Bruceae Fructus



- A. Bruceae Fructus B. Magnified image of fruit
- C. Magnified image of transverse section of fruit
- D. Magnified image of seed

空江南 Chrysanthemi Indici Flos 作節参 Lycoridis Radiatae Pericarpium Melicopes Pteleifoliae Caulis 三叉苦 Rhapontic Smilacis Chinae Rhizoma 豆蔻 漏產 和omi Fructus Rotundus Tinosporae Radix 季陵菜 T 古 ざんたっし ゴ ヘアリ際

Bruceae Fructus

1. NAMES

Official Name: Bruceae Fructus

Chinese Name: 鴉膽子

Chinese Phonetic Name: Yadanzi

2. SOURCE

Bruceae Fructus is the dried ripe fruit of *Brucea javanica* (L.) Merr. (Simaroubaceae). The fruit is collected in autumn when ripe, foreign matter removed, then dried under the sun to obtain Bruceae Fructus.

3. DESCRIPTION

Ovoid to ellipsoid, slightly flat, 3-10 mm long, 2-8 mm in diameter. Externally yellowish-green, yellowish-brown, dark green to dark brown, with raised polygonal reticulate wrinkles. Apex acuminate, base with a dented fruit stalk scar, two sides with obvious ridges. Shell hard but fragile, seed 1, ovoid, externally whitish to yellowish-white, endosperm yellowish-white, cotyledon green, oily. Odour slight but characteristic; taste extremely bitter (Fig. 1).

4. **IDENTIFICATION**

4.1 Microscopic Identification (Appendix III)

Transverse Section

Outermost layer of exocarp consists of 1 layer of epidermal cells, covered with cuticle, with 1-2 layers of subsquare cells beneath the epidermal cells. Mesocarp consists of subrounded parenchymatous cells, with scattered clusters of calcium oxalate, and collateral vascular bundles bordering the endocarp. Outer side of endocarp wavy with protruding peaks, consisting of 2 layers of stone cells, forming annular bands, and 1 layer of crystal-containing cells. Outer band of stone cells consist of 1 to several layers of stone cells with distinct lumen; middle band is a crystal cells layer, consisting of 1 to several layers of cells with slightly thickened wall, usually containing prisms of calcium oxalate; inner band of stone cells relatively broad, made up of stone cells with indistinct boundaries. Testa consists of 1 layer of cells, boundaries indistinct. Endosperm cells polygonal, filled with aleurone grains. Cotyledon cells relatively small, filled with aleurone grains (Fig. 2).

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Powder

Colour yellowish-brown to dark brown. Stone cells of inner endocarp mostly in clumps, pale yellow to bright yellow, irregular in shape, walls thick, boundaries indistinct; bright yellow under the polarized microscope. Stone cells of outer endocarp scattered or in groups, colourless or pale yellow, subrounded, subpolygonal or irregular in shape, 15-119 µm long, 14-84 µm in diameter, with distinct pits and pit canals; yellowish-white under the polarized microscope. Crystal cells in groups, yellow to yellowish-brown, subpolygonal or subrounded, walls slightly beaded-thickened, usually containing prisms of calcium oxalate; crystal cells dark yellowish-brown, prisms of calcium oxalate polychromatic under the polarized microscope. Prisms of calcium oxalate scattered or present in crystal cells, 4-37 µm in diameter; polychromatic under the polarized microscope. Clusters of calcium oxalate scattered or present in parenchymatous cells, 6-36 µm in diameter; polychromatic under the polarized microscope. Epidermal cells of exocarp colourless or pale yellowish-brown, subpolygonal in surface view, sometimes with slightly beaded-thickened walls, stomata rare, anomocytic. Epidermal cells of testa with granular surface, boundaries indistinct. Oil droplets numerous, colourless or pale yellow (Fig. 3).





Figure 2 Microscopic features of transverse section of Bruceae Fructus

A. Sketch B. Section illustration C. Magnified image of exocarp D. Magnified image of endocarp

- 1. Exocarp 2. Mesocarp 3. Vascular bundle 4. Stone cells band of outer endocarp
- 5. Crystal cells of endocarp 6. Stone cells band of inner endocarp 7. Testa 8. Endosperm
- 9. Cotyledon 10. Cuticle 11. Epidermis 12. Cluster of calcium oxalate
- 13. Prism of calcium oxalate





Figure 3 Microscopic features of powder of Bruceae Fructus

- 1. Stone cells of inner endocarp 2. Stone cell of outer endocarp 3. Crystal cells
- 4. Prisms of calcium oxalate 5. Cluster of calcium oxalate 6. Epidermal cells of exocarp (-> stoma)
- 7. Epidermal cells of testa 8. Oil droplets
- a. Features under the light microscope b. Features under the polarized microscope

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

Standard solution

Brucein D standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, methanol, formic acid and water (15:1:1:1, v/v).

Test solution

Weigh 1.0 g of the freshly powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (270 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 10 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 4 mL of methanol. Filter through a 0.45-µm nylon filter.

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 brucein D standard solution (1.5 µL) and the test solution (7 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 10 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 (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).



Figure 4 Chemical structure of brucein D

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Figure 5 A reference HPTLC chromatogram of Bruceae Fructus extract observed under UV light (254 nm)

1. Brucein D 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 brucein D (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Brucein D standard solution for fingerprinting, Std-FP (25 mg/L) Weigh 0.25 mg of brucein D CRS and dissolve in 10 mL of methanol (50%).

Test solution

Weigh 1.0 g of the freshly powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with 10 mL of methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 µm particle size, 100 Å pore size, 16% carbon loading and 325 m²/g surface area). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Time (min)	0.4% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0-6	95	5	isocratic
6 - 30	$95 \rightarrow 90$	$5 \rightarrow 10$	linear gradient
30 - 35	90	10	isocratic
35-40	$90 \rightarrow 82$	$10 \rightarrow 18$	linear gradient

Table 1	Chromatographic system	conditions

System suitability requirements

Perform at least five replicate injections, each using 20 μ L of brucein D Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of brucein D should not be more than 5.0%; the RSD of the retention time of brucein D peak should not be more than 2.0%; the column efficiency determined from brucein D peak should not be less than 30000 theoretical plates.

The *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject brucein D Std-FP and the test solution (20 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of brucein D peak in the chromatogram of brucein D Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify brucein D peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of brucein D Std-FP. The retention times of brucein D 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 Bruceae Fructus extract are listed in Table 2.



Peak No.	RRT	Acceptable Range
1	0.42	± 0.03
2	0.52	± 0.03
3	0.66	± 0.03
4	0.83	± 0.03
5 (marker, brucein D)	1.00	-

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



Figure 6 A reference fingerprint chromatogram of Bruceae Fructus extract

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. 6).

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*): not more than 1.0%.
- 5.6 Ash (Appendix IX)

Total ash: not more than 6.5%. Acid-insoluble ash: not more than 0.5%.



5.7 Water Content (Appendix X)

Oven dried method: not more than 10.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (cold extraction method): not less than 12.0%. Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution

Brucein D standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 mg of brucein D CRS and dissolve in 10 mL of methanol (50%).

Brucein D standard solution for assay, Std-AS

Measure accurately the volume of the brucein D Std-Stock, dilute with methanol (50%) to produce a series of solutions of 3, 6, 12, 25, 50 mg/L for brucein D.

Test solution

Weigh accurately 1.0 g of the freshly powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with 10 mL of methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 µm particle size, 100 Å pore size, 16% carbon loading and 325 m²/g surface area). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

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Time (min)	0.4% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0-6	95	5	isocratic
6-30	$95 \rightarrow 90$	$5 \rightarrow 10$	linear gradient
30 - 35	90	10	isocratic
35 - 40	$90 \rightarrow 82$	$10 \rightarrow 18$	linear gradient

Table 3	Chromatographic sy	stem conditions
		r

System suitability requirements

Perform at least five replicate injections, each using 20 μ L of brucein D Std-AS (12 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of brucein D should not be more than 5.0%; the RSD of the retention time of brucein D peak should not be more than 2.0%; the column efficiency determined from brucein D peak should not be less than 30000 theoretical plates.

The R value between brucein D 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 brucein D Std-AS (20 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of brucein D against the corresponding concentrations of brucein D Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 μ L of the test solution into the HPLC system and record the chromatogram. Identify brucein D peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of brucein D Std-AS. The retention times of brucein D 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 brucein D in the test solution, and calculate the percentage content of brucein D in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.070% of brucein D ($C_{20}H_{26}O_9$), calculated with reference to the dried substance.

Bruceae Fructus (鴉膽子)



Figure 1 A reference assay chromatogram of Bruceae Fructus extract