Liquidambaris Fructus

Figure 1 A photograph of Liquidambaris Fructus

A. Liquidambaris Fructus B. Magnified small capsules

1. NAMES

Official Name: Liquidambaris Fructus

Chinese Name: 路路通

Chinese Phonetic Name: Lulutong

2. SOURCE

Liquidambaris Fructus is the dried ripe infructescence of *Liquidambar formosana* Hance (Hamamelidaceae). The ripe infructescence is collected in winter when the fruit is ripe, foreign matter removed, then dried under the sun to obtain Liquidambaris Fructus.

3. DESCRIPTION

Infructescence spheroidal, 20-50 mm in diameter, composed of numerous coalescing, spiny, small fruit-capsules, with a fruit stalk at the base. Externally greyish-brown to brown, bearing numerous acute and beaked spines, 0.5-1 mm long, usually broken. Small capsules splitted at apex, showing small honeycomb-shaped holes. Texture hard and light in weight, uneasily broken. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour dark brown. Fibres mostly broken, the ends subrounded, 8-89 μm in diameter, walls sinuous, lignified, lumens vary in diameter, usually contain yellowish-brown substances. Non-glandular hairs unicellular, curved, 39-182 μm long, base 8-31 μm wide, containing yellowish-brown substances. Stone cells of pericarp subsquare, spindle-shaped, branched or irregular, 21-178 μm in diameter, walls thickened, pit canals branched. Epidermal cells of pericarp polygonal, walls thickened, with pit canals, containing yellowish-brown substances (Fig. 2).

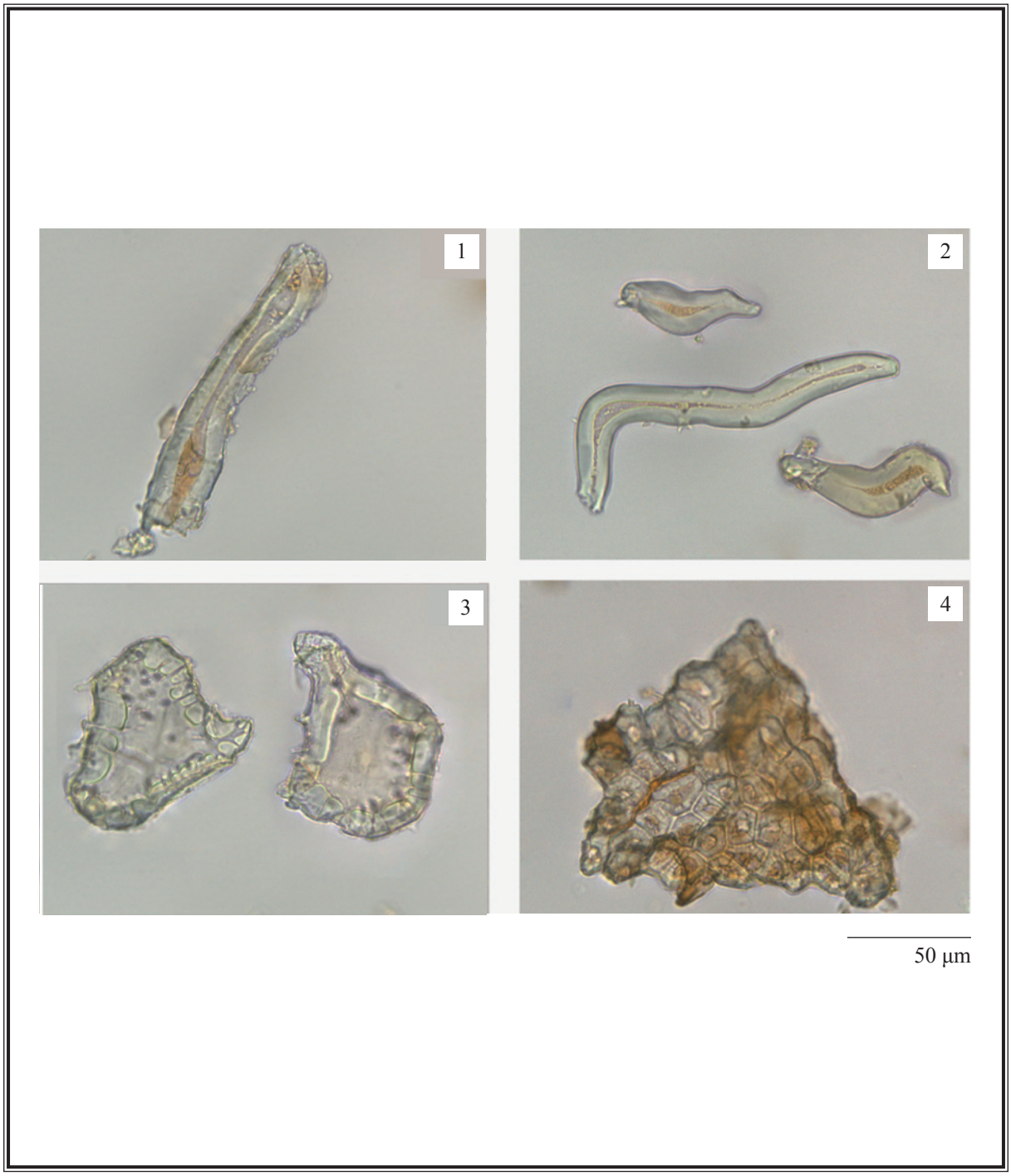

Figure 2 Microscopic features of powder of Liquidambaris Fructus (under the light microscope)

1. Fibre 2. Unicellular non-glandular hairs 3. Stone cells 4. Epidermal cells of pericarp

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

Standard solution

Liquidambaric acid (Betulonic acid) standard solution

Weigh 2.0 mg of liquidambaric acid CRS (Fig. 3) and dissolve in 2 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and formic acid (8:2:0.1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol and dissolve 1.0 g of vanillin.

Test solution

Weigh 1.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethyl acetate. Sonicate (150 W) the mixture for 30 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 liquidambaric 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 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 2 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

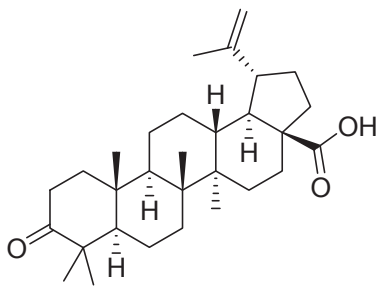

Figure 3 Chemical structure of liquidambaric acid (betulonic acid)

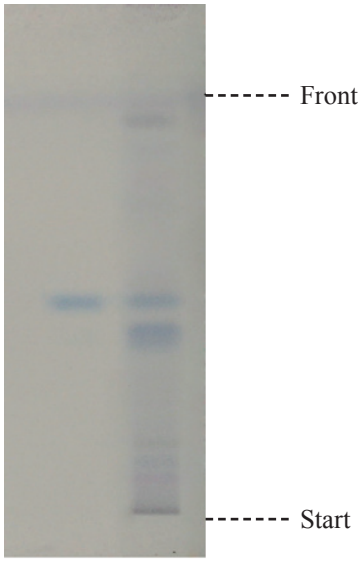

Figure 4 A reference HPTLC chromatogram of Liquidambaris Fructus extract observed under visible light after staining

1. Liquidambaric 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 liquidambaric acid (Fig. 4).

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

Standard solution

Liquidambaric acid (Betulonic acid) standard solution for fingerprinting, Std-FP (250 mg/L)

Weigh 2.5 mg of liquidambaric acid CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of ethanol. 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 (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. 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 – 25	64	36	isocratic
25 – 40	64 → 80	36 → 20	linear gradient
40 – 60	80	20	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of liquidambaric acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of liquidambaric acid should not be more than 5.0%; the RSD of the retention time of liquidambaric acid peak should not be more than 2.0%; the column efficiency determined from liquidambaric acid peak should not be less than 70000 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. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Liquidambaris Fructus extract

Peak No.	RRT	Acceptable Range
1	0.48	± 0.03
2 (marker, liquidambaric acid)	1.00	-
3	1.05	± 0.03

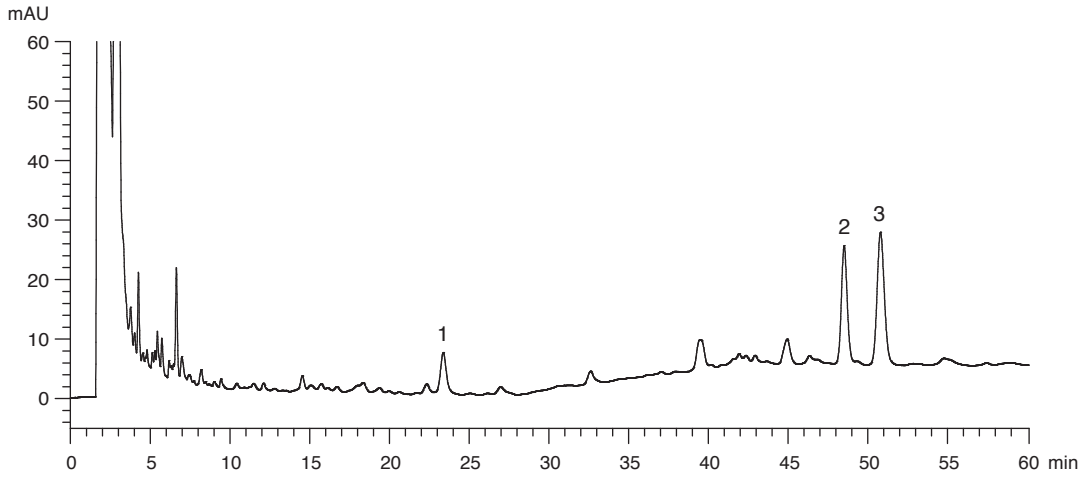

Figure 5 A reference fingerprint chromatogram of Liquidambaris Fructus extract

For positive identification, the sample must give the above three 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 4.0%.

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 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

Standard solution

Liquidambaric acid (Betulonic acid) standard stock solution, Std-Stock (300 mg/L)

Weigh accurately 3.0 mg of liquidambaric acid CRS and dissolve in 10 mL of ethanol.

Liquidambaric acid standard solution for assay, Std-AS

Measure accurately the volume of the liquidambaric acid Std-Stock, dilute with ethanol to produce a series of solutions of 30, 60, 120, 180, 240 mg/L for liquidambaric acid.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 7 mL of ethanol. Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol. Combine the solutions and make up to the mark with ethanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (195 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.1% phosphoric acid and acetonitrile (20:80, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of liquidambaric acid Std-AS (120 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of liquidambaric acid should not be more than 5.0%; the RSD of the retention time of liquidambaric acid peak should not be more than 2.0%; the column efficiency determined from liquidambaric acid peak should not be less than 7500 theoretical plates.

The *R* value between liquidambaric 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 liquidambaric acid Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of liquidambaric acid against the corresponding concentrations of liquidambaric 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 liquidambaric acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of liquidambaric acid Std-AS. The retention times of liquidambaric 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 liquidambaric acid in the test solution, and calculate the percentage content of liquidambaric acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.27% of liquidambaric acid ($C_{30}H_{46}O_3$), calculated with reference to the dried substance.