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Traditional Chinese medicine — *Dendrobium officinale* stem

Médecine traditionnelle chinoise — Tige de Dendrobium officinale

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
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 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

Dendrobium officinale stem, the dried stem of *Dendrobium officinale* Kimura et Migo, is a widely used herb medicine in China and many other Asian countries. In traditional Chinese medicine, this herb is used to treat fever, retching and excessive thirst. Modern pharmacological studies also demonstrate its great potential in diabetes treatment and immuno-enhancement. Therefore, the market for *Dendrobium officinale* has developed very rapidly, as indicated by the increase in yield, production output and trade volume.

However, there remain many challenges, such as adulteration of similar species and lack of suitable testing methods for quality assessment. In addition, though *Dendrobium officinale* has been recorded in several pharmacopoeias and standards, such as Chinese Pharmacopoeia[4], Hong Kong Chinese Materia Medica Standards[5] and Taiwan Herbal Pharmacopoeia[6], specifications and quality requirements of 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 *Dendrobium officinale*.

As national implementation may differ, National Standards Bodies are invited to modify the values given in 5.2.3, 5.2.4 and 5.2.5 in their national standards. Examples of national and regional values are given in Annex E.

Traditional Chinese medicine — *Dendrobium officinale* stem

1 Scope

This document specifies minimum requirements and test methods for *Dendrobium officinale* stem that is derived from cultivated *Dendrobium officinale* Kimura et Migo.

It is applicable to *Dendrobium officinale* stem that is sold and used as Chinese materia medica (whole medicinal materials) 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 1575, *Tea — Determination of total ash*

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

ISO 20409, *Traditional Chinese medicine — Panax notoginseng root and rhizome*

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

CAC/MRL01, *Maximum Residue Limits for Pesticides in Foods*

CODEX STAN 229, *Analysis of pesticide residues: Recommended methods*

World Health Organization. *Quality control methods for herbal materials, General advice on sampling*, 2011

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 http://www.electropedia.org/

3.1
fresh stem
stem that is newly harvested and has not undergone any processing

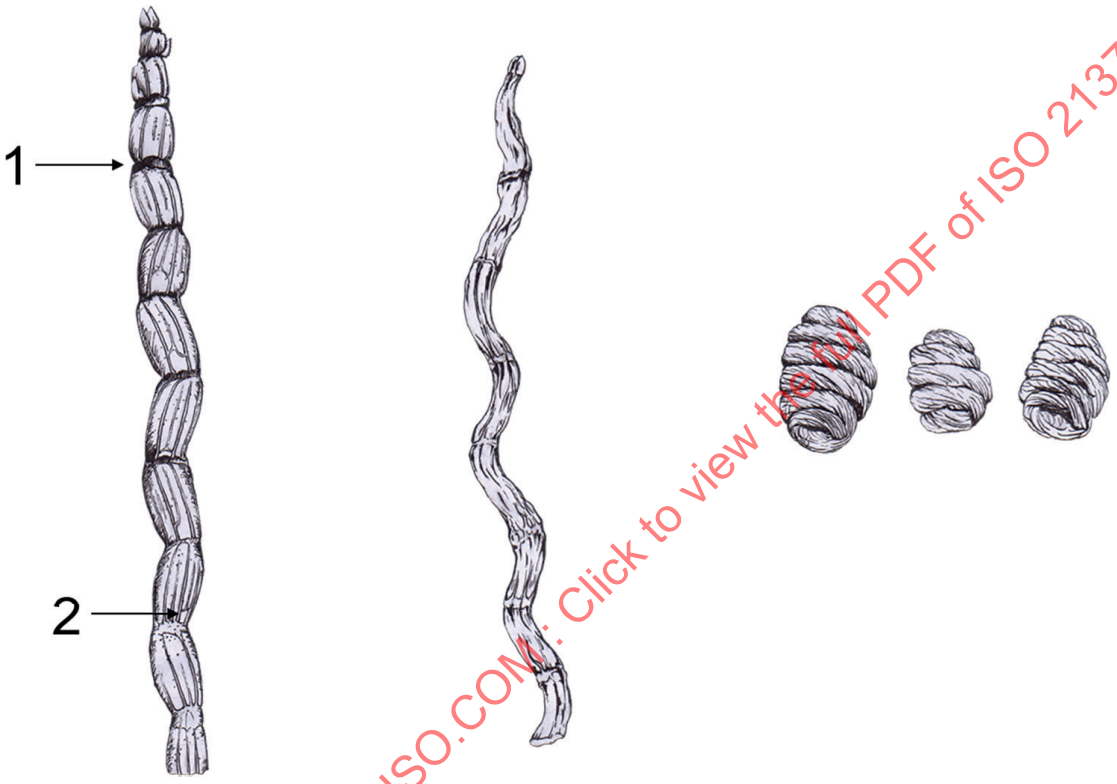
3.2
dried stem
stem prepared by cleaning, cutting and then heating or sun-drying

3.3
processed stem
stem processed by trimming off the fibrous root and leaves, stir-baking while twisting to a spiral or spring form and drying

Note 1 to entry: This is the common trade form of dried stem of *Dendrobium officinale*. It is usually called Tie Pi Feng Dou in Chinese.

4 Descriptions

Dendrobium officinale stem generally has three trade forms, including fresh stem, dried stem and processed stem (Tie Pi Feng Dou), as shown in Figure 1.



a) Fresh stem **b) Dried stem** **c) Processed stem (Tie Pi Feng Dou)**

Key
1 node
2 leaf sheath

Figure 1 — Structure of *Dendrobium officinale*

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Dendrobium officinale* stem shall be clean and free from foreign matter.

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

5.2 *Dendrobium officinale* stem

5.2.1 Morphological features

5.2.1.1 Fresh stem

Cylindrical, circular for cross section. Nodes distinct, unbranched. Purple-dotted leaf sheath. Externally yellowish-green, striated longitudinally. Odour slight. Taste mild, then sweet and viscous on chewing.

5.2.1.2 Dried stem

Cylindrical, differ in length. Externally greyish-green, yellowish-green or golden yellow, striated longitudinally, sometimes grey leaf sheath remained. Odour slight. Taste mild, then viscous on chewing.

5.2.1.3 Processed stem

Spiral or spring-like, usually with two to six spires. Texture compact, easily broken, fracture even, grey to greyish-green. Externally yellowish-green or golden yellow, striated longitudinally, sometimes grey leaf sheath remained. Odour slight. Taste mild, then viscous on chewing.

5.2.2 Thin-layer chromatogram (TLC) identification

The identification of *Dendrobium officinale* stem by thin-layer chromatogram (TLC) shall present spots or bands with the same colour and position corresponding to those of reference standard solution.

5.2.3 Moisture

The mass fraction of moisture should not be more than 14,0 %.

NOTE The fresh stem is exempt from this test.

5.2.4 Total ash

The mass fraction of total ash should not be more than 7,0 %.

NOTE The fresh stem is exempt from this test.

5.2.5 Ethanol-soluble extractives

The mass fraction of ethanol-soluble extractives should not be less than 5,0 %.

NOTE The fresh stem is exempt from this test.

5.2.6 Marker compounds

Marker compounds such as polysaccharides, mannose and peak area ratio of mannose to glucose shall be determined.

NOTE The fresh stem is dried before testing.

5.2.7 Heavy metals

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

5.2.8 Pesticide residues

The content of pesticide residues such as Benzex, DDT (dichloro-diphenyl-trichloroethane) and quintozene shall be determined.

6 Sampling

Sampling of *Dendrobium officinale* stem shall be in accordance with the World Health Organization's *Quality control methods for herbal materials.*

7 Test methods

7.1 Macroscopic identification

Samples not less than 500 g are taken from each batch randomly. These samples are examined by the naked eye, smell and taste.

7.2 TLC identification

See Annex A for additional information.

7.3 Determination of moisture content

The testing method specified in ISO 20409 shall apply.

7.4 Determination of total ash

The testing method specified in ISO 1575 shall apply.

7.5 Determination of ethanol-soluble extractives

See Annex B for additional information.

7.6 Determination of marker compounds

See Annexes C and D for additional information.

7.7 Determination of heavy metals

The testing method specified in ISO 18664 shall apply.

7.8 Determination of pesticide residues

The testing method specified in CODEX STAN 229 and CAC/MRL01 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(s) used;

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 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 product shall be stored in a dry and cool place.

The product shall be protected from light, moisture, pollution and the entry of foreign substances during long-distance delivery.

10 Marking and labelling

The requirements specified in ISO 21371 shall apply. The following items shall be marked or labelled on the packages:

a) product name;

b) category of the product in the marketed country or region;

c) net mass/quantity;

d) contact information;

e) name of raw materials;

f) warning statements, if any;

g) expiry date;

h) storage method;

i) batch/lot number;

j) CITES conformity;

k) miscellaneous.

Annex A
(informative)

TLC identification

A.1 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 1 g of the powder, add 15 ml of trichloromethane-methanol (9:1), ultrasonicate for 20 min and take supernatant as the test solution.

A.2 Preparation of reference standard solution

Weigh 1 g of *Dendrobium officinale* stem reference drug powder, and treat it in the same manner as in A.1 to prepare the reference standard solution.

A.3 Developing solvent system

Prepare a mixture of methylbenzene, ethyl formate and formic acid in the volume ratio of 6:3:1 as the mobile phase.

A.4 Identification by TLC

Apply 5 µl each of the test solution and the reference standard solution on the same TLC plate (silica gel) previously dried at 110 °C for 15 min in the oven. Develop the plate using the mobile phase, then remove the plate from the chamber and dry. Treat with 10 % sulfuric acid-ethanol solution, and heat for about 3 min at 95 °C. Immediately examine under UV light at 365 nm. The chromatogram of the sample solution exhibits bands corresponding in colour and position to similar bands in the chromatogram of the reference standard solution. Typical TLC chromatograms of *Dendrobium officinale* stem are shown in Figure A.1. The reference standard solution exhibits blue to blue-green bands.

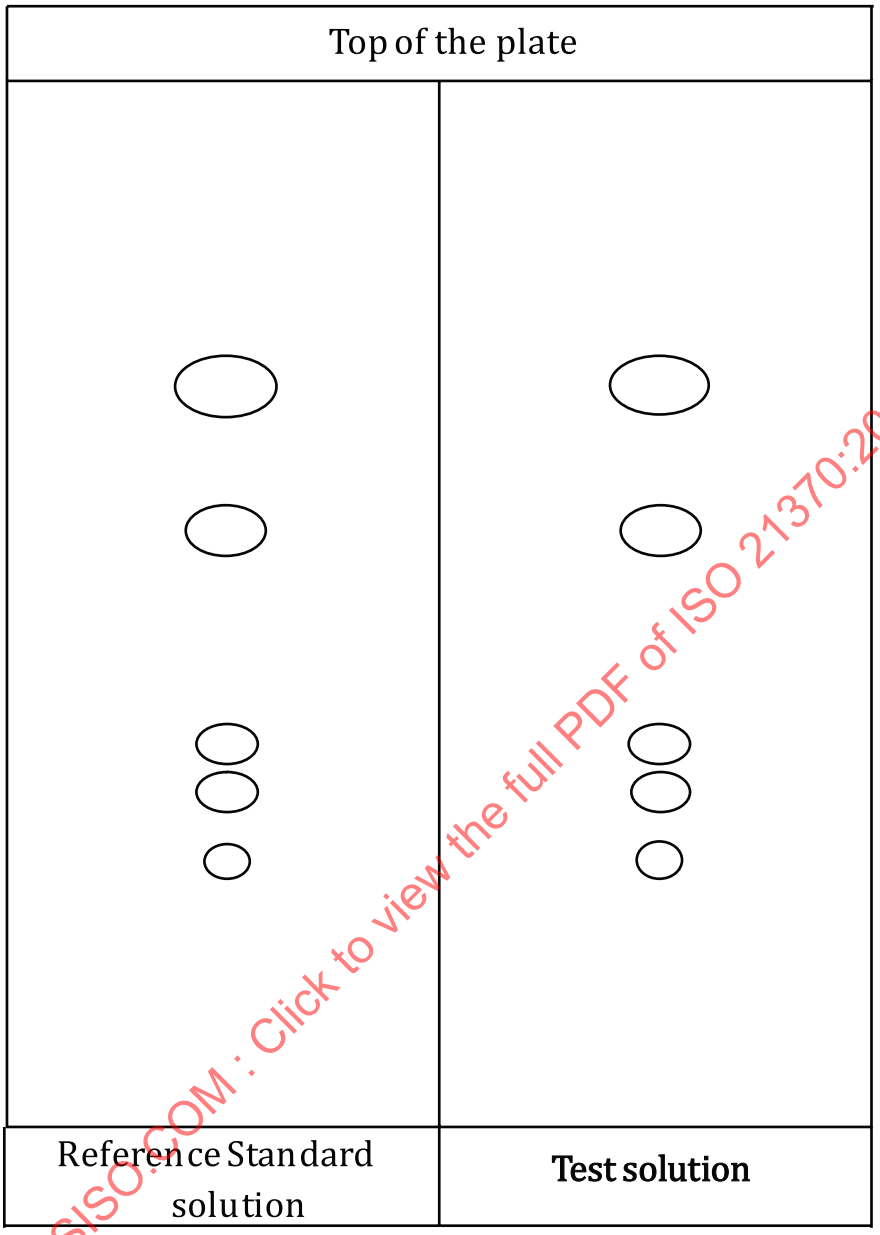


Figure A.1 — Schematic diagram of typical TLC chromatograms of *Dendrobium officinale* stem

Annex B
(informative)

Determination of ethanol-soluble extractives

a) Weigh 250 g of the sample to grind and pass it through a 24-mesh or coarse sieve. Weigh approximately 4 g of the powder into a 250-ml stopper conical flask. Accurately add 50 ml ethanol. Weigh and allow to stand for 1 h.

b) Heat it under reflux to slightly boil on a water bath for 1 h. Cool and weigh again. Replenish the loss of mass with ethanol, mix well and filter.

c) Weigh a dried evaporating dish. Transfer 25 ml of the successive filtrate into the evaporating dish. Evaporate the filtrate to dryness on a water bath.

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

e) Calculate the mass fraction of ethanol-soluble extractives, m_{ese}, on the dried basis (%) with Formula (B.1).

$$m_{ese} = (m_1 - m_0) \times 2 / m_s \times 100 \quad \text{(B.1)}$$

where

m_1 is the mass of the evaporating dish and residue after drying (g);

m_0 is the mass of the evaporating dish (g);

m_s is the mass of the sample (g).

Annex C
(informative)

Determination of polysaccharides

C.1 Principle of the test method

The phenol-sulfuric acid method is employed to determine the content of polysaccharides. In this method, the concentrated sulfuric acid breaks down the polysaccharides to monosaccharides. Pentoses (5-carbon monosaccharides) are then dehydrated to furfural, and hexoses (6-carbon monosaccharides) to hydroxymethyl furfural. These compounds then react with phenol to produce orange-yellow complexes that can be measured spectrophotometrically. Glucose is generally used as the reference standard in this method. Therefore, in this method, the content of the monosaccharide (glucose) can reflect the content of polysaccharides.

C.2 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80-mesh or finer sieve. Accurately weigh 0,3 g of the powder and transfer to a flask. Add 200 ml of water, heat and reflux for 2 h, and cool. Transfer the solution to a 250-ml volumetric flask, rinse the flask three times with 5 ml of water, and transfer the washings to the volumetric flask. Dilute with water to volume, mix and filter. Discard the first portion of the filtrate, and transfer 2 ml of the filtrate to a 15-ml centrifugal tube. Add 10 ml of ethanol to the centrifugal tube, mix and chill for 1 h. Centrifuge for 20 min, discard the supernatant and wash the precipitate with 8 ml of 80 % ethanol twice. Centrifuge again and discard the supernatant. Dissolve the precipitate with hot water and transfer the solution to a 25-ml volumetric flask. Cool, dilute with water to volume and mix.

C.3 Preparation of reference standard solution

Accurately weigh a quantity of anhydrous glucose to a measuring flask, then dissolve in water to prepare a solution containing 90 µg anhydrous glucose per millilitre as the reference standard solution.

C.4 Construction of calibration curve

Separately transfer 0,2 ml, 0,4 ml, 0,6 ml, 0,8 ml and 1,0 ml of the reference standard solution to 10-ml test tubes with glass stopper, dilute with water to 1,0 ml, add 1,0 ml of freshly prepared 5 % phenol solution and mix. Add 5,0 ml of sulfuric acid and mix. Heat for 20 min in a boiling water bath then cool the tube in an ice bath for 5 min. Determine the absorbance of the samples at 488 nm using an ultraviolet-visible spectrophotometer. Construct the calibration curve by plotting the absorbance (y-axis) against the concentration of the glucose (x-axis).

C.5 Content of polysaccharides

Transfer 1,0 ml of the sample solution to a 10-ml test tube with glass stopper, and determine the absorbance according to the method in C.4 (beginning from “add 1,0 ml of freshly prepared 5 % phenol

solution"). Calculate the content of glucose in test solutions using the calibration curve. The content of polysaccharides, W_{pol} (%), is calculated with Formula (C.1):

$$W_{pol} = \frac{\frac{(a-b)}{c} \times 250 \times 25}{m_s \times 2 \times 10^6 \times (1 - W_m)} \times 100 \quad \text{(C.1)}$$

where

a is the absorbance of the test solution;

b is the intercept of the calibration curve;

c is the slope of the calibration curve;

m_s is the mass of the sample (g);

W_m is the moisture content of the sample (%).

Annex D
(informative)

Determination of mannose and peak area ratio of mannose to glucose

D.1 Principle of the test method

Polysaccharides of *Dendrobium officinale* are mainly composed of mannose and glucose. The content of mannose and peak area ratio of mannose to glucose can be used to distinguish *Dendrobium officinale* from other Dendrobium species.

D.2 Preparation of internal standard solution

Accurately weigh a quantity of glucosamine hydrochloride to a measuring flask, dissolve in water to prepare a solution containing 6 mg per ml as the internal standard solution.

D.3 Preparation of reference standard solution

Transfer 10 mg of mannose and 10 mg of glucose, accurately weighed, to a 100-ml volumetric flask, accurately add 1 ml of the internal standard solution, dilute with water to volume and mix. Transfer 400 µl of the solution to a tube, separately add 400 µl of 0,5 mol/l PMP (1-phenyl-3-methyl-5-pyrazolone) in methanol and 0,3 mol/l NaOH to the tube and mix. Heat for 100 min in a water bath at 70 °C. Add 500 µl of 0,3 mol/l hydrochloric acid solution and mix. Wash with 2 ml of trichloromethane three times and discard the trichloromethane. Centrifuge and take 10 µl of supernatant for injection.

D.4 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80-mesh or finer sieve. Accurately weigh 0,12 g of the powder and transfer to a soxhlet extractor. Add q.s. 80 % ethanol solution, heat under reflux for 4 h, discard the ethanol solution and evaporate the residue to dryness. Transfer the residue and the filter paper to a beaker, accurately add 100 ml of water and 2 ml of the internal reference standard solution, heat for 1 h and stir well. Cool the beaker, add water to 100 ml, mix and centrifuge. Transfer 1 ml of the supernatant to a headspace vial, add 0,5 ml of 3,0 mol/l hydrochloric acid solution, seal and mix. Heat for 1 h at 110 °C for hydrolysis, cool and adjust the pH value to 7 with 3,0 mol/l NaOH. Transfer 400 µl of the solution to a tube and prepare the sample solution according to the method in D.3 (begin from "separately add 400 µl of 0,5 mol/l PMP in methanol and 0,3 mol/l NaOH to the tube").

D.5 Chromatographic condition

D.5.1 Column.

D.5.1.1 Stationary phase: octadecylsilane bonded silica gel or equivalent.

D.5.1.2 Size: l = 250 mm, $Ø$ = 4,6 mm, particle size = 5 µm.

D.5.1.3 Theoretical plates: not less than 4 000.

D.5.1.4 Column temperature: 25 °C.