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Traditional Chinese medicine — Gardenia jasminoides fruit

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Gardenia jasminoides fruit, the dried fruit of *Gardenia jasminoides* Ellis (Fam. Rubiaceae), is widely used in China, Japan, Korea, other southeast Asian countries and North American with a very long application history as herbal medicine. It is commonly used for draining fire and treatment of certain febrile conditions, cooling blood and eliminating stasis to activate blood circulation. Clinically, owing to its medicinal properties, it plays an important role in diuretic, cholagogue, anti-inflammatory, antioxidant and antipyretic effects.

G. jasminoides fruit has also been applied as an important natural colourant such as gardenia yellow and gardenia blue pigments in China, Japan, Korea, India and North American. Recently, there is an increasingly greater demand for *G. jasminoides* fruit in China and abroad, and the average annual demand for *G. jasminoides* fruit is about 1 500 tons in the latest five years (2015-2020). *G. jasminoides* fruit is ranked as No. 59 in ISO 23975 which has urgent need for developing an international standard.

G. jasminoides fruit, as a medicinal and ornamental plant, has been cultivated widely in the tropical and subtropical regions of the world, and grows on mountain slope or on road sides. However, the quality of *G. jasminoides* fruit provided from different areas or by different cultivators is quite different. In addition, though *G. jasminoides* fruit has been recorded in several pharmacopeias and standards, specifications and quality requirements in these standards vary. Thus, there is a clear and urgent need to develop an international standard for harmonizing the existing standards, as well as ensuring the safety and effectiveness of *G. jasminoides* fruit.

As national implementation may differ, national standards bodies are invited to modify the values given in [5.4](#) and [5.5](#) in their national standards. Examples of national and regional values are given in [Annex D](#).

Traditional Chinese medicine — *Gardenia jasminoides* fruit

1 Scope

This document specifies the quality and safety requirements and test methods of *Gardenia jasminoides* fruit, which is derived from the dried fruit of *Gardenia jasminoides* Ellis (Fam. Rubiaceae).

This document is applicable to *Gardenia jasminoides* fruit that are sold and used as natural medicines in international trade, including Chinese materia medica (whole medicinal material) and decoction pieces derived from this plant.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 18664, *Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine*

ISO/TS 21310, *Traditional Chinese medicine — Microscopic examination of medicinal herbs*

ISO 21371, *Traditional Chinese medicine — Labelling requirements of products intended for oral or topical use*

ISO 22217, *Traditional Chinese medicine — Storage requirements for raw materials and decoction pieces*

ISO 22258, *Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography*

ISO 23723, *Traditional Chinese medicine — General requirements for herbal raw material and materia medica*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

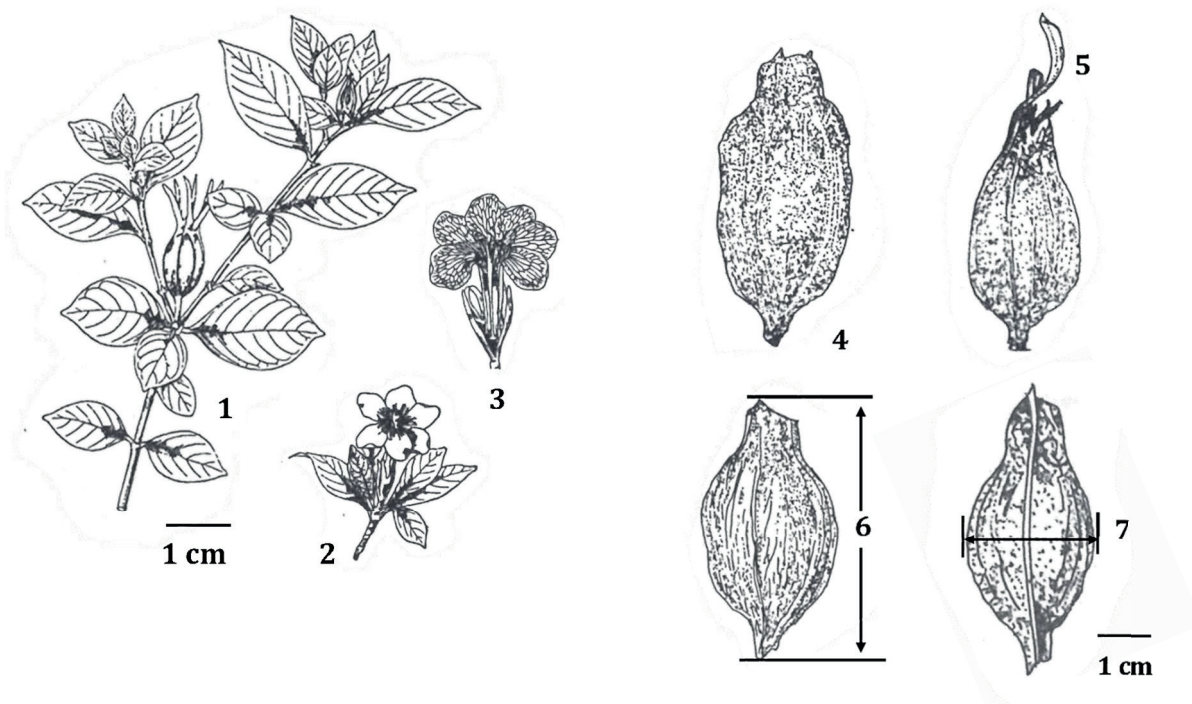
3.1

***Gardenia jasminoides* fruit**

dried ripe fruit of *Gardenia jasminoides* Ellis (Fam. Rubiaceae)

4 Descriptions

In this document, the structure of *Gardenia jasminoides* Ellis and the dried ripe fruit are shown in Figure 1. Different features such as leaves, flowers, fruits in *Gardenia jasminoides* Ellis and *Gardenia jasminoides* var. *grandiflora* Nakai, and methods for differentiating these two species, are given in [Annex C](#).



a) Plant part of *Gardenia jasminoides* Ellis

b) Dried ripe *Gardenia jasminoides* fruit

Key

- 1 fructing branch
- 2 flowering branch
- 3 corolla and calyx
- 4 peduncle
- 5 persistent calyx
- 6 length
- 7 diameter

Figure 1 — Structure of the plant part and fruit of *Gardenia jasminoides* Ellis

5 Requirements

5.1 General

The following requirements shall be met before the sampling refer to Caluse 6.

- a) *Gardenia jasminoides* fruit shall be clean and free from leaves, stems and foreign matters.
- b) The presence of living inserts, mouldy fruit and external containinants which are visible to the naked eye shall not be permitted.

5.2 Morphological features

5.2.1 Appearance

The fruit is ovate to long ovate or elliptical (see [Figure 1b](#)). The external fruit surface usually has 6 antesepalous raised and longitudinal winged ridges. The basally gamosepalous persistent calyx (key 5)

or its scar are visible at the upper end of the fruit, and sometimes has the peduncle (key 4) remaining at the lower end of the fruit. The inner surface of pericarp is brittle with smooth and lustrous, and it is internally divided into 2 or 3 rows of protruding membranes, which contain seeds.

5.2.2 Colour

The smooth or slightly rough outer surface is yellowish, reddish-yellow or brownish-red. The pericarp is pale yellow or brownish-yellow inner surface.

5.2.3 Dimensions

The fruit is 15 mm to 35 mm in length measured from the base to the end of the fruit, and 10 mm to 15 mm in diameter measured at the middle of the fruit.

5.2.4 Fracture

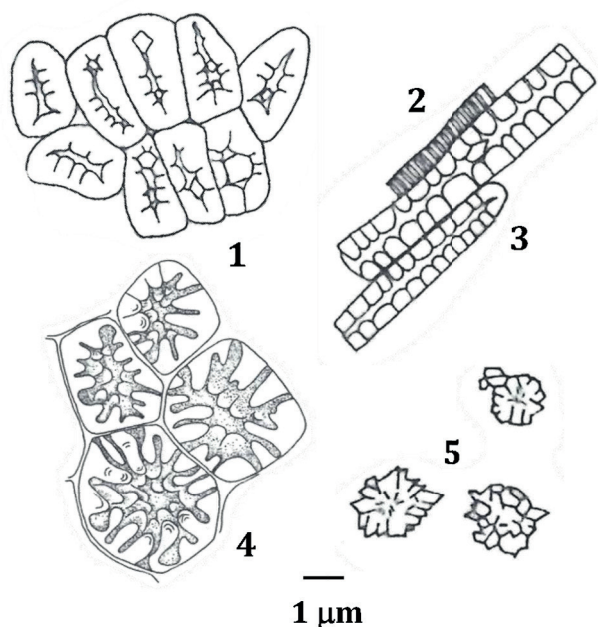
The pericarp is thin and easily broken. The seeds are flattened ovate, several seeds gathered together to form masses. They are deep red or yellow-red with a dense arrangement of thin, small strumae on the external surface.

5.2.5 Odour and taste

Odour is slight and characteristic, and the taste is bitter.

5.3 Microscopical characteristics

As shown in [Figure 2](#), the powder is reddish-brown or yellow-brown; sclereid of endocarp (key 1) is subrounded or polygonal, about 17 μm to 31 μm in diameter, with thickened walls, and lumina containing crystals of calcium oxalates that about 8 μm in diameter; fragments of mesocarp consisting of thin-walled parenchymatous cells associated with fibres and yellowish spiral or annular vessels (key 2); endocarp fibres (key 3) are long and thin, about 10 μm in diameter and up to 100 μm long, obliquely mosaic-arranged; fragments of testa consisting of largely irregularly shaped sclereids (key 4), 58 μm to 150 μm in diameter and up to 260 μm long, with irregularly thickened yellowish walls, wide pits and containing a reddish-brown substance; endosperm with cells containing scattered clusters of calcium oxalate (key 5).



Key

- 1 sclereid of endocarp
- 2 annular vessel
- 3 endocarp fibre
- 4 sclereid of testa
- 5 calcium oxalate

Figure 2 — Microscopical characteristics of *Gardenia jasminoides* fruit powder

5.4 Moisture

The moisture content in percentage mass should not be more than 13,0%.

5.5 Total ash

The total ash content in percentage mass should not be more than 6,0%.

5.6 Thin-layer chromatogram (TLC) identification

The identification of extracts of *Gardenia jasminoides* fruit with thin-layer chromatogram (TLC) should present the spot or band with the same color and position corresponding to those of reference solution.

5.7 Marker compound(s)

The content of marker compound, such as geniposide ($C_{17}H_{24}O_{10}$), should be determined.

5.8 Heavy metals

The contents of heavy metals, such as arsenic, mercury, lead and cadmium should be determined.

5.9 Pesticide residues

The contents of pesticide residues, such as DDT, endrin, BHC, aldrin, dieldrin, shall be determined.

6 Sampling

Sampling of *Gaedenia jasminoides* fruit shall be carried out in accordance with Clause 8 of ISO 23723.

7 Test methods

7.1 Macroscopic identification

Samples not less than 500 g are taken from each batch randomly. These samples are examined by naked eyes observation in sunlight, smell and taste.

7.2 Microscopical identification

The testing method specified in ISO/TS 21310 shall apply.

7.3 Determination of moisture content

The testing method specified in the Clause 7.2.3 of ISO 23723 shall apply.

7.4 Determination of total ash content

The testing method specified in the Clause 7.2.3 of ISO 23723 shall apply.

7.5 Thin-layer chromatogram (TLC) identification

See [Annex A](#) for additional information.

7.6 Determination of marker compound(s)

See [Annex B](#) for additional information.

7.7 Determination of heavy metals contents

The testing method specified in ISO 18664 shall apply.

7.8 Determination of pesticide residues contents

The testing method specified in ISO 22258 shall apply.

8 Test report

For each test method, the test report shall specify the following:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used;
- c) the test method used, with reference to this international standard;
- d) the test result(s) obtained;

- e) all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- f) any unusual features (anomalies) observed during the test;
- g) the date of the test.

9 Packaging, storage and transportation

The packaging and transportation shall not transmit any odour or flavour to the product and shall not contain substances which may damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

The storage requirements for *Gardenia jasminoides* fruit shall refer to ISO 22217.

The products shall be protected from light, moisture, pollution and entry of foreign substances during long distance delivery. Carriers should be well ventilated to keep dry and moisture-proof.

10 Marking and labeling

See the method specified in ISO 21371. The following items shall be marked or labeled on the packages:

- a) all quality features indicated in [clause 5](#), determined in accordance with methods specified in [clause 7](#);
- b) gross weight and net weight of the package;
- c) country of origin and province / state of the products;
- d) date of production and expiry date of the products;
- e) storage method;
- f) any items required by regulatory bodies of destination country.

Annex A (informative)

Thin-layer chromatogram (TLC) identification

A.1 Preparation of test solution

Weigh 1.0 g of sample to grind and pass it through a 24 mesh or coarse sieve, add 50 mL 50% methanol and extract with ultrasonic for 30 min, filter and the filtered solution as the test solution.

A.2 Preparation of reference drug solution

Weigh 1.0 g of reference drug of *Gardenia jasminoids* fruit to grind and pass it through a 24 mesh or coarse sieve, and treat it in the same manner as in [B.1](#) as the reference drug solution.

A.3 Preparation of reference substance solution

Dissolve a quantity of geniposide CRS with ethanol in a brown volumetric flask to produce a solution containing 4.0 mg of each per mL as the reference solution.

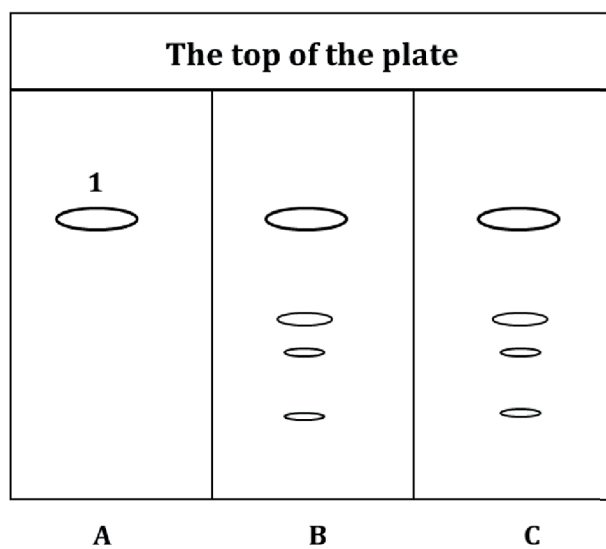
A.4 Developing solvent system

Prepare a mixture of ethyl acetate—acetone—formic acid—water (5: 5: 5: 1, v/v/v/v) as the mobile phase.

A.5 Procedure

Apply 2.0 µL each of the reference drug solution and the test solutions on the same TLC plate (silical gel G) previously dried at 110°C for 15 min in the oven. Develop the plate in the mobile phase described in [A.4](#), then move the plate and dry in air for determination of the colour of spots. Subsequently, examine the plate under spraying with 10% sulfuric acid ethanol solution, and then heat at 110°C until spots appear clearly. Identify the spots of the test solutions by comparing the positions and colours with those of the reference drug solution.

Typical reference TLC chromatograms are shown in Figure A.1.



Key

- 1 geniposide
- A reference solution
- B *Gardenia jasminoids* fruit reference drug solution
- C *Gardenia jasminoids* fruit test solution

Figure A.1 — Schematic diagram of typical TLC chromatogram of *Gardenia jasminoides* fruit

Annex B (informative)

Determination of geniposide contents

B.1 Principle of the test method

The high-performance liquid chromatography (HPLC) method is employed to determine the content of geniposide. The HPLC system consists of a quaternary pump, continuous vacuum degasser, thermostated auto-sampler and column compartment coupled to a variable wavelength diode-array detector.

B.2 Preparation of reference standard solution

Dissolve a quantity of geniposide CRS with ethanol in a brown volumetric flask to produce a solution containing 0,3 mg of each per mL as the reference solution.

B.3 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80 mesh. Weigh accurately 0,1 g of the powder in a stopper conical flask. Accurately add 25 mL of methanol. Weigh and extract under ultrasonic for 20 min. Cool and weigh again. Replenish the loss of mass with methanol and mix well. Filter and use the successive filtrate, pipet 10 mL of the filtrate, add methanol to make exactly 25 mL, and use this solution as the final test solution. The supernatant was then filtered through a 0,45 µm Millipore filter unit before HPLC analysis.

B.4 Chromatographic system

Column.

- a) Stationary phase: octadecylsilane bonded silica gel as analysing column or equivalent;
- b) Size: $l = 250$ mm, $\Phi = 4,6$ mm;
- c) Theoretical plates: not less than 1 500.

Mobile phase:

- a) Mobile phase A: water of chromatography grade.
- b) Mobile phase B: acetonitrile of chromatography grade.
- c) Isocratic elution: a mixture of mobile phases A and B (85: 15).

Flow rate: 1,0 ml/min.

Detector: 238 nm.

Column temperature: 30 °C.

Injection volume: 10 µl.

B.5 Content calculation of geniposide

B.5.1 The content of geniposide, C_{gen} (%) is calculated with [Formula C.1](#).

$$C_{\text{gen}} = \frac{c_s \times 10^{-3} \times 100}{M \times (1 - C_m)} \times 100 \% \quad (\text{C.1})$$

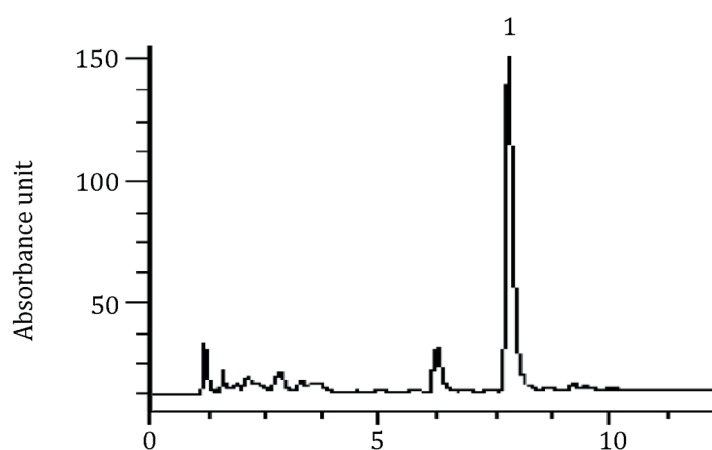
where

C_s is the average content of the sample (mg/ml);

M is the mass of *Gardenia jasminoides* fruit taken to prepare the sample solution (g);

C_m is the moisture content of samples (%).

B.5.2 A typical reference HPLC chromatogram is shown in Figure B.1.



Key

1 Geniposide

Figure B.1 — Typical HPLC chromatograms of *Gardenia jasminoides* fruit

Annex C (informative)

Referenced information and methods for differentiating *Gardenia jasminoides* Ellis and *Gardenia jasminoides* var. *grandiflora* Nakai

Different features such as leaves, flowers, fruits in *Gardenia jasminoides* Ellis and *Gardenia jasminoides* var. *grandiflora* Nakai, and methods for differentiating these two species, are shown in Table C.1.

Table C.1 — Reference information and methods for differentiating *Gardenia jasminoides* Ellis and *Gardenia jasminoides* var. *grandiflora* Nakai [5]

Items		<i>G. jasminoides</i> Ellis (Mountain- <i>Gardenia jasminoides</i> fruit)	<i>G. jasminoides</i> var. <i>grandiflora</i> Nakai (Water- <i>Gardenia jasminoides</i> fruit)
Morphological features	Leaves	Oblong-lanceolate or broad-lanceolate; Length: 5,0 cm to 11,0 cm	Oblong-lanceolate or ovate-lanceolate; Length: 7,0 cm to 14,0 cm
	flower	Smaller; diameter \leq 7,0 cm	Bigger; diameter \geq 7,0 cm
	fruit	Length: 1,5 cm to 3,5 cm; Diameter: 1,0 cm to 1,5 cm	Length: 3,0 cm to 7,0 cm; Diameter: 1,0 cm to 1,5 cm
Application		Medicinal use	Ornamental, natural edible pigments
Methods for differentiating		RAPD (random amplified polymorphic DNA) ^[5]	

Annex D (informative)

Referenced information of *Gardenia jasminoides* fruit in different national and regional requirements

Different countries and regions give their quality requirement items on *Gardenia jasminoides* fruit as shown in Table D.1.

Table D.1 — National and regional quality requirement items of *Gardenia jasminoides* fruit

Items	Authority regulation	Chinese Pharmacopoeia (ChP 2020)	European Pharmacopoeia (EP 10 th)	Japanese Pharmacopoeia (JP 18 th)	Korean Pharmacopoeia (KP 12 th)
Plant origin		<i>Gardenia jasminoides</i> Ellis	<i>Gardenia jasminoides</i> Ellis	<i>Gardenia jasminoides</i> Ellis	<i>Gardenia jasminoides</i> Ellis
Identification	Description	√	√	√	√
	Microscopy	√	√	√	√
	TLC	√	√	√	√
Examination	Moisture	≤ 8,5 %	≤ 8,5 %	≤ 13,0 %	≤ 10,0 %
	Total ash	≤ 6,0 %	≤ 6,0 %	≤ 6,0 %	≤ 6,0 %
Heavy metals	Lead	≤ 5,0 mg/kg	≤ 5,0 ppm	-	≤ 5,0 ppm
	Cadmium	≤ 1,0 mg/kg	≤ 1,0 ppm		≤ 0,3 ppm
	Mercury	≤ 0,2 mg/kg	≤ 0,1 ppm		≤ 0,2 ppm
	Arsenic	≤ 2,0 mg/kg	-		≤ 3,0 ppm
Residual pesticides	Total DDT	33 kinds of pesticide residues such as DDT, BHC, Endrin shall be detected	≤ 1,0 ppm	-	≤ 0,1 ppm
	Endrin		≤ 0,05 ppm	-	≤ 0,01 ppm
	Total BHC		-	-	≤ 0,2 ppm
	Aldrin		≤ 0,05 ppm	-	≤ 0,01 ppm
	Dieldrin			-	≤ 0,01 ppm
Assay	Geniposide	≥ 1,8 %	≥ 2,0 %	≥ 2,7 %	≥ 3,0 %
Key					
√ The index is set in the pharmacopoeia;					
- The index is not set in the pharmacopoeia.					

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