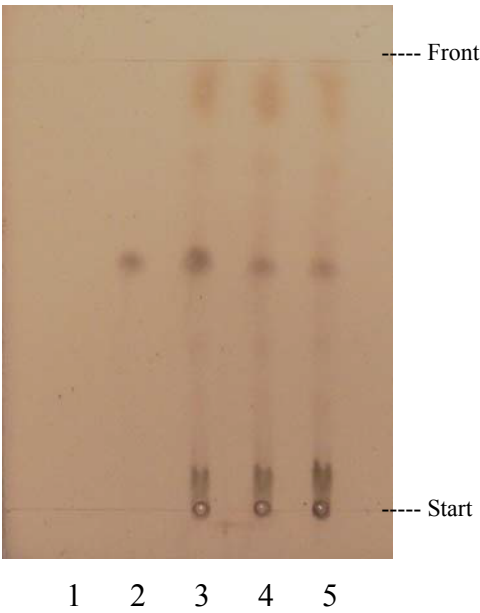
Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify salidroside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of salidroside Std-AS. The retention times of salidroside peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of salidroside in the test solution, and calculate the percentage content of salidroside in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.039% of salidroside ($C_{14}H_{20}O_7$), calculated with reference to the dried substance.

Fructus Ligustri Lucidi (女貞子)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Salidroside)	Salidroside positive
3	Spiked sample (Sample plus salidroside)	Salidroside positive
4	Sample (Fructus Ligustri Lucidi)	Salidroside positive
5	Sample duplicate (Fructus Ligustri Lucidi)	Salidroside positive

Figure 1 TLC results of Fructus Ligustri Lucidi extract observed under visible light after staining