Garciniae Resina (unprocessed)

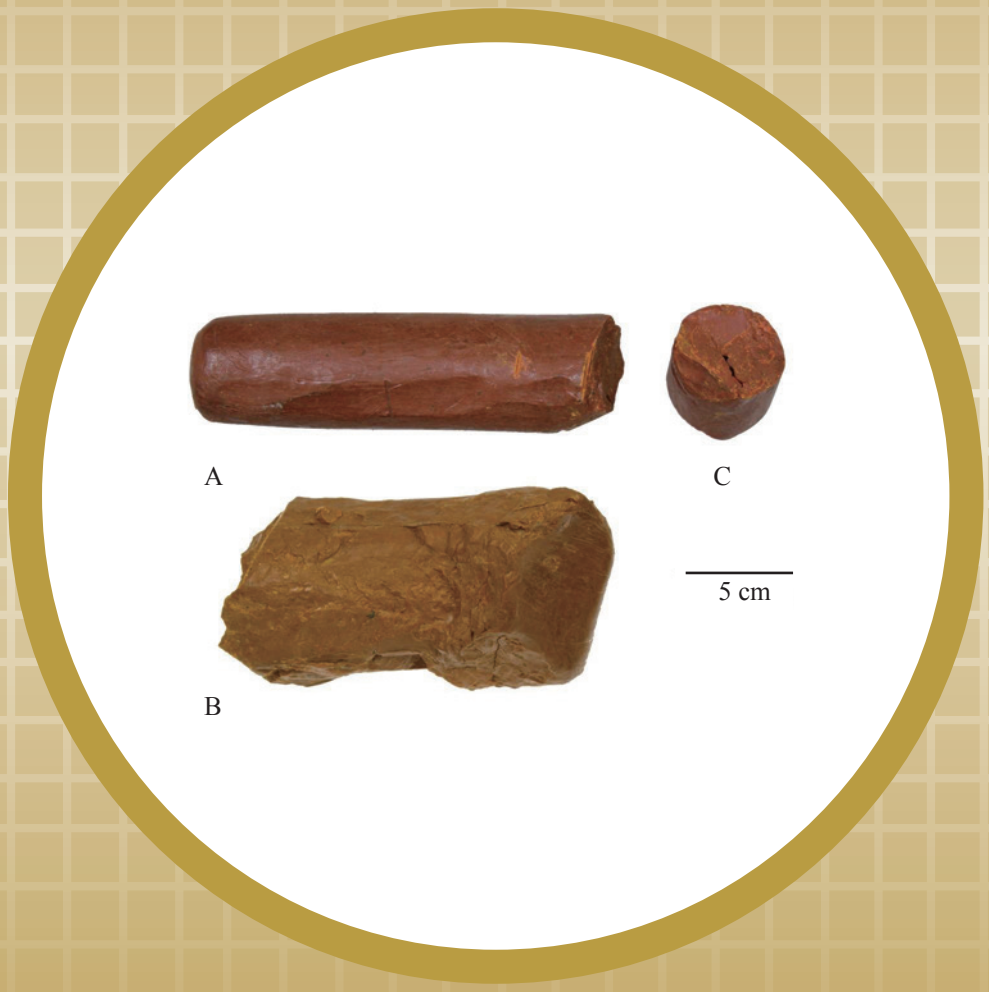

Figure 1 A photograph of Garciniae Resina (unprocessed)

A. Garciniae Resina (unprocessed) (cylindrical)

B. Garciniae Resina (unprocessed) (irregular mass)

C. Fracture

1. NAMES

Official Name: Garciniae Resina (unprocessed)

Chinese Name: 藤黃 (生)

Chinese Phonetic Name: Tenghuang (sheng)

2. SOURCE

Garciniae Resina (unprocessed) is the unprocessed gum-like resin of *Garcinia hanburyi* Hook. f. (Clusiaceae). The resin is collected in August to September before flowering. The stem 3 m above the ground is cut spirally, in order to make emulsions leak from the wound, emulsions collected, solidified, and dried under the sun; melted and molded into a cylindrical object or other forms, then dried under the sun to obtain Garciniae Resina (unprocessed).

3. DESCRIPTION

Cylindrical or irregular masses, up to 34 cm long, 3-16 cm in diameter. Externally reddish-yellow to orange-yellow, sometimes covered with yellowish-green powder, slightly sticky when held. Texture fragile. Fracture smooth, reddish-yellow to orange-yellow, with wax-like lustre, some have cavities at the centre. Odour slightly aromatic (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

No significant identification feature was found.

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

Standard solutions

Gambogenic acid standard solution

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

Gambogic acid standard solution

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

Remarks: Gambogic acid exists as a mixture of *R*- and *S*-form.

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate, methanol and diethylamine (5:3:2:1, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 150-mL conical flask, then add 50 mL of ethyl acetate. Sonicate (160 W) the mixture for 30 min. Stand for 10 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate [Immerse in methanol and dry in air. Heat the plate at 105ºC for 30 min.], a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately gambogenic acid standard solution, gambogic acid standard solution and the test solution (6 μL each) 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 1 h. 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 15 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

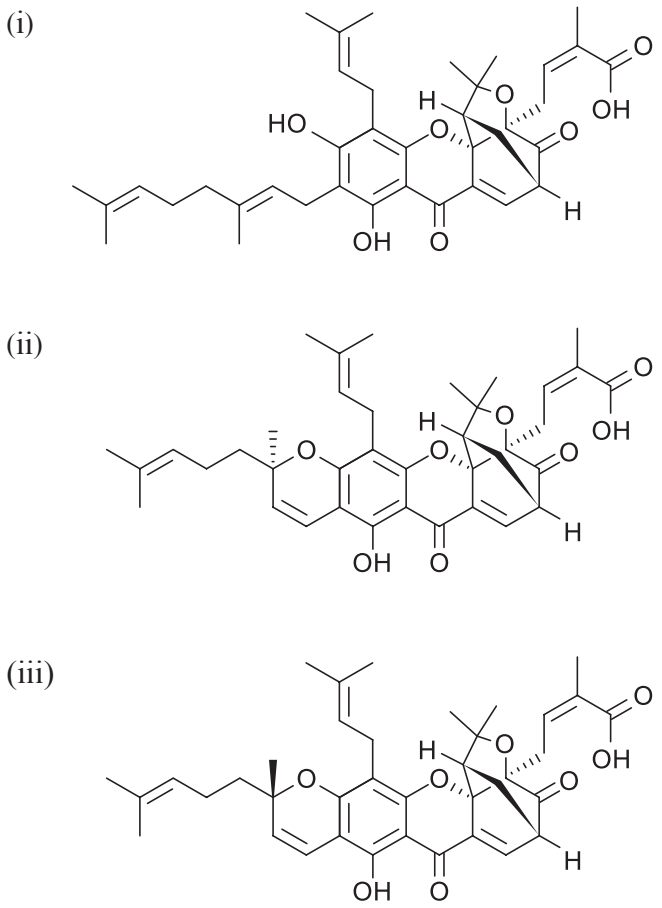

Figure 2 Chemical structures of (i) gambogenic acid (ii) *R*-gambogic acid and (iii) *S*-gambogic acid (epigambogic acid)

Remarks: Gambogic acid exists as a mixture of *R*- and *S*-form.

Figure 3 A reference HPTLC chromatogram of Garciniae Resina (unprocessed) extract observed under UV light (254 nm)

1. Gambogenic acid standard solution
2. Gambogic acid standard solution (lower band: *R*-gambogic acid; upper band: *S*-gambogic acid)
3. Test solution

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 gambogenic acid and gambogic acid (Fig. 3).

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

Standard solutions

Gambogenic acid standard solution for fingerprinting, Std-FP (75 mg/L)

Weigh 1.5 mg of gambogenic acid CRS and dissolve in 20 mL of acetonitrile.

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

Weigh 2.0 mg of gambogic acid CRS and dissolve in 10 mL of acetonitrile.

Remarks: Gambogic acid exists as a mixture of *R*- and *S*-form.

Test solution

Weigh 0.15 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of acetonitrile. Sonicate (160 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue for four times each with 5 mL of acetonitrile. Combine the solutions and make up to the mark with acetonitrile. Pipette 1 mL of the solution to a 10-mL volumetric flask and make up to the mark with acetonitrile. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (360 nm) and a column (4.6 × 150 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 28ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.1% formic acid and acetonitrile (27.5:72.5, v/v). The elution time is about 45 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of gambogenic acid Std-FP and gambogic acid Std-FP. Gambogic acid elutes as *R*-gambogic acid and *S*-gambogic acid isomeric peaks in the chromatogram of gambogic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of gambogenic acid, *R*-gambogic acid and *S*-gambogic acid should not be more than 5.0%; the RSD of the retention times of gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks should not be more than 2.0%; the column efficiencies determined from gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks should not be less than 6000 theoretical plates.

The *R* value between peak 2 and the closest peak; the *R* value between peak 3 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 1.5 (Fig. 4).

Procedure

Separately inject gambogenic acid Std-FP, gambogic acid Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks in the chromatograms of gambogenic acid Std-FP, gambogic acid Std-FP and the retention times of the four characteristic peaks (Fig. 4) in the chromatogram of the test solution. Identify gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of gambogenic acid Std-FP and gambogic acid Std-FP. The retention times of gambogenic acid, *R*-gambogic acid and *S*-gambogic 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 Garciniae Resina (unprocessed) extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Garciniae Resina (unprocessed) extract

Peak No.	RRT	Acceptable Range
1	0.54	± 0.03
2 (marker, gambogenic acid)	1.00	-
3 (*R*-gambogic acid)	1.75	± 0.03
4 (*S*-gambogic acid)	1.86	± 0.03

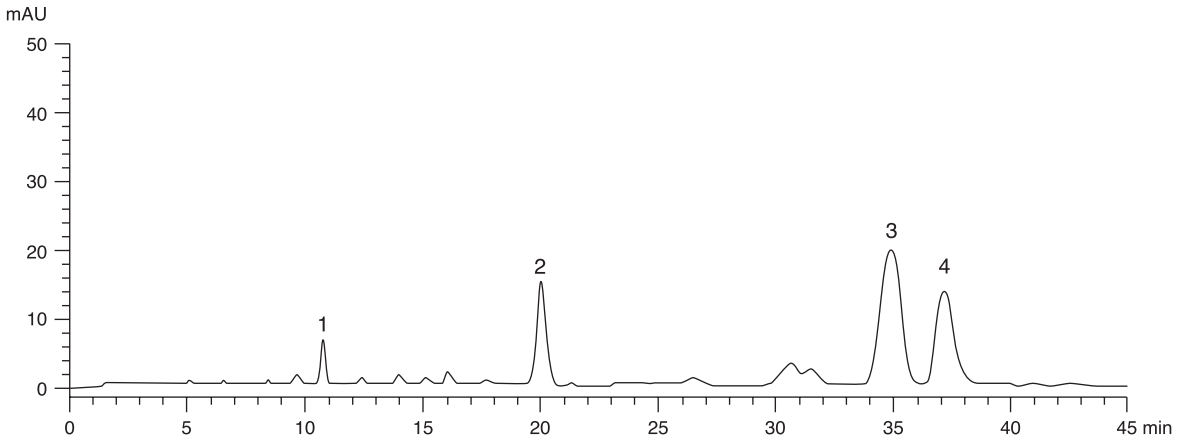

Figure 4 A reference fingerprint chromatogram of Garciniae Resina (unprocessed) extract

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. 4).

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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*
Note: No foreign matter requirement is proposed due to it is resin.

5.6 Ash *(Appendix IX)*

Total ash: not more than 0.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 7.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 37.0%.
Ethanol-soluble extractives (hot extraction method): not less than 69.0%.

7. ASSAY

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

Standard solution

Mixed gambogenic acid and gambogic acid standard stock solution, Std-Stock (400 mg/L for gambogenic acid and 1000 mg/L for gambogic acid)

Weigh accurately 4.0 mg of gambogenic acid CRS and 10.0 mg of gambogic acid CRS, and dissolve in 10 mL of acetonitrile.

Mixed gambogenic acid and gambogic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed gambogenic acid and gambogic acid Std-Stock, dilute with acetonitrile to produce a series of solutions of 10, 40, 80, 120, 200 mg/L for gambogenic acid and 25, 100, 200, 300, 500 mg/L for gambogic acid.

Remarks: Gambogic acid exists as a mixture of *R*- and *S*-form.

Test solution

Weigh accurately 0.15 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of acetonitrile. Sonicate (160 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue for four times each with 5 mL of acetonitrile. Combine the solutions and make up to the mark with acetonitrile. Pipette 1 mL of the solution to a 10-mL volumetric flask and make up to the mark with acetonitrile. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (360 nm) and a column (4.6 × 150 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 28ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.1% formic acid and acetonitrile (27.5:72.5, v/v). The elution time is about 45 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed gambogenic acid and gambogic acid Std-AS (80 mg/L for gambogenic acid and 200 mg/L of gambogic acid). Gambogic acid elutes as *R*-gambogic acid and *S*-gambogic acid isomeric peaks in the chromatogram of mixed gambogenic acid and gambogic acid Std-AS. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of gambogenic acid, *R*-gambogic acid and *S*-gambogic acid should not be more than 5.0%; the RSD of the retention times of gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks should not be more than 2.0%; the column efficiencies determined from gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks should not be less than 6000 theoretical plates.

The *R* value between gambogenic acid peak and the closest peak; the *R* value between *R*-gambogic acid peak and the closest peak; and the *R* value between *S*-gambogic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Calibration curves

Inject a series of the mixed gambogenic acid and gambogic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of gambogenic acid and the sum of peak area of *R*-gambogic acid and *S*-gambogic acid against the corresponding concentrations of the mixed gambogenic acid and gambogic 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 gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks (Fig. 5) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed gambogenic acid and gambogic acid Std-AS. The retention times of gambogenic acid, *R*-gambogic acid and *S*-gambogic acid peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of gambogenic acid and gambogic acid in the test solution, and calculate the percentage contents of gambogenic acid and gambogic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 38% of the total content of gambogenic acid ($C_{38}H_{46}O_8$) and gambogic acid ($C_{38}H_{44}O_8$), calculated with reference to the dried substance.

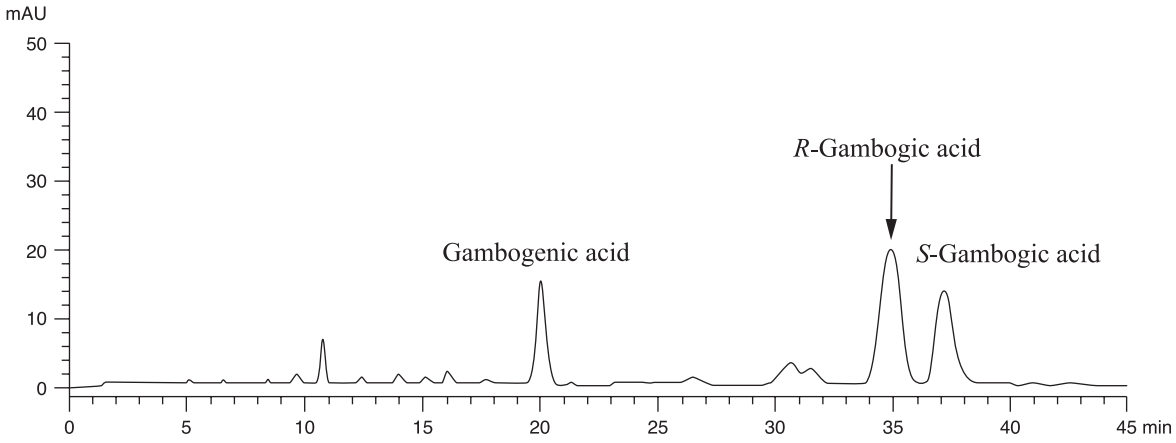

Figure 5 A reference assay chromatogram of Garciniae Resina (unprocessed) extract

8. CAUTION

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.