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Traditional Chinese medicine — *Pinellia ternata* tuber

Médecine traditionnelle chinoise — Tubercule de Pinellia ternata

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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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 document 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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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

Pinellia ternata tuber, the dried tuber of *Pinellia ternata* (Thunb.) Breit. (Fam. Araceae), has a long history of medicinal use in China, Japan, Korea and other Southeast Asian countries. *P. ternata* tuber is commonly used for drying dampness, resolving phlegm and descending counterflow to relieve nausea and vomiting.

Owing to its effectiveness, there has recently been an increasing demand for *P. ternata* tuber in China and worldwide. According to data from Chinese customs, the average annual demand for *P. ternata* tuber from 2015 to 2019 was about 1 800 tons and overseas trade counts for as much as USD 1 million per year. *P. ternata* tuber is ranked no. 3 in the priority list of single herbal medicines for developing standards (see ISO/TR 23975).

P. ternata tuber has a pungent taste with a numbing and irritating sensation in the gastrointestinal mucosa, throat and oral cavity. Its properties are warm, pungent and toxic. Indeed, unprocessed *P. ternata* tuber, or its insufficiently boiled decoction, causes acrid irritation of the oral and laryngopharynx mucosa when taken by mistake. The toxicity and side effects of raw *P. ternata* tuber can be reduced dramatically with proper processing and dose control. Three kinds of processed *P. ternata* tuber with different processing methods are available and traded on the market, as well as applied in clinical treatments: liquorice-limewater-processed *P. ternata* tuber, ginger-alum-processed *P. ternata* tuber and alum-processed *P. ternata* tuber. However, a unified International Standard regarding the characteristics of and test methods for raw and processed *P. ternata* tuber is not yet available. The regulatory authorities in many countries have not adequately differentiated high-toxic forms of *P. ternata* tuber from less-toxic forms (or even non-toxic forms). Additionally, the quality of raw and processed *P. ternata* tuber provided from different areas varies a lot. Therefore, an International Standard for raw and processed *P. ternata* tuber in terms of quality control of this herb and its products is urgently required to ensure the safe use of these medicinal materials.

This document aims to build a systematic and practical International Standard for *P. ternata* tuber to control and supervise its stable quality, to ensure its safe and effective application in clinics, to regulate the trade in the global market and to reduce cases of Pinellia poisoning.

As national implementation can differ, national standards bodies are invited to modify the values given in 5.4, 5.5 and 5.8 in their national standards. Examples of national and regional values are given in Annex E.

Traditional Chinese medicine — *Pinellia ternata* tuber

1 Scope

This document specifies the quality and safety requirements and test methods of *Pinellia ternata* tuber, including raw and processed *Pinellia ternata* tuber [dried tuber of *Pinellia ternata* (Thunb.) Breit.].

This document does not cover processing methods of *Pinellia ternata* tuber.

This document is applicable to raw and processed *Pinellia ternata* tuber that are sold and used as natural medicines in international trade, including Chinese materiamedica (whole medicinal material) and decoction pieces derived from this plant.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 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 22467, *Traditional Chinese medicine — Determination of microorganisms in natural products*

ISO 22590, *Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration*

ISO 23723:2021, *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 terminology 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
raw *Pinellia ternata* tuber
dried tuber of *Pinellia ternata* (Thunb.) Breit

3.2
processed *Pinellia ternata* tuber
dried tuber of *Pinellia ternata* (Thunb.) Breit after traditional processing

Note 1 to entry: Commonly used varieties of processed Pinellia ternata tuber include liquorice-limewater-processed *Pinellia ternata* tuber, ginger-alum-processed *Pinellia ternata* tuber and alum-processed *Pinellia ternata* tuber.

3.3
liquorice-limewater-processed *Pinellia ternata* tuber
processed *Pinellia ternata* tuber in liquorice and limewater

Note 1 to entry: Liquorice-limewater-processed *Pinellia ternata* tuber is processed with the following method: soak the dried raw *Pinellia ternata* tuber in water until the tuber is fully wet, decoct proper liquorice (*Glycyrrhiza* root and rhizome) with water twice, mix the decoction solutions then pour into the limewater; add the soaked *Pinellia ternata* tuber and stir it one to two times per day, maintaining a pH value over 12,0; gradually prolong the time for processing until the colour of the longitudinal section becomes evenly yellow and tasting it leaves the tongue slightly numb; take it out then wash and dry it in the shade or an oven.

Note 2 to entry: The mass ratio of raw *Pinellia ternata* tuber, liquorice and quicklime powder is 20:3:2.

3.4
ginger-alum-processed *Pinellia ternata* tuber
processed *Pinellia ternata* tuber in ginger and alum

Note 1 to entry: Ginger-alum-processed *Pinellia ternata* tuber is processed with the following method: soak the dried raw *Pinellia ternata* tuber in water until the tuber is fully wet then remove; decoct ginger slices to prepare the ginger solution; add raw *Pinellia ternata* tuber and alum to boil thoroughly, then take it out and dry in the air or cut it into slices and then dry in the air.

Note 2 to entry: The mass ratio of raw *Pinellia ternata* tuber, ginger and alum is 8:2:1.

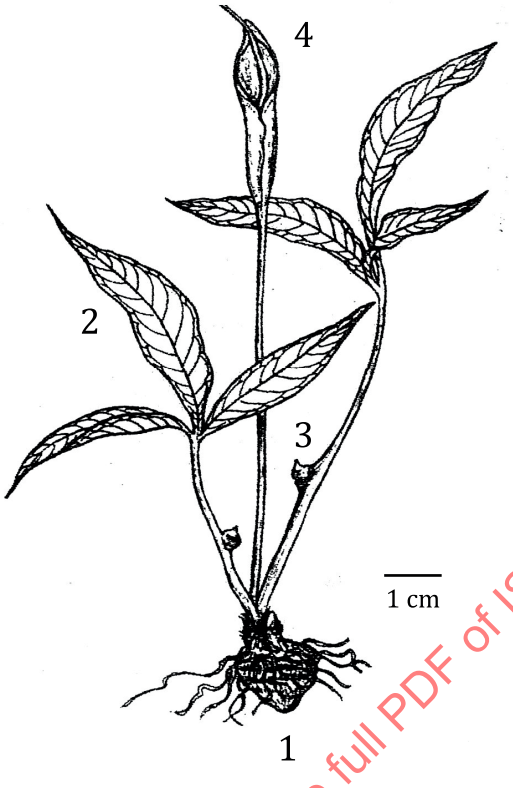
3.5
alum-processed *Pinellia ternata* tuber
processed *Pinellia ternata* tuber in alum

Note 1 to entry: Alum-processed *Pinellia ternata* tuber is processed with the following method: soak the dried raw *Pinellia ternata* tuber in 8 % alum solution until the tuber is fully wet and tasting it leaves the tongue slightly numb; take it out, wash it, cut into thick slices and dry in the air.

Note 2 to entry: The mass ratio of raw *Pinellia ternata* tuber and alum is 5:1.

4 Description

The features of *Pinellia ternata* (Thunb.) Breit. plant in the family of Araceae are shown in Figure 1.



Key

1 tuber
2 leaf
3 bulbil
4 spadix

Figure 1 — Structure of *Pinellia ternata* plant

5 Quality and safety requirements and recommendations

5.1 General characteristics

The following requirements shall be met before sampling.

a) *Pinellia ternata* tuber shall be clean and free from fibrous roots and foreign matter.

b) The presence of living insects, mouldy tuber and external contaminants which are visible to the naked eye shall not be permitted.

5.2 Morphological features of the tuber

5.2.1 Raw *Pinellia ternata* tuber

Tubers have a spheroidal shape, some being slightly oblique, and of 1 cm to 1,5 cm in diameter. Externally, they are white or pale yellow, with a dented stem scar at the apex, densely surrounded by pocked and dotted root scars; the base is obtuse, rounded and relatively smooth. Its texture is hard and the cross-section of the root is white and starchy. Its odour is slight and the taste is pungent, with a numbing and irritating sensation [see Figure 2 a)].

5.2.2 Liquorice-limewater-processed *Pinellia ternata* tuber

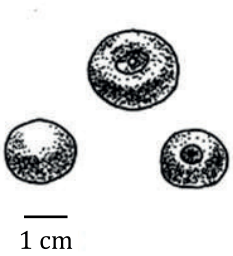
The tuber is spheroidal or broken into irregular granules. Externally, the tuber is pale yellowish-white, yellow or brownish yellow. The texture is fragile and loose or hard, the granules are relatively hard and fragile and the fracture is yellow or pale greyish yellow. The odour is slight and the taste is slightly sweet and numbing [see Figure 2 b)].

5.2.3 Ginger-alum-processed *Pinellia ternata* tuber

The tuber is slice-shaped with irregular grains, or spheroidal. Externally, the tuber is brown or dark brown. The texture is hard and fragile. The fracture is pale yellowish-brown, frequently horny and lustrous. The odour is slightly aromatic and the taste is bland and slightly numbing; the texture is viscous while chewing [see Figure 2 c)].

5.2.4 Alum-processed *Pinellia ternata* tuber

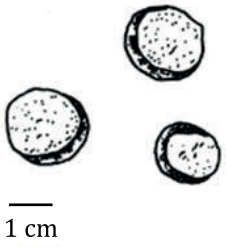
The tuber has elliptical, rounded or irregular slices. The vertical section is pale grey or greyish-white, showing greyish-white and dotted or short line-shaped vascular bundles, sometimes with purplish-red dapples under the remaining cork. The texture is fragile and easily broken; the fracture is slightly cutin-like. The odour is slight and the taste astringent, with a slight numbing sensation [see Figure 2 d)].



a) Raw *P. ternata* tuber

b) Liquorice-limewater-processed *P. ternata* tuber

c) Ginger-alum-processed *P. ternata* tuber



d) Alum-processed *P. ternata* tuber

Figure 2 — Illustration of a *Pinellia ternata* tuber

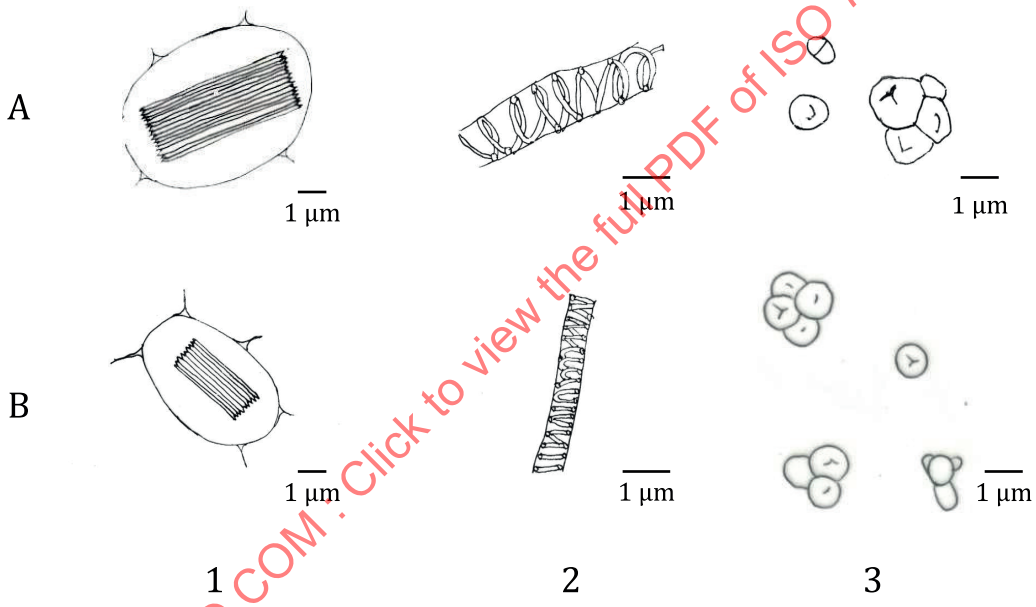
5.3 Microscopic characteristics

5.3.1 Raw *Pinellia ternata* tuber

The powder is almost white. Starch granules are abundant; simple granules are rounded, semi-circular or rounded-polygonal, 2 µm to 20 µm in diameter, hilum slit-shaped, V-shaped or stellate; compound granules consist of two to six components. Raphides of calcium oxalate are embedded in elliptical mucilage cells or scattered throughout; needle crystals are 20 µm to 144 µm long. Spiral vessels have a diameter of 10 µm to 24 µm (see Figure 3, key A).

5.3.2 Processed *Pinellia ternata* tuber

The powder is yellowish-brown to pale yellowish-brown gelatinized starch granules in parenchymatous cells. Raphides of calcium oxalate are embedded in elliptical mucilage cells or scattered throughout; needle crystals are 20 µm to 144 µm long. The spiral vessels have a diameter of 10 µm to 24 µm (see Figure 3, key B).



Key

A raw *Pinellia ternata* tuber
B processed *Pinellia ternata* tuber
1 acicular crystal
2 vascular
3 starch granules

Figure 3 — Microscopic characteristics of raw and processed *Pinellia ternata* tuber

5.4 Moisture

5.4.1 Raw *Pinellia ternata* tuber

The moisture content in percentage mass should not be more than 14,0 %.

5.4.2 Processed *Pinellia ternata* tuber

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

5.5 Total ash

5.5.1 Raw *Pinellia ternata* tuber

The total ash content in mass fraction percentage should not be more than 4,0 %.

5.5.2 Liquorice-limewater-processed *Pinellia ternata* tuber

The total ash content in mass fraction percentage should not be more than 9,0 %.

5.5.3 Ginger-alum-processed *Pinellia ternata* tuber

The total ash content in mass fraction percentage should not be more than 7,5 %.

5.5.4 Alum-processed *Pinellia ternata* tuber

The total ash content in mass fraction percentage should not be more than 4,0 %.

5.6 Water-soluble extract

5.6.1 Raw *Pinellia ternata* tuber

The mass fraction of water-soluble extract should be determined.

5.6.2 Processed *Pinellia ternata* tuber

The mass fraction of water-soluble extract should be determined.

5.7 Thin-layer chromatogram identification

The identification of extracts of *Pinellia ternata* tuber with thin-layer chromatogram (TLC) should present the spot or band with the same colour and position as those of the reference solutions.

5.8 Alum

5.8.1 Ginger-alum-processed *Pinellia ternata* tuber

The content of alum should not be more than 8,5 %.

5.8.2 Alum-processed *Pinellia ternata* tuber

The content of alum should not be more than 10,0 %.

5.9 Heavy metals

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

5.10 Pesticide residue

The mass fraction of pesticide residues shall be determined.

5.11 Sulfur dioxide

The mass fraction of sulfur dioxide should be determined.

5.12 Microbial contamination

The mass fraction of microbial contamination should be determined.

5.13 Grading information

If grades are needed, see Annex D.

6 Sampling

Sampling of raw and processed *Pinellia ternata* tuber shall be carried out in accordance with ISO 23723:2021, Clause 8.

7 Test methods

7.1 Macroscopic identification

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

7.2 Determination of moisture content

The testing method specified in ISO 23723 shall apply.

7.3 Determination of total ash content

The testing method specified in ISO 23723 shall apply.

7.4 Water-soluble extractives

See Annex A for information on water-soluble extractives.

7.5 Thin-layer chromatogram identification

See Annex B f or information TLC.

7.6 Determination of alum contents

To determine alum content, see Annex C.

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.

7.9 Determination of sulfur dioxide contents

The testing method specified in ISO 22590 shall apply.

7.10 Determination of microbial contamination

The testing method specified in ISO 22467 shall apply.

8 Test report

For each test method, the following contents shall be specified in the test report in accordance with ISO 23723:2021, Clause 9:

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 document;

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 could 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 can damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

The storage requirements for *Pinellia ternata* tuber specified in ISO 22217 shall apply.

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 them dry and moisture-proof.

10 Labelling

The requirements of labelling specified in ISO 23723 shall apply.

Annex A
(informative)

Determination of water-soluble extractives

A.1 Weigh 250 g of sample to grind and pass it through a 24-mesh or coarser sieve. Dry the powder in a desiccator to a constant weight. Weigh approximately 4 g of the dried powder into a 250 ml stoppered conical flask and add 100 ml water.

A.2 Allow the mixture of powder and water to stand at room temperature for 18 h. Stir the mixture from time to time during the first 6 h, then filter rapidly with a dry filter.

A.3 Weigh a dried evaporating dish. Transfer 25 ml of the successive filtrate into the evaporating dish. Evaporate the filtrate to dryness in a water bath.

A.4 Dry at 105 °C for 3 h and allow to cool for 30 min in a desiccator. Weigh the extracts rapidly and accurately.

A.5 Calculate the mass fraction of water-soluble extractives, M_{wse}, on the dried basis (%) using Formula (A.1):

$$M_{wse}(\%) = \frac{(M_1 - M_0) \times 4}{M_s} \times 100 \quad \text{(A.1)}$$

where

M_s is the mass of the sample, in g;

M_0 is the mass of the evaporating dish, in g;

M_1 is the mass of the evaporating dish and residue after drying, in g.

Annex B
(informative)

Thin-layer chromatogram identification

B.1 Preparation of test solution

Weigh 1,0 g of sample to grind and pass it through a 24-mesh or coarser sieve. Add 10 ml ethanol and reflux for 30 min, then filter and evaporate the solution to about 0,5 ml as the test solution.

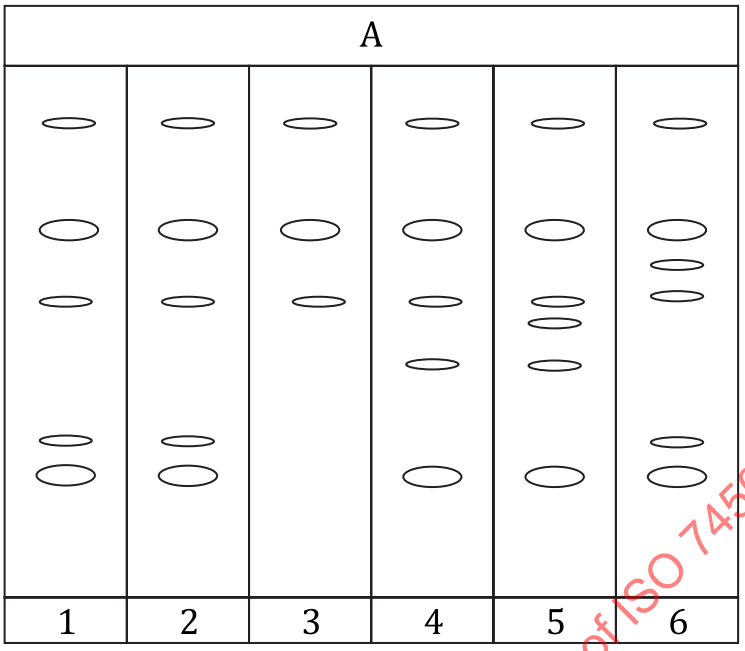
B.2 Preparation of reference solution

Weigh 1,0 g of reference drug of raw *Pinellia ternata* tuber to grind and pass it through a 24-mesh or coarse sieve. Add 10 ml ethanol and reflux for 30 min, then filter and evaporate the solution to about 0,5 ml as the reference solution.

B.3 Procedure

Apply 5,0 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 composed of the volume ratio of petroleum ether (60 °C to 90 °C), ethylacetate, acetone and formic acid (30: 6: 4: 0,5), then move the plate and dry in air. Examine the plate after spraying with 10 % sulfuric acid ethanol solution and then heat at 105 °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 B.1.



Key

1 reference drug of raw *Pinellia ternata* tuber
2 raw *Pinellia ternata* tuber
3 liquorice-limewater-processed *Pinellia ternata* tuber
4 ginger-alum-processed *Pinellia ternata* tuber
5 alum-processed *Pinellia ternata* tuber
6 *Pinellia pedatisecta* Schott tuber (adulterant)
A top of the plate

Figure B.1 — Typical TLC chromatograms of *Pinellia ternata* tuber

Annex C
(informative)

Determination of alum contents

C.1 Principle of the test method

The ethylenediamine tetraacetic acid disodium salt (EDTA-2Na) method is employed to determine the contents of alumn in ginger-alum-processed *Pinellia ternata* tuber or alum-processed *Pinellia ternata* tuber.

C.2 Procedure

a) Weigh accurately 5,0 g of the powder of ginger-alum-processed *Pinellia ternata* tuber or alum-processed *Pinellia ternata* tuber to a crucible. Heat gradually up to 450 °C and incinerate for 4 h until completely carbonized.

b) Add 10 ml of dilute hydrochloric acid to the crucible, cover with a watch glass and heat in a water bath for 10 min. Wash the watch glass with 5 ml of hot water, then wash the residue and crucible with 50 ml of water in stages.

c) Combine the filtrate and scrubbing solution, add one drop of methyl red (0,025 %) in ethanol, then add dropwise ammonia test solution until the colour of the solution is slightly yellow.

d) Add 20 ml of buffer solution (pH 6,0) of acetic acid-ammonium acetate and 25 ml of disodium edetate indicator solution (0,05 mol/l), boil for 3 min to 5 min and cool. Add 1 ml of xylenol orange indicator solution and titrate with zinc titration solution (0,05 mol/l) until the colour changes from yellow to red. Perform a blank determination in the same manner to correct the test result.

C.3 Content of alum

1,0 ml of EDTA-2Na titration solution (0,05 mol/l) corresponds to 23,72 mg of potassium aluminium sulfate [$KAl(SO_4)_2 \cdot 12H_2O$]. The cotent of alum (%) is calculated with the actual content of $KAl(SO_4)_2 \cdot 12H_2O$.

Annex D
(informative)

Example of traditional grades information of *Pinellia ternata* tuber

D.1 Principle

The requirements of commercial grading are established according to the traditional grading system of processed *Pinellia ternata* tuber used in the market. The grading requirements are normally influenced by morphological features and the size of raw *Pinellia ternata* tubers.

The grading requirements of raw *Pinellia ternata* tuber have no clear relationship with the efficacy and safety of tubers but do impact the market price of these products.

The same grade of raw *Pinellia ternata* tuber should be made into the same grade of processed *Pinellia ternata* tuber. For example, first-grade products of liquorice-limewater-processed *Pinellia ternata* tuber, ginger-alum-processed *Pinellia ternata* tuber or alum-processed *Pinellia ternata* tuber should be made with first-grade raw *Pinellia ternata* tuber.

D.2 Grading requirements

Samples of not less than 500 g are taken from each batch randomly. The samples are weighed together accurately to 0,1 g and counted. The number of tubers per 500 g is calculated as follows: 500 g multiplied by the number of samples, then divided by the weight of samples. The recommended grading requirements are shown in Table D.1.

Table D.1 — Recommended grading requirements of *Pinellia ternata* tuber

Grade	Tubers per 500 g
First	≤500
Second	500 to 1 000
Third	≥1 000