

Draconis Sanguis

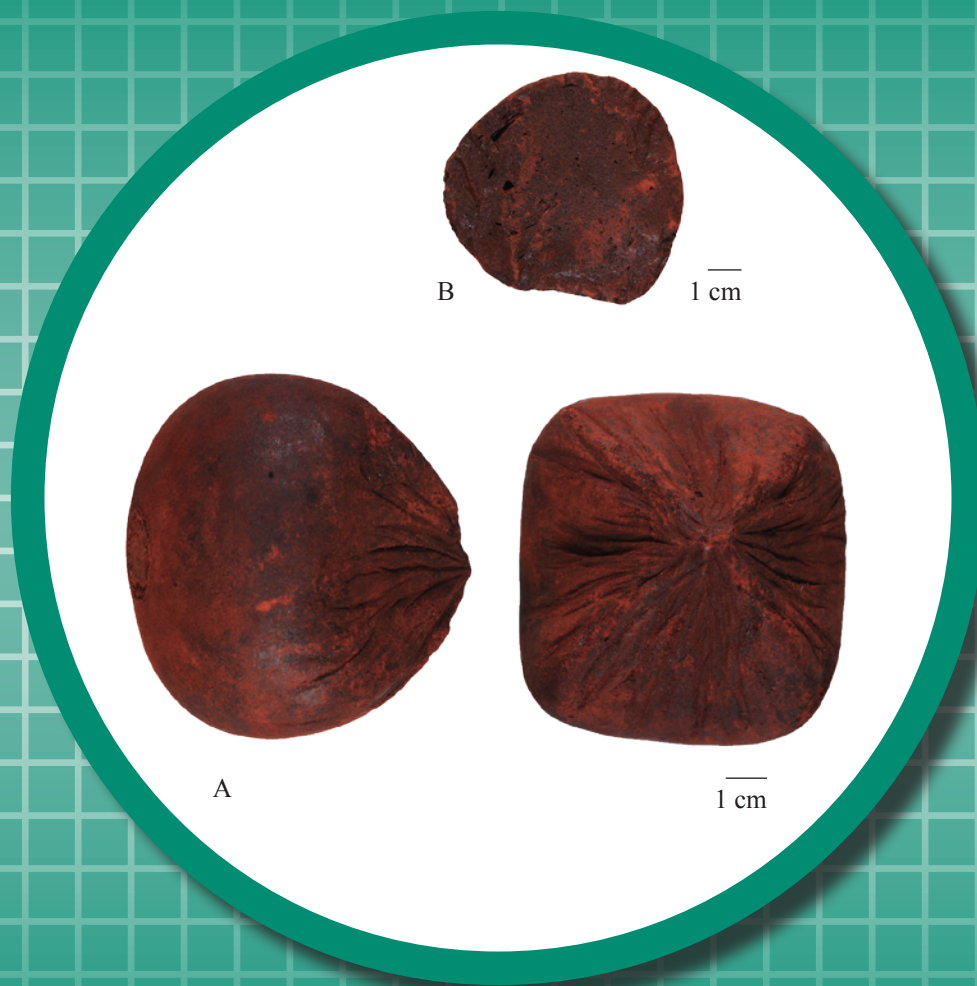


Figure 1 A photograph of Draconis Sanguis

A. Draconis Sanguis B. Transverse section of Draconis Sanguis

Draconis Sanguis**1. NAMES**

Official Name: Draconis Sanguis

Chinese Name: 血竭

Chinese Phonetic Name: Xuejie

2. SOURCE

Draconis Sanguis is the prepared resin of the fruit of *Daemonorops draco* Bl. (Arecaceae). The fruit is collected when ripe with densely hard squamae, red resin leaking from squamae; then dried under the sun, foreign matter removed, softened in hot water, and then taken out in a shaded area to obtain raw resin. The raw resin is then mixed with an excipient, dammar gum (a resin from trees of the Dipterocarpaceae family), to obtain Draconis Sanguis.

3. DESCRIPTION

Subglobular to cuboidal, 60-80 mm in diameter, 7-8 cm high. Externally dark red, lustrous, attached with friction-caused red powder. Texture hard and fragile, broken surface red, ground powder brick red. Odour slight; taste bland. Insoluble in water, softened in hot water (Fig. 1).

4. IDENTIFICATION**4.1 Thin-Layer Chromatographic Identification** [Appendix IV(A)]**Standard solution**

Dracorhodin perchlorate standard solution

Weigh 2.5 mg of dracorhodin perchlorate CRS (Fig. 2) and dissolve in 5 mL of ethanol.

Developing solvent system

Prepare a mixture of ethyl acetate and ethanol (20:1, v/v).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of ethanol.

Sonicate (150 W) the mixture for 10 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately dracorhodin perchlorate standard solution (0.5 μL) and the test solution (10 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. Examine the plate under UV light (366 nm). Calculate the *R_f* value by using the equation as indicated in Appendix IV (A).

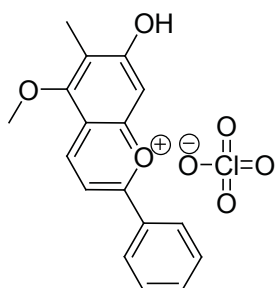


Figure 2 Chemical structure of dracorhodin perchlorate

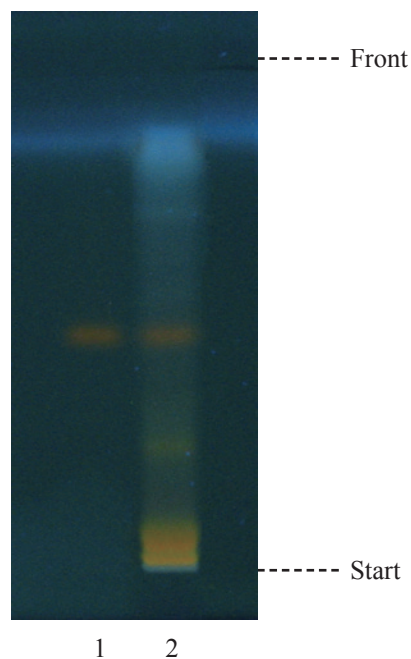


Figure 3 A reference HPTLC chromatogram of *Draconis Sanguis* extract observed under UV light (366 nm)

1. Dracorhodin perchlorate 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 dracorhodin (Fig. 3).

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

Standard solution

Dracorhodin perchlorate standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of dracorhodin perchlorate CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol. Sonicate (150 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 5 min. Filter through a 0.45- μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (440 nm) and a column (4.6 \times 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% Trifluoroacetic acid (% v/v)	Elution
0 – 40	15 → 80	85 → 20	linear gradient
40 – 60	80	20	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 µL of dracorhodin perchlorate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of dracorhodin should not be more than 5.0%; the RSD of the retention time of dracorhodin peak should not be more than 2.0%; the column efficiency determined from dracorhodin peak should not be less than 100000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 4).

Procedure

Separately inject dracorhodin perchlorate Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention time of dracorhodin peak in the chromatogram of dracorhodin perchlorate Std-FP and the retention times of the three characteristic peaks (Fig. 4) in the chromatogram of the test solution. Identify dracorhodin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dracorhodin perchlorate Std-FP. The retention times of dracorhodin 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 Draconis Sanguis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Draconis Sanguis extract

Peak No.	RRT	Acceptable Range
1 (marker, dracorhodin)	1.00	-
2	2.05	± 0.03
3	2.22	± 0.03

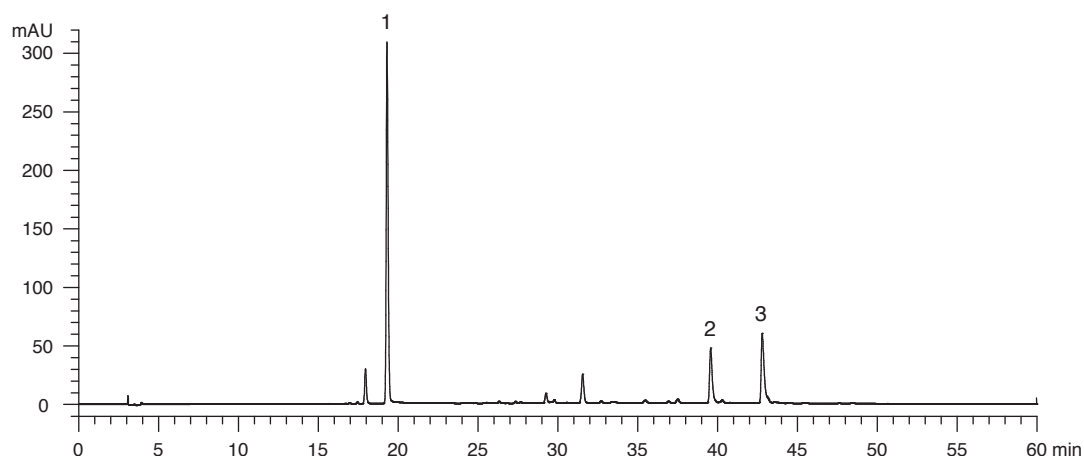
Draconis Sanguis

Figure 4 A reference fingerprint chromatogram of Draconis Sanguis 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. 4).

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 Ash (*Appendix IX*)

Total ash: not more than 3.0%.

Acid-insoluble ash: not more than 2.0%.

5.6 Water Content (*Appendix X*)

Oven dried method: not more than 7.0%.

5.7 Ethanol-insoluble matter

Weigh 2.0 g of the powdered sample and place it in a weighted cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 200 mL of ethanol to a 500-mL round-bottomed flask. Perform the soxhlet extraction until the mixture become colourless. Cool down to room temperature. Collect the thimble and dry it at about 105°C for 4 h. Weigh accurately and calculate the percentage of ethanol-insoluble matter with reference to the dried substance. The amount of ethanol-insoluble matter in the sample should not be more than 25.0%.

5.8 Limit of rosin

Weigh 0.1 g of the powdered sample and place it in a test tube, then add 10 mL of petroleum ether (60-80°C). Shake well for 10 min. Filter and transfer 5 mL of the filtrate to another test tube. Add 5 mL of freshly prepared 0.5% (w/v) copper acetate solution and allow to stand for 10 min. No green colour should be observed in the upper layer of the sample solution.

6. EXTRACTIVES (*Appendix XI*)

Ethanol-soluble extractives (hot extraction method): not less than 39.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 2.0 mg of dracorhodin perchlorate CRS and dissolve in 5 mL of ethanol.

Dracorhodin perchlorate standard solution for assay, Std-AS

Measure accurately the volume of the dracorhodin perchlorate Std-Stock, dilute with ethanol to produce a series of solutions of 10, 40, 100, 200, 400 mg/L for dracorhodin perchlorate.

Test solution

Weigh accurately 0.2 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 $3000 \times g$ for 10 min. Transfer the supernatant to a 25-mL volumetric flask. 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 (440 nm) and a column (4.6 \times 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 0.1% trifluoroacetic acid and acetonitrile (63:37, v/v). The elution time is about 15 min.

System suitability requirements

Perform at least five replicate injections, each using 5 μL of dracorhodin perchlorate Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of dracorhodin should not be more than 5.0%; the RSD of the retention time of dracorhodin peak should not be more than 2.0%; the column efficiency determined from dracorhodin peak should not be less than 10000 theoretical plates.

The *R* value between dracorhodin 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 dracorhodin perchlorate Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of dracorhodin against the corresponding concentrations of dracorhodin perchlorate 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 dracorhodin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dracorhodin perchlorate Std-AS. The retention times of dracorhodin 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 dracorhodin in the test solution, and calculate the percentage content of dracorhodin (the percentage content of dracorhodin perchlorate \times 0.726, where 0.726 is the molar mass ratio of dracorhodin and dracorhodin perchlorate) in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.0% of dracorhodin ($\text{C}_{17}\text{H}_{14}\text{O}_3$), calculated with reference to the dried substance.